SULPHASALAZINE IN RHEUMATOID ARTHRITIS

THOMAS PULLAR MB ChB (Commendation) MRCP (UK)

Thesis submitted for the degree of MD

University of Glasgow

Centre for Rheumatic Diseases
University Department of Medicine,
Royal Infirmary,
Glasgow

July 1986

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

The following papers based on work in this thesis have been published or accepted for publication:-

1. Pullar T, Hunter J A, Capell H A. Sulphasalazine in rheumatoid arthritis: a double blind comparison of sulphasalazine with placebo and sodium aurothiomalate. Br Med J 1983; 287: 1102-1104.

2. Pullar T, Capell H A. Sulphasalazine a "new" antirheumatic drug. Br J Rheumatol 1984; 23: 26-34.

3. Pullar T, Capell H A. A rheumatological dilemma: Is it possible to alter the course of rheumatoid arthritis? Can we answer the question? Ann Rheum Dis 1985; 44: 134-40.

4. Pullar T, Hunter J A, Capell H A. Which is the active component of sulphasalazine in rheumatoid arthritis? Br Med J 1985; 290: 1535-1538.

5. Pullar T, Hunter J A, Capell H A. Effect of acetylator phenotype on the efficacy and toxicity of sulphasalazine in rheumatoid arthritis. Ann Rheum Dis 1985; 44: 831-837.

6. Pullar T, Hunter J A, Capell H A. Sulphasalazine in the treatment of rheumatoid arthritis: relationship of dose and serum levels to efficacy. Br J Rheumatol 1985; 24: 269-276.

7. Pullar T, Capell H A. Selection of suitable patients for second line therapy in rheumatoid arthritis. Br J Rheumatol (in press).

The following papers on this subject have been presented by myself at the following scientific meetings:-

1. Sulphasalazine as a second line agent in rheumatoid arthritis. Xth European Congress of Rheumatology, Moscow, July 1983.

2. Sulphasalazine in rheumatoid arthritis: Results at one year. Scottish Rheumatology Club, Aberdeen, November 1983.

3. Studies of sulphasalazine in rheumatoid arthritis. Symposium on Current Immunological Concepts in Rheumatology. Budapest, September 1984.

4. Pharmacokinetics of sulphasalazine in elderly rheumatoid patients. Sulphasalazine Workshop, Birmingham, October 1984.

5. Effect of sulphasalazine on scavengers of oxygen derived free radicals in rheumatoid arthritis. British Society for Rheumatology, AGM, London, November 1984.

6. Relationship of dose and serum levels to efficacy of sulphasalazine in rheumatoid arthritis. British Society for Rheumatology, AGM, London, November 1984.

7. Toxicity of sulphasalazine in patients with rheumatoid arthritis. British Society for Rheumatology, Manchester, March 1985.

8. Which is the active moiety of sulphasalazine in rheumatoid arthritis? British Society for Rheumatology, Manchester, March 1985.

9. Practical problems in studies of second line antirheumatic drugs: can they be overcome? Northern Universities Clinical Pharmacology Group, Leeds, May 1985.

10. Does previous second line therapy influence the clinical response to sulphasalazine in rheumatoid arthritis? British Society for Rheumatology, AGM, London, Nov 1985.

Table of Contents

List of Tables

Table XLIX — Pattern of change in inflammatory indices in patients who had received no previous second line drugs, in patients who had received 1 previous second line drug and in patients who had received 2 or more previous second line drugs and a comparison between the percent change in the 3 groups (Kruskal-Wallis).

Table L — Pattern of change in inflammatory parameters over 24 weeks in patients with ESR $\leq$30mm/hr and in patients with ESR >30mm/hr and comparison of the percent change between weeks 0 and 24 in the two groups (Mann-Whitney U Test).

Table LI — Pattern of change in inflammatory indices over 24 weeks for patients with DAI $\leq$14 and those with DAI >14 and comparison of the percent change in the two groups (Mann-Whitney U Test).

Table LII — Medians (ranges) and p values (Wilcoxon) for inflammatory parameters in sulphasalazine treated patients who had thiols measured at wks 0, 6, 12 and 24 (n = 22).

Table LIII — Changes in scavengers of oxygen derived free radicals in patients completing 24 weeks sulphasalazine therapy (n = 22).

Table LIV — Medians (and ranges) for various parameters at week 0 and week 24 for sulphapyridine and 5-ASA treated patients.

Table LV — Reasons for and time of discontinuation of therapy in sulphapyridine and 5-ASA treated patients.

Table LVI — p values for weeks 0 versus 24 in both patient groups (Wilcoxon matched-pairs signed-rank test) and for sulphapyridine week 24 versus 5-ASA week 24 values (Mann-Whitney U Test).

Table LVII — p values for Wilcoxon matched-pairs signed-rank test wk 0 versus wk 6 and wk 0 versus wk 12 for both treatments.

Table LVIII — Pattern of leucopenia in sulphasalazine treated patients.

List of Illustrations

Fig. XVII Serum sulphasalazine (SASP) levels in patients allocated to high dose (3g/day) and low dose (1.5g/day).

Fig. XVIII Correlation between actual 24 week dose of sulphasalazine expressed as mg/kg body weight and serum sulphasalazine (SASP) levels (Spearman Rank).

Fig. XIX Correlation between actual 24 week sulphasalazine (SASP) dose expressed as mg/kg body weight and serum levels of unmetabolised sulphapyridine (SP) (Spearman Rank).

Fig. XX Relationship of serum levels of unmetabolised sulphapyridine (SP) to acetylator phenotype in high and low dose groups.

Fig. XXI Relationship of serum levels of acetylated sulphapyridine (ASP) to acetylator phenotype in high and low dose groups.

Fig. XXII Relationship of serum levels of total sulphapyridine (total SP) to acetylator phenotype in high and low dose groups.

Fig. XXIII Study 3. Median ESR wks 0 and 24 in fast acetylator/high dose group and slow acetylator/low dose group.

Fig. XXIV Reasons for discontinuing therapy in the fast and slow acetylator groups.

Fig. XXV Sulphasalazine concentration-time curves on two separate occasions for patient No 6 (Study 4).

Fig. XXVI Unmetabolised sulphapyridine concentration-time curves on two separate occasions for patient No 6 (Study 4).

Fig. XXVII Total sulphapyridine concentration-time curveson two separate occasions for patient No 6 (Study 4).

Fig. XXVIII Mean sulphasalazine concentration-time curves for those patients who continued treatment and those who stopped because of side effects.

Fig. XXVIX Mean unmetabolised sulphapyridine concentration-time curves for those patients who continued treatment and those who stopped because of side effects.

Fig. XXX Mean total sulphapyridine concentration-time curves for those patients who continued therapy and those who stopped because of side effects.

Fig. XXXI Reasons for discontinuing therapy in the elderly and the younger age groups.

Fig. XXXII Effect of access to prochlorperazine on drop-out rate and achievement of allocated dose in the sulphasalazine treated patients.

Fig. XXXIII Median (range) red cell lysate superoxide dismutase (SOD) activity in 22 sulphasalazine treated patients over a 24 week treatment period.

Fig. XXXIV Median (range) red cell lysate thiol levels in 22 sulphasalazine treated patients over a 24 week treatment period.

Fig. XXXV Median (range) plasma thiol levels in 22 sulphasalazine treated patients over a 24 week treatment period.

Fig. XXXVI Correlation between the change in total plasma thiol and the change in ESR in 22 sulphasalazine treated patients over a 24 week treatment period (Spearman Rank).

Fig. XXXVII Sulphasalazine and its azo cleavage products.

Fig. XXXVIII Change in ESR over 24 week period in sulphapyridine and 5-ASA treated patients.

Fig. XXXIX Change in haemoglobin over 24 week period in sulphapyridine and 5-ASA treated patients.

Fig. XL Change in hand grip strength over 24 week period in sulphapyridine and 5-ASA treated patients.

ACKNOWLEDGEMENTS

I should like to thank the following people without whose help this thesis could not have been completed:-

Dr R D Sturrock, Dr H A Capell, Dr J Hunter and Professor A C Kennedy for their invaluable support and advice, for allowing me to study their patients, for giving me the time to do so and for nurturing my interests in rheumatology. The late Dr J Markson for his many useful comments. Sister A Thomson for her metrology help.

Mr R M Murdoch, Dr D Hole, Mrs R Campbell and Mrs D McKnight for their help and advice with statistics and computing.

Professor D H Lawson for his advice on the interpretation of the pharmacokinetic study.

Mr F Khan, Dr W E Smith and Dr D Brown for measuring thiol and superoxide dismutase levels and for teaching me something about free radical chemistry.

Dr P Smith and colleagues from Pharmacia GB and Pharmacia AB for supplying sulphasalazine and placebo tablets, for financial support for Sister A Thomson and for measuring drug levels.

Tillot Laboratories and May and Baker Ltd for supplies of 5-aminosalicylic acid and sulphapyridine respectively.

The Biochemistry, Haematology and Immunology Departments of the Royal Infirmary, Gartnavel General Hospital and the Western Infirmary, Glasgow and especially Dr A Shenkin, Mr J Kneppel, Dr D Neithercut and Professor K Whaley for carrying out laboratory measurements, routine and otherwise.

Ms B Burns, Ms J McDonald and Mr F Spiers for their graphics and photography.

Dr B McConkey for providing photographs of himself and Professor Svarz.

Miss D Crawford for typing the manuscript, reading my writing and uncomplainingly making innumerable alterations.

My parents and Irene for putting up with me while working on this thesis.

Finally and most importantly I should like to give my warmest thanks to all the patients who agreed willingly to give up their time to participate in these studies.

Acknowledgement of collaborative work:

The work presented in this thesis was carried out and the thesis composed by myself with the following exceptions.

The protocol for Study 4 (pharmacokinetics in the elderly) was designed by me in collaboration with Dr M Ryde of Pharmacia AB and serum concentrations of sulphasalazine and its metabolites were measured by Dr Ryde's colleagues at Pharmacia.

Approximately 20% of the patients in Studies 1, 2 and 3 and in the comparison of sulphapyridine with 5-amino salicylic acid were managed at Gartnavel General Hospital by Dr J A Hunter; data from these patients were collected analysed and interpreted by myself.

Measurements of oxygen-derived free radicals (in Chapter 9) were carried out at the University of Strathclyde by Mr F Khan, Dr W E Smith and Dr D Brown.

SUMMARY

Recent open studies of sulphasalazine have suggested that it may have "second line" antirheumatic activity. Previous experience with sulphasalazine in the treatment of inflammatory bowel disease has also suggested that it may be less toxic than the commonly used second line agents. In addition, the complex pharmacology of sulphasalazine may allow manipulation of a number of variables to improve the efficacy:toxicity ratio and may also shed some more light on the rheumatoid disease process. I, therefore, decided to investigate further the use of this drug in rheumatoid arthritis. My specific aims were: (1) to investigate by means of a placebo controlled trial the efficacy of sulphasalazine; (2) to document its toxicity and relate toxicity and efficacy to a number of variables; (3) to investigate its single dose pharmacokinetics; (4) to define its optimal clinical use; (5) to examine for an effect on free radical scavengers as a potential mode of action; (6) to identify its active therapeutic moiety.

The first study described is a double blind comparison of sulphasalazine 3g/day, placebo and sodium aurothiomalate (GST) in 90 patients randomised to one of the three treatments. At 24 weeks significant improvement was seen in inflammatory indices in sulphasalazine and GST but not placebo groups and the degree of improvement in the sulphasalazine and GST groups was also greater than that in the placebo group. Improvement was apparent by 6 weeks and was maintained until at least week 48. The drop out rate in the sulphasalazine group was similar to that in the GST group. The commonest reason for discontinuing sulphasalazine was nausea/vomiting

but potentially serious toxicity did occur.

In an attempt to relate dose and serum levels to efficacy a further 60 patients were randomised to sulphasalazine 3.0g/day or sulphasalazine 1.5g/day. There was a statistically non-significant trend towards greater efficacy and toxicity in the 3.0g/day group. When analysis was carried out with dose expressed as mg/kg body weight, however, a direct relationship was demonstrated between dose and degree of improvement and the optimum therapeutic dose was in excess of 40mg/kg/day. No direct relationship could be demonstrated between efficacy and serum levels of sulphasalazine and its metabolites.

Analysis of the 60 patients who had been randomised to 3.0g/day in the above two studies demonstrated no difference in efficacy between slow and fast acetylators. In a further prospective study of 60 patients, fast acetylators (40 patients) were assigned to 3.0g/day and slow acetylators (20 patients) to 1.5g/day. No significant difference was demonstrated between the groups but there was a trend towards greater improvement in the fast acetylator group. These results imply that any tendency for fast acetylators to do less well (as may have been expected) is unlikely and if it does exist is of minimal importance in comparison to the effect of dose. Overall, acetylator phenotype results were available in 149 patients and nausea/vomiting was more common among the slow acetylators. No excess serious toxicity was apparent in slow acetylators.

Eight elderly patients underwent single dose pharmacokinetic studies before chronic dosing was commenced. Four patients subsequently discontinued therapy because of nausea/vomiting and these patients had achieved higher peak levels and areas under the curve (AUCs) for

sulphasalazine and its metabolites. The elderly patients, however, excreted in their urine, as sulphasalazine and its metabolites, a greater proportion of the ingested dose than a previously documented group of young normal volunteers.

Of the total of 158 patients treated with sulphasalazine 31 were $\geq$65 years old. Significantly more elderly patients had to stop because of adverse effects. No single adverse reaction could be identified as more common in those patients.

Further analysis of these 158 sulphasalazine treated patients demonstrated no association between gender, disease duration, previous second line drugs or disease activity and either toxicity or efficacy. In this group, potentially serious toxicity was common (7 leucopenias, 2 hepatitis, 1 thrombocytopenia). These problems, however, all occurred within the first 12 weeks of treatment.

Twenty-two consecutively treated patients who achieved 24 weeks therapy had serial measurements of red cell superoxide dismutase activity, red cell thiol levels and plasma thiol levels carried out. Changes in these parameters similar to those seen with other second line drugs were seen. This suggests that, although it lacks an intrinsic aliphatic thiol group, sulphasalazine affects the oxygen derived free radical scavenging system. This may represent a mode of action of sulphasalazine.

Finally, in an attempt to separate efficacy from toxicity and also, perhaps, to give further information on the aetiological and pathological mechanisms of rheumatoid arthritis, a further 60 patients were randomised to sulphapyridine or 5-aminosalicylic acid (5-ASA).

Significant improvement was seen in the sulphapyridine but not the 5-ASA group and by the end of 24 weeks (although initially compatible) the sulphapyridine patients had less active disease.

From the data provided in this thesis I can draw the following conclusions:-

1) In a placebo controlled trial sulphasalazine was an effective second line agent.

2) Potentially serious toxicity may occur but tends to occur within the first 12 weeks of treatment and the most intensive monitoring can be concentrated over this period.

3) Sulphasalazine exhibits a dose/response relationship in rheumatoid arthritis and the optimum dose is >40mg/kg/day. No relationship can be demonstrated between efficacy and serum levels of sulphasalazine or its metabolites.

4) Other than dose there are no clinical predictors of efficacy.

5) No clinical predictors of potentially serious toxicity can be demonstrated although elderly patients experience a greater overall drop out rate because of toxicity. Slow acetylators have a higher incidence of upper gastrointestinal side effects and patients who experience such symptoms tend to achieve higher peak levels and AUCs for sulphasalazine and its metabolites in single dose studies.

6) Sulphasalazine, although it contains no thiol group, alters the oxygen derived free radical scavenging system and the redox status of red blood cells. This may represent a mode of action.

7) Sulphapyridine and not 5-ASA is the active moiety of sulphasalazine in rheumatoid arthritis. This finding may be of interest in the aetiopathogenesis of the disease.

CHAPTER 1

Sulphasalazine - a "new" drug for the treatment of rheumatoid arthritis. A review of its pharmacology.

Section 1 Introduction and historical background.

Section 2 Chemistry and metabolism.

Section 3 Mode of action in inflammatory bowel disease and immunological effects of sulphasalazine.

Section 4 Adverse effects.

Section 5 Drug interactions.

Section 6 Conclusions.

Summary

Section 1

Introduction and historical background

Rheumatoid arthritis affects approximately 3% of the Western population. In its most severe form it is a relentlessly progressive disease which leads to destruction and failure of the joint. No practical form of therapy, pharmacological or physical, has been shown convincingly in placebo controlled studies consistently to slow or prevent this progression. The substances which have shown most promise in this respect, however, are the so-called second and third line agents such as gold salts, d-penicillamine, chloroquine, levamisole, cyclophosphamide and azathioprine. In addition, these drugs doubtlessly produce major symptomatic relief which cannot be obtained using first line agents (non-steroidal anti-inflammatory drugs) alone. All of these currently used second and third line agents were initially introduced into medicine for the treatment of conditions other than rheumatoid arthritis. One of the major factors limiting the use of these agents is their potential toxicity. Less than 30% of patients commencing such a drug will still be receiving the same agent 4 years later although approximately 66% of those ceasing treatment will have received at least one similar agent and some will require as many as 5 such drugs over a 4 year period (1) (Figs I, II, III). The number of available second and third line drugs is at present severely limited and there is a clear need for additional such agents, preferably with a lower overall toxicity and in particular a lower incidence of potentially serious side effects.

Sulphasalazine (SASP), a drug well known to most physicians for the treatment of inflammatory bowel disease and which is generally

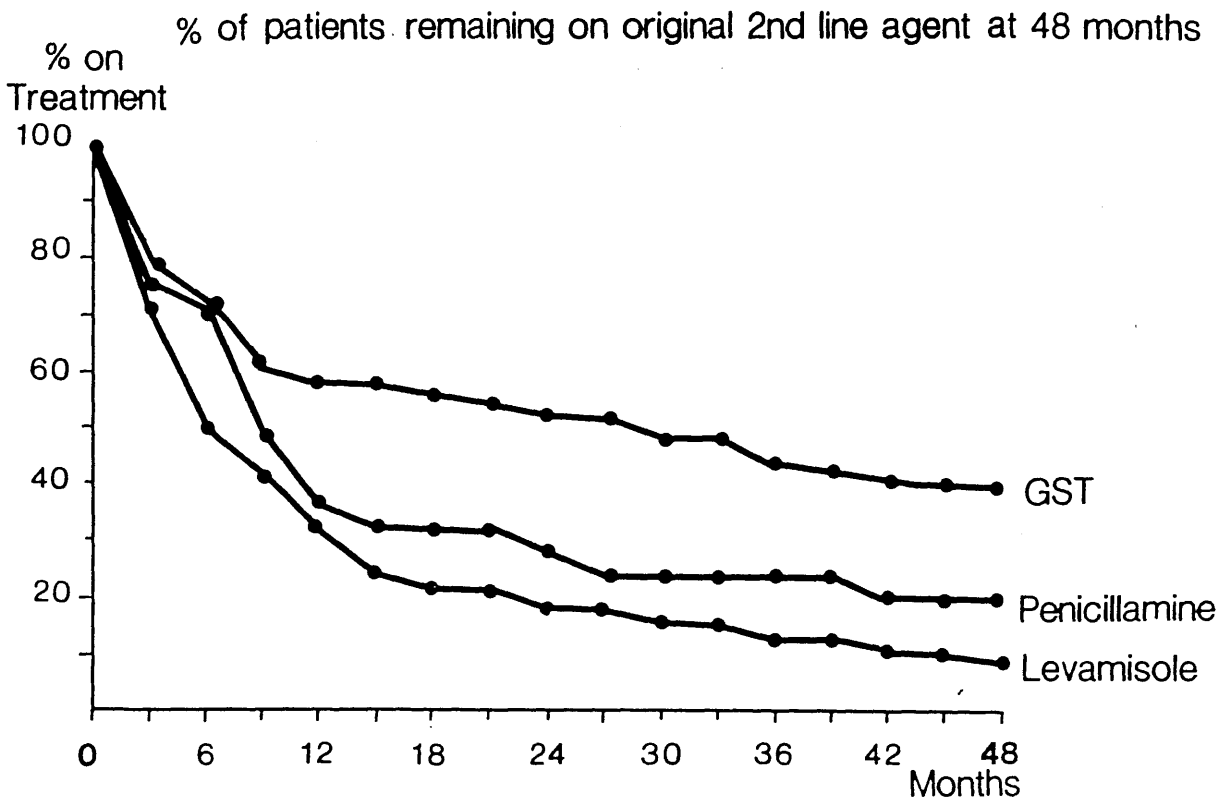


Fig. I Life table showing the rate of drop out from individual second line drugs over a 48 month period.

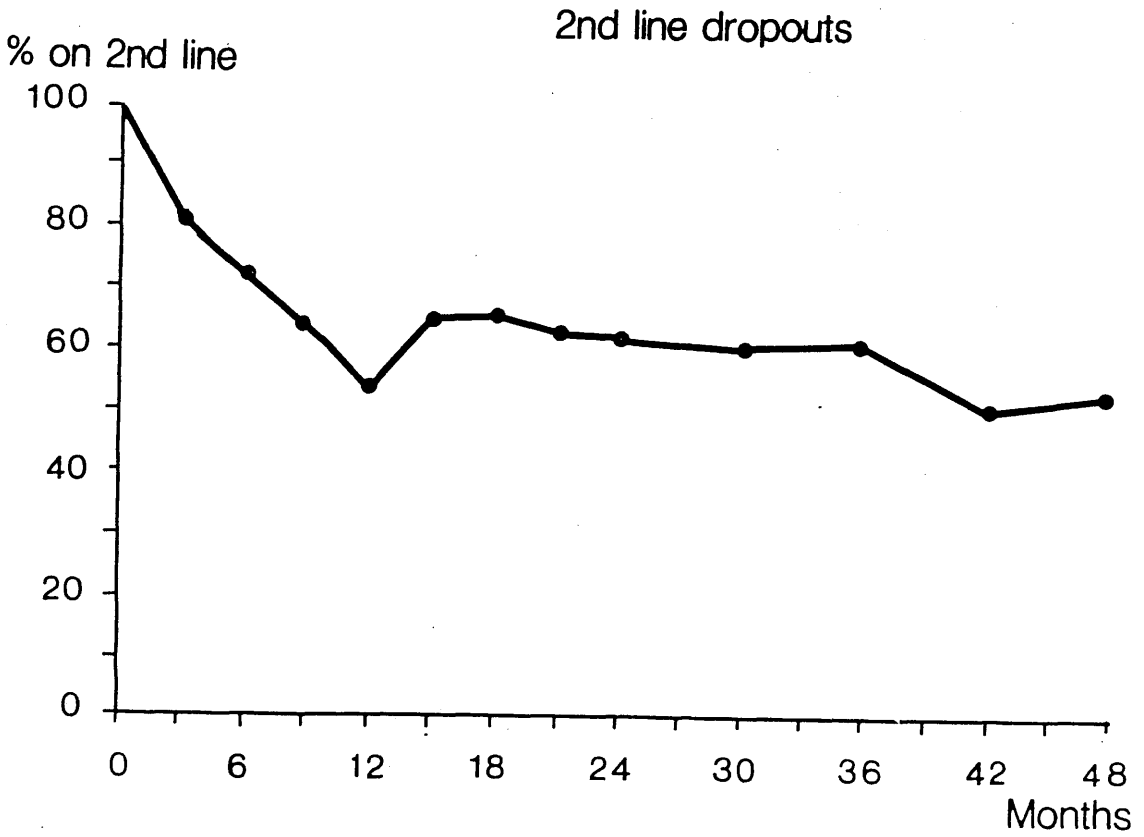


Fig. II Graph showing the percentage of rheumatoid patients commenced on a second line drug who are receiving any second line agent during 4 year follow up.

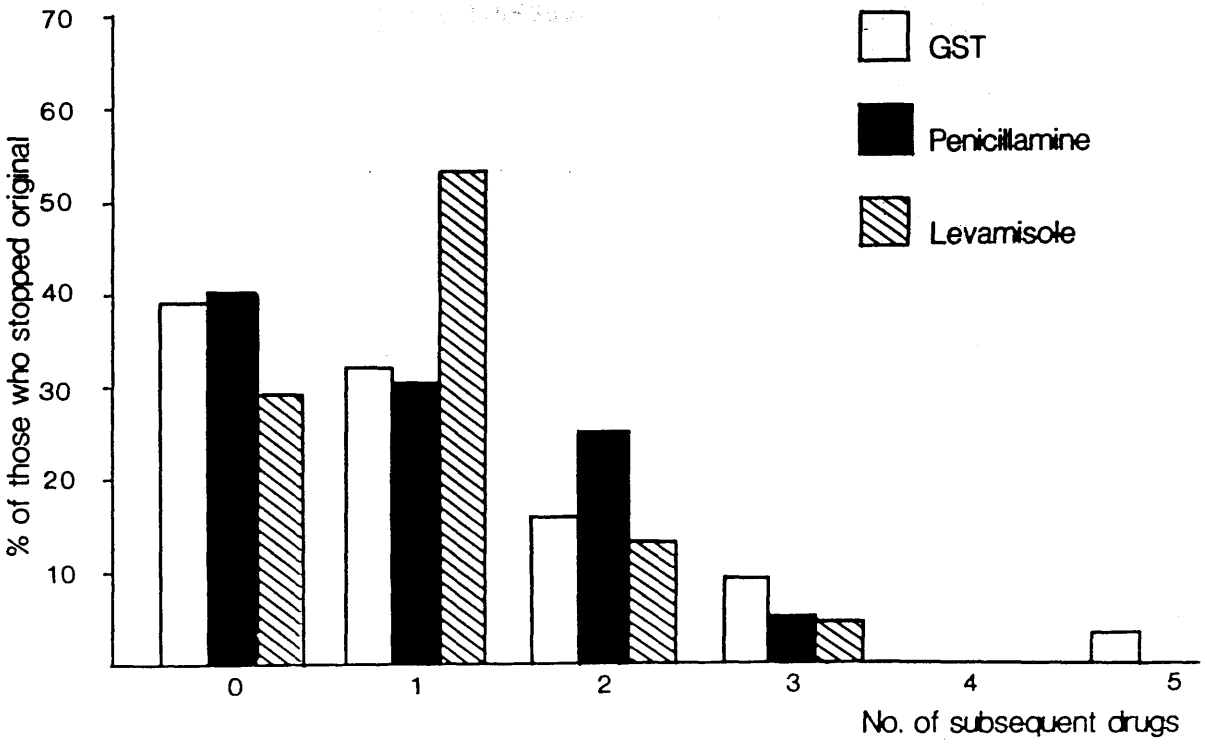


Fig. III Bar chart showing the percentage of patients who progress to further second line drugs after discontinuing their first second line drug.

accepted as a safe, relatively non-toxic drug in this condition, was first introduced by Nana Svarz in 1941 for the treatment of "rheumatic polyarthritis". Nana Svarz believed "rheumatic polyarthritis" had an infective aetiology and thought that the combination of an antibiotic (sulphapyridine) and a salicylate (5-aminosalicylic acid) would be of value. The first report in the English language was published the following year (2). She described 20 selected patients (11 with polyarthritis and 9 with ulcerative colitis) who had responded well to oral sulphasalazine in a dose of 4-6g daily with a subsequent maintenance dose of 1.5-3g/day. She published another study of this drug in arthritis in 1948 (3) with similar results, and postulated that its efficacy might be related to its "affinity for connective tissue". Neither of these studies, however, contained a control group and her polyarthritis group included a wide range of arthritic conditions such as rheumatoid arthritis, ankylosing spondylitis and Reiter's syndrome.

In 1949 Sinclair and Duthie (4) published a study in 60 rheumatoid patients comparing sulphasalazine with intramuscular gold and with no specific treatment (20 in each group). An initial high incidence of toxicity with sulphasalazine forced them to use a smaller maintenance dose than that employed by Svarz. This study showed neither sulphasalazine nor gold to be any better than non-specific treatment for patients with rheumatoid arthritis. All patients, however, were initially hospitalised for at least 4 weeks (median 9 weeks) and during this time received a non-specific regimen of bed rest, dietary supplements, splinting and physiotherapy. Furthermore many of the patients were assessed many months after cessation of drug therapy. These confounding factors make it difficult to draw definite

conclusions from this study. Despite these shortcomings, and the results of a further controlled study published one year later (5) which showed a beneficial effect of sulphasalazine in polyarthritis, the use of this drug declined. Notwithstanding its undoubted benefit in inflammatory bowel disease (6, 7) it remained out of favour in rheumatology for 30 years until McConkey et al published results of an uncontrolled trial which suggested that sulphasalazine might be an effective second line agent (8, 9). Figure IV shows a meeting between Dr McConkey and Professor Nana Svarz in the late 1970's.

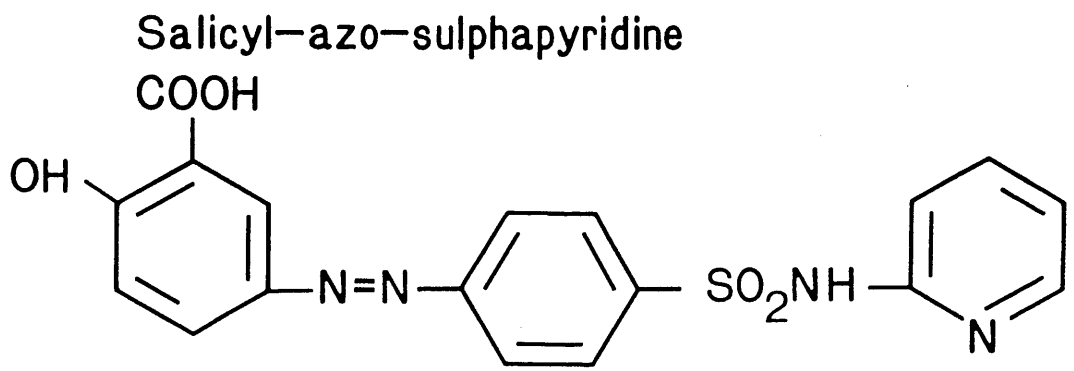
Section 2

Chemistry and metabolism

Sulphasalazine (4- pyridyl-(2)-aminosulphonyl-3-carboxy-4-hydroxybenzol) (Fig. V) is an azo compound of sulphapyridine and 5-aminosalicylic acid (5-ASA). It is a brown-yellow powder which is difficult to dissolve in water and dilute acid but is soluble in alkali and in strong acids (2).

Sulphasalazine is detectable in the serum one and a half hours after ingestion of a single oral dose. In healthy volunteers peak levels are reached after 3-5 hours, it displays a mean serum half-life of 5.7 hours (this rises to 7.6 hours with repeated dosing) and it is almost completely absent from the serum after 24 hours (10, 11). Sulphasalazine is not absorbed from the stomach as it is insoluble in weak acids. Absorption from the small intestine is variable between individuals. Usually less than 5% (no more than 20.5%) of the ingested dose is recoverable unchanged from urine or bile, whereas about 80% of the dose may be excreted as sulphapyridine or its metabolites (11,

Fig. IV Dr Brian McConkey, who reintroduced sulphasalazine into rheumatology, meeting Professor Nana Svarz who originally synthesised the compound.



4 Pyridyl–(2) aminosulphonyl–3–carboxy–4'–hydroxybenzol (sulphasalazine)

Fig. V The structure of sulphasalazine.

12). This implies that a large proportion of ingested sulphasalazine is split into its separate components either before or after absorption. Patients with ileostomies provide a useful model for investigating this question further and in these patients, although the amount of unaltered sulphasalazine obtained from the urine is similar to normal individuals, only about 7% of the ingested dose of sulphasalazine is recoverable from the urine either as sulphapyridine or its metabolites. Biliary excretion of sulphapyridine and its metabolites is negligible. The above data suggest that very little sulphasalazine is metabolised in the small bowel or liver and that most ingested sulphasalazine reaches the caecum unaltered (12, 13). This is confirmed by the finding of about 70% of an ingested dose of sulphasalazine unchanged and another 14% as free sulphapyridine in the ileostomy effluent (13, 14). In the colon sulphasalazine is broken down by a process of azo reduction by the colonic bacteria to sulphapyridine and 5-aminosalicylic acid (5-ASA). The finding that serum and urine levels of sulphapyridine and its metabolites are significantly higher in patients with transverse colostomies than patients with ileostomies suggests that most of the cleavage takes place in the caecum or proximal colon. Van Hees (16) has shown that less than 1% of an oral 3g dose of sulphasalazine is recoverable in its original form from the faeces in normal subjects and less than 8% can be recovered as sulphapyridine. In contrast to sulphapyridine at least 50% of the 5-ASA component of the original sulphasalazine is excreted in the faeces. Thus almost all the sulphasalazine which reaches the colon is split into its two components and while the sulphapyridine is largely absorbed the 5-ASA remains mainly within the colonic lumen. Serum levels of 5-ASA (primarily in free form) in

ulcerative colitis patients reach only 1-4ug/ml and urinary excretion (mainly as acetyl-5-ASA) is usually around 22% (range 1-40%) of the ingested quantity (12, 17). Sulphapyridine is not identifiable in the serum until 3-5 hours after ingestion of sulphasalazine and reaches peak levels after about 24 hours (11) whereas, with an equimolar dose of sulphapyridine, peak serum concentration is reached after only one and a half hours; this lag is explained by the delay in absorption of the sulphapyridine moiety of sulphasalazine (10). Steady state concentration of sulphapyridine is reached by 5 days (10). Once absorbed, sulphapyridine, which is highly protein bound, undergoes N-acetylation and ring hydroxylation in the liver and is finally conjugated with glucuronic acid. Unchanged sulphapyridine plus acetylsulphapyridine comprise approximately 75-90% of total circulating sulphapyridine (18). Metabolites of sulphapyridine are excreted by the kidneys more rapidly than unmetabolised sulphapyridine. The rate of acetylation is genetically determined, there being two types of acetylator:- fast (autosomal dominant) and slow (autosomal recessive). This polymorphism governs the activity of the hepatic acetyl transferase enzyme (19, 20) (Fig. VI). In Western Europe approximately 60% of the population are slow acetylators and 40% fast acetylators (20). Elimination half-life of sulphapyridine is 5.5 hours in fast acetylators and 15.3 hours in slow acetylators (21).

There is some controversy regarding the effect of acetylator status on steady state serum sulphapyridine levels. Schroder et al (22) found no significant difference in serum total sulphapyridine levels between slow and fast acetylators after 72 hours of sulphasalazine treatment, although they did demonstrate a difference after 24 hours and showed a greater serum concentration of acetylsulphapyridine and lower

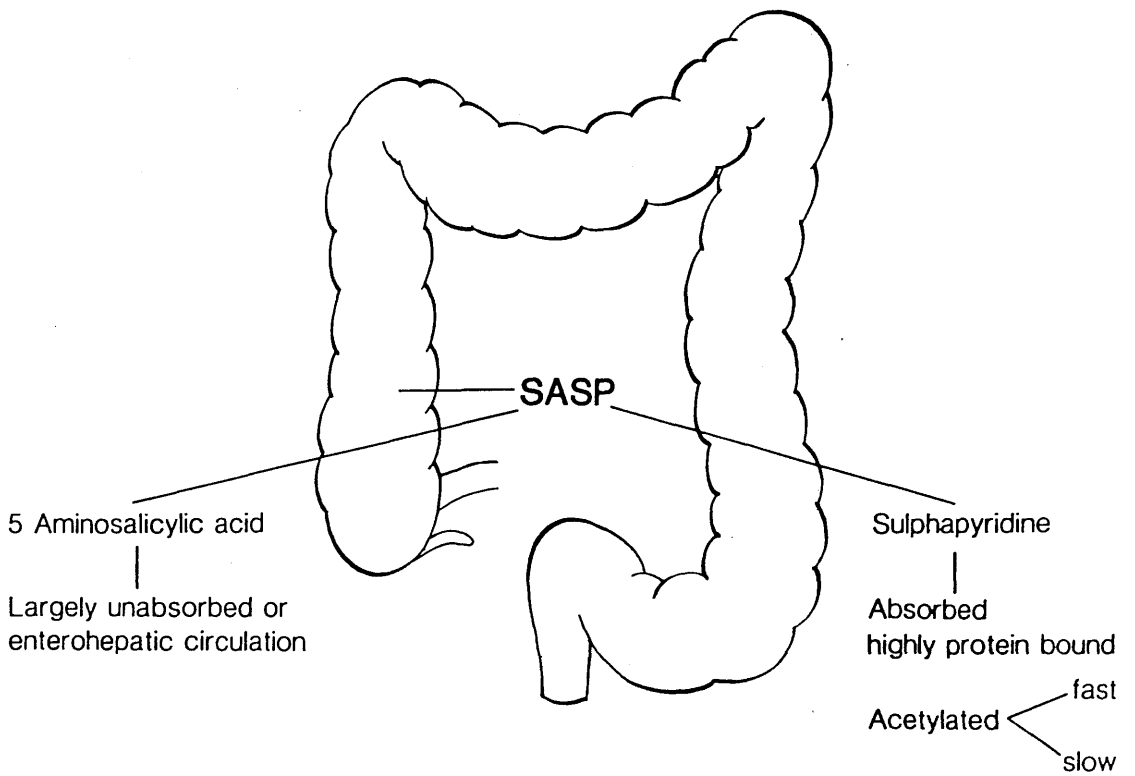


Fig. VI The fate of the sulphasalazine molecule after it reaches the large bowel.

concentration of unmetabolised sulphapyridine in fast acetylators after both 24 and 72 hours. On the other hand, Van Hees (16), Das et al (17) and Azad Khan et al (23, 24) showed a significantly lower steady state concentration of total sulphapyridine in fast acetylators. As sulphapyridine is more rapidly excreted as its acetyl metabolite (acetylsulphapyridine) one might expect total serum sulphapyridine to be greater in slow acetylators. There is good evidence that normal volunteers and patients with ulcerative colitis who are slow acetylators have a higher incidence of adverse effects (23, 25).

After administration of sulphasalazine orally, sulphasalazine, sulphapyridine, N-acetylsulphapyridine, sulphapyridine-O-glucuronide, acetylsulphapyridine-O-glucuronide, N-acetyl-5-OH-sulphapyridine-O-glucuronide, 5-ASA and acetyl-5-ASA can all be detected in urine (18); 2.5% of the total dose is excreted in the bile as sulphasalazine and a further 0.45% as sulphapyridine or its derivatives (12).

Section 3

Mode of action in inflammatory bowel disease and immunological effects of sulphasalazine

Sulphasalazine was first introduced because of the theoretical beneficial effects of a combined antibacterial and anti-inflammatory agent in ulcerative colitis and rheumatoid arthritis, both of which were thought, possibly, to have an infective aetiology. This was despite the findings of earlier studies showing sulfanilamide compounds alone to have no effect in either of these conditions (26). One reason which was postulated at this time for the effectiveness of

sulphasalazine was its "affinity for connective tissue and thus its ability to deliver its active ingredients to the required sites" (3, 27).

It is unclear whether it is the sulphasalazine, sulphapyridine, 5-ASA or one of their metabolites which is the active agent in ulcerative colitis.

Less than one third of ingested sulphasalazine is absorbed (12) and serum levels of sulphasalazine bear no relationship to the efficacy of the drug in ulcerative colitis (17). This suggests that circulating sulphasalazine is unlikely to be the active component in this condition.

Das et al (17), claimed a relationship between circulating sulphapyridine levels and the efficacy of sulphasalazine in ulcerative colitis. They reached this conclusion, however, by the observation that patients who did not respond to therapy had lower serum total sulphapyridine levels than responders and on increasing the dose of sulphasalazine clinical improvement was accompanied by a concomitant rise in blood levels of sulphapyridine. This relationship could be explained by reduced sulphapyridine absorption in the active phase of the disease and this explanation is supported by Azad Khan who demonstrated no relationship between circulating total sulphapyridine levels and the relapse rate in ulcerative colitis (24).

It has been suggested that <u>in vivo</u> sulphasalazine alters the immune process by decreasing the number of activated monocytes and B-lymphocytes and increasing T-lymphocyte numbers and function although these findings could not be repeated <u>in vitro</u> using sulphasalazine or

its metabolites (28). Thayer et al (29), although they demonstrated a reduction in the numbers of complement receptor bearing B-lymphocytes, could show no effect on T-lymphocytes. Other workers have demonstrated that, in vitro, sulphasalazine and sulphapyridine inhibit the natural killer activity of peripheral mononuclear cells whereas even at high concentrations 5-ASA produces only minimal inhibition of this function (30). In vitro sulphasalazine also almost completely inhibits the synthesis of 5-hydroxy-6, 8, 11, 14-eicosatetranoic acid (5-HETE) and 5, 12-dihydroxy-6, 8, 11, 14-eicosatetranoic acid (5, 12-di-HETE) (leucotriene B4) by human neutrophils (31), and blocks binding at neutrophil derived peptides that activate chemotaxis and superoxide production (32). Similar but less marked effects were found with 5-ASA with only minimal inhibition by sulphapyridine. In addition sulphasalazine has been shown, in an animal model, to suppress specific antibody production in the intestine and also, possibly, to produce non-immunologically active antigen binding substance (33). It would seem, therefore, that sulphasalazine possesses immune regulating properties but the exact mechanisms require clarification.

Patients with ulcerative colitis have been shown to have raised faecal and colonic venous blood prostaglandin E_2 (PGE_2) and in vitro both sulphasalazine and 5-ASA by itself have been shown similarly to inhibit either prostaglandin synthesis or the interaction of PGE_2 with receptors (34).

It has also been shown that 5-ASA suppositories or retention enemas are of benefit in ideopathic proctitis (35, 36) and that oral 5-ASA appears as effective as sulphasalazine in ulcerative colitis (37).

Finally, sulphasalazine has been shown to alter bowel flora in patients with ulcerative colitis (38) and this is most likely to be a function of the sulphapyridine component. The mode of action of sulphasalazine in inflammatory bowel disease is, therefore, unclear although a local anti-inflammatory activity of 5-ASA would seem most likely.

Section 4

Adverse Effects

Svartz (2) found side effects (with the exception of fever and rash) to be rare and she listed nausea and vomiting, "cyanosis", nephrolithiasis, anaemia, fever and exanthem as observed side effects. She also described a fatal case of "agranulocytosis" from another hospital. Das et al (25) and Van Hees (16) have added to this list:- frank haemolysis, transient reticulocytosis, macrocytosis, leucopenia, pancytopenia, thrombocytopenia, folate deficiency, headaches, dizziness, anorexia, and epigastric discomfort. Other workers have reported male infertility (39, 40), hepatic hypersensitivity (41, 42, 43), systemic granulomatous reactions (44), late hepatic toxicity (45), pancreatitis (46), neurotoxicity (47), pulmonary involvement (48, 49, 50, 51, 16) and drug induced systemic lupus erythematosus (52, 53, 54) as possible toxic effects.

Das (18) reviewed a number of studies of adverse reactions to sulphasalazine and quoted an overall incidence ranging from 15-70%. The actual incidence appears to be related to the dose of sulphasalazine, total sulphapyridine serum levels and acetylator status (16, 23, 25).

Nausea and Vomiting

Das (25) found nausea or vomiting within a few days of starting treatment in 6 out of 34 patients with inflammatory bowel disease. Two patients developed their symptoms after the first dose and they proved to be fast acetylators. The other 4 developed symptoms over the next few days and they were all slow acetylators. In these patients symptoms were related to high serum levels of total sulphapyridine. The fact that these symptoms are related to serum levels of sulphapyridine would suggest a central mechanism for nausea and vomiting.

"Cyanosis"

The exact nature of the blue discolouration which affects some patients on sulphasalazine is not clear. It is said not to be related to haemoglobin oxygenation, or to the formation of methaemoglobin or sulphaemoglobin (2). It is closely linked to the dose of sulphasalazine and serum levels of total sulphapyridine (25). This side effect has not been reported during more recent studies of sulphasalazine therapy.

Nephrolithiasis

Apart from a mention in Svartz's paper (2) this does not appear to have been a problem.

Anaemia

The major cause of anaemia during sulphasalazine therapy appears to be haemolysis. Although frank haemolytic anaemia is relatively rare, evidence of haemolysis is commonly detected in about 50% of patients receiving sulphasalazine. Haemolysis during sulphasalazine treatment is due to decreased membrane stability and is associated with an increase in methaemoglobin and occasionally the production of Heinz bodies. Haemolysis seems to be related to total serum sulphapyridine levels and those with frank anaemia may be those with the highest levels (16, 55). Folate deficiency has also been described in patients taking sulphasalazine and Franklin et al (56) suggest that sulphasalazine acts as a competitive inhibitor of folate absorption. Selhub et al (57) also found sulphasalazine to be a competitive inhibitor of dihydrofolate reductase, methylenetetrahydrofolate reductase and serine transhydroxymethylase activity (all 3 enzymes are involved in folate metabolism).

Van Hees (16), on the other hand, failed to confirm this mechanism and suggests that folate deficiency is secondary to chronic haemolysis resulting in increased folate utilisation.

Leucopenia and agranulocytosis

This is a rare complication but probably the most serious. Less than 30 cases have been reported but at least 5 deaths have occurred (16). One case of pancytopenia has also been reported.

Skin Rashes

The incidence of skin rash with sulphasalazine is poorly recorded but appears to vary between 0-10% (16, 22, 25) and, as with agranulocytosis, is not dose related. The rashes are usually mild but occasionally more serious rashes, eg, Lyell's Syndrome have been reported (58, 59).

Hepatotoxicity

Acute hepatitis, usually associated with fever, rash and lymphadenopathy is known to occur with sulphasalazine (41, 42, 43) and this appears to be immunologically mediated. A systemic granulomatous reaction affecting the liver has also been described (44). Recently a case of hepatitis after 15 years sulphasalazine treatment has been described (45).

Male infertility

Sulphasalazine has been shown to reduce the absolute sperm count and cause morphological abnormalities in spermatozoa in a high proportion of patients (39, 40). This appears to be completely reversible and there are no reports of increased incidence of foetal abnormalities or perinatal morbidity/mortality in babies whose fathers were on sulphasalazine at the time of conception.

Lung disease

Eight patients with infiltrative lung disease, often with systemic features of fever, weight loss and eosinophilia, have been described (16, 48, 49, 50, 51). Most cases have reversed although at least 1 fatality has occurred.

Drug induced lupus erythematosus

A number of cases of drug induced lupus erythematosus have been described in ulcerative colitis patients and most of these were receiving sulphasalazine. Eight cases of possible lupus syndrome were described by Alacron-Segovia et al (52). All of these cases appeared to be associated with the administration of sulphasalazine or sulpha drugs. LE cells were present in all these cases but the clinical features in some of the cases were not consistent with a diagnosis of drug induced lupus. At the time of this report anti-nuclear and anti-DNA antibody estimations were not available.

A further case (53) was accompanied by a raised DNA binding although the temporal relationship with sulphasalazine was convincing. In classic drug induced lupus, however, DNA binding is normal and this case may, therefore, represent an exacerbation of spontaneous systemic lupus erythematosus which is well recognised with sulphonamides. The case described by Crisp and Hoffbrand (54) again displayed a "slightly raised" DNA binding but again the temporal relationship with sulphasalazine administration was convincing.

Desensitisation

Patients who have experienced mild idiosyncratic reactions to sulphasalazine may benefit from a desensitisation regimen starting with lmg orally per day and slowly increasing the dose. This may facilitate the eventual re-introduction of the drug (60, 61).

Pregnancy

Retrospective studies have failed to show a deleterious effect due to sulphasalazine on the outcome of pregnancy (62, 63).

In a series of patients with Crohn's disease only 3-4% had to discontinue sulphasalazine because of adverse events (6). In an other series of 200 patients with ulcerative colitis only 13 dropped out because of side effects (Truelove S.C., personal communication).

Section 5

Drug interactions

Drug interactions may take place either within the gut or following absorption.

Interactions within the intestinal lumen

Concomitant administration of antibiotics leads to a reduction in the degradation of sulphasalazine in the large bowel resulting in a lower serum sulphapyridine level and increased faecal excretion of sulphasalazine (15, 16).

Cholestyramine, which is occasionally given to patients with inflammatory bowel disease, binds both sulphasalazine and its azo reduction products in the gut. This results in slower intestinal passage of sulphasalazine with a reduction in sulphasalazine cleavage by the bacteria of the large bowel. Cholyestyramine binding of 5-ASA may also inhibit its local anti-inflammatory effect (16). Sulphasalazine is also known to chelate iron and when sulphasalazine and ferrous sulphate are administered together absorption of

sulphasalazine but not sulphapyridine is reduced (11). Calcium gluconate, although it retards absorption of sulphasalazine, has no effect on the overall quantity absorbed (11). Mention has already been made of the possible inhibition of folate absorption.

Interaction after absorption

Concomitant phenobarbitone administration results in a decrease in serum acetyl-sulphapyridine, an increase in serum sulphapyridine-o-glucuronide levels and a reduction in serum sulphasalazine levels. The clinical significance of these findings is unknown (13).

Theoretically, as it is highly protein bound, sulphapyridine might be expected to displace other highly bound drugs such as warfarin or the sulphonylureas. To date, however, there is no firm evidence of significant clinical interaction.

Conclusions

Sulphasalazine is a drug with a complex pharmacology and apparently low toxicity which merits further investigation as a second line anti-rheumatic drug.

Summary

Chapter 1

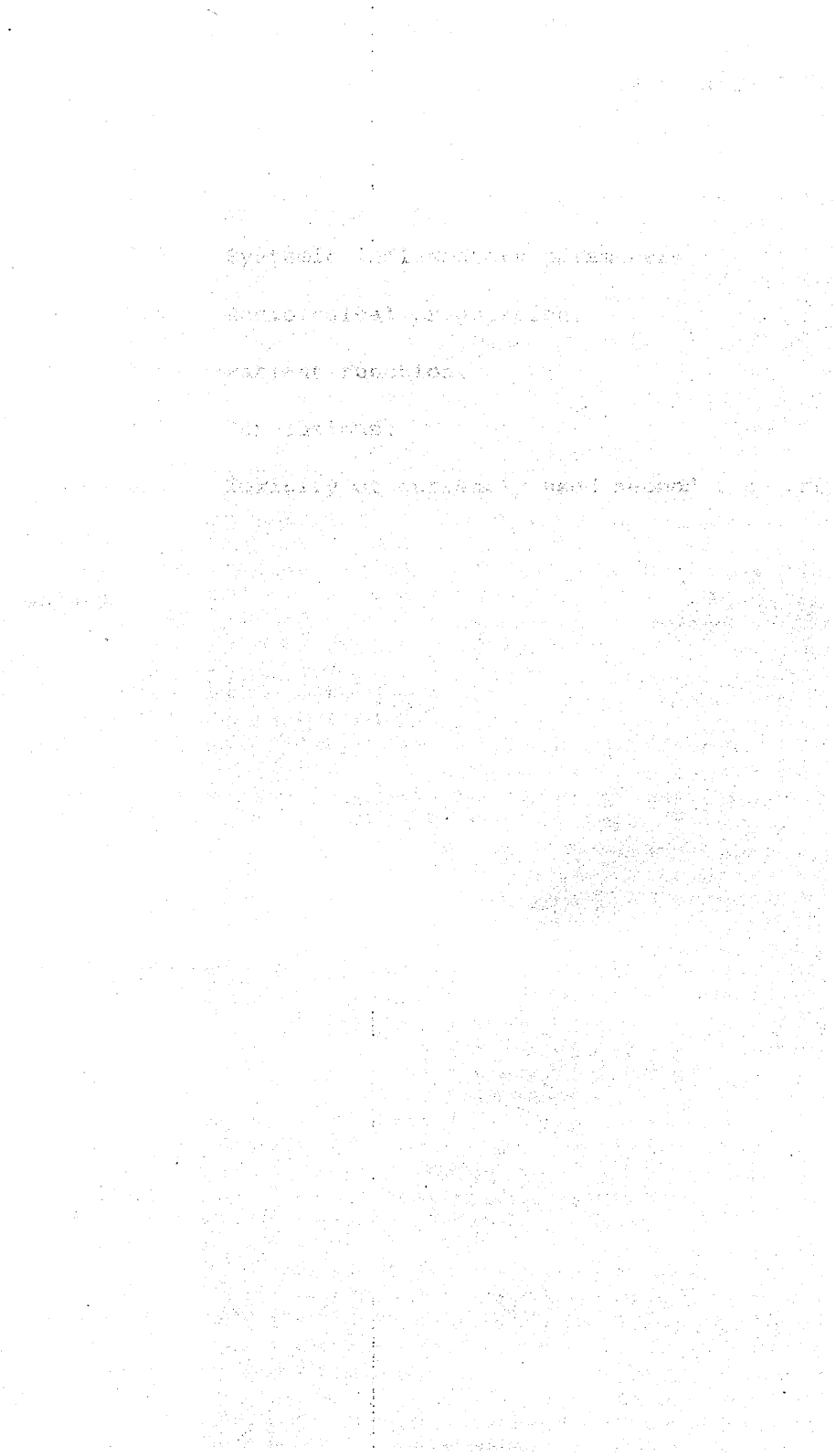
Rheumatoid arthritis is a common, progressive, potentially crippling condition. Because of its relentlessly progressive nature and the toxicity of the second line drugs available at present to treat it there is a need for more effective, less toxic drugs. Although sulphasalazine was first introduced for the treatment of "rheumatic polyarthritis" its use in this condition rapidly declined and it remained in use only as a treatment for inflammatory bowel disease. A number of uncontrolled studies have recently suggested, once more, that it has second line anti-rheumatic properties.

Only a small proportion of an ingested dose of sulphasalazine is absorbed as such and most reaches the large intestine intact where it is split by bacterial action to sulphapyridine and 5-amino salicylic acid (5-ASA). The 5-ASA remains largely within the bowel lumen and is excreted unchanged in the faeces. The sulphapyridine is almost completely absorbed, undergoes hepatic metabolism (the rate of which depends upon the genetically determined acetylator phenotype) and is then excreted in the urine.

Sulphasalazine or its metabolites affect a number of biological systems and can produce antimicrobial, immunoregulating and anti-prostaglandin effects.

Although nausea is a common side effect of sulphasalazine, serious side effects (agranulocytosis, hepatitis and pulmonary infiltrates) are rare and it is relatively free of drug interactions. Some of its adverse effects are thought to be related to acetylator phenotype.

Sulphasalazine is, therefore, a suitable drug to investigate further for anti-rheumatic properties.



CHAPTER 2

Assessment of second line drug activity

Section 1: What is a second line drug?

Section 2: Methods of assessing activity of second line drugs.

2.1 Classification of drug response.

2.2 Measures of symptoms and local joint inflammation.

2.3 Systemic inflammatory parameters.

2.4 Radiological progression.

2.5 Patient function.

2.6 Conclusions.

Section 3: Toxicity of currently used second line drugs.

Summary

CHAPTER 2

Section 1

What is a second line drug?

Drugs used in the treatment of rheumatoid arthritis are conventionally classified as first line drugs ("non-steroidal anti-inflammatory drugs (NSAIDs)", "aspirin like drugs", "cyclo-oxygenase inhibitors") or second line drugs ("disease modifying antirheumatic drugs", "specific antirheumatic drugs", "slow acting drugs", "d-penicillamine like drugs"). In addition cytotoxic drugs or corticosteroids are occasionally employed and these are sometimes referred to as third line or even fourth line drugs. Most patients suffering from rheumatoid arthritis require only first line therapy. A minority, however, with progressive generalised inflammatory disease which is not adequately controlled by first line drugs alone require the addition of second line drugs such as gold salts, d-penicillamine or chloroquine. These drugs differ from first line drugs in their relatively slow onset of action, their frequently prolonged beneficial effect when treatment is stopped and their effect on a number of systemic indices of inflammation (Table I). It is this latter property, in addition to a clinical benefit, which most readily distinguishes second line drugs. It has also been suggested that these drugs slow the progression of joint destruction (67, 68, 69, 70). Numerous effects of second line drugs at a cellular level have been described and many of these effects have been proposed as possible modes of action. Examples of these effects are shown in Table II. No single mode of action has been shown to explain their therapeutic action, although most proposed mechanisms have

Erythrocyte sedimentation rate (ESR)

Haemoglobin level

Platelet count

C-reactive protein

Haptoglobin

Conconavalin-A binding

Rheumatoid factor titre

Serum IgG levels

Serum IgM levels

Serum IgA levels

Serum thiols

Plasma viscosity

Serum histidine

Table I Laboratory parameters which may be altered by second line drugs (64,65,66).

Drug	Authors	Proposed mode of action of second line drug
Sodium aurothiomalate	Lipsky & Ziff (1977)	Inhibition of lymphocyte proliferation (71)
Sodium aurothiomalate	Hopkins, Jayson & Van der Zeil (1983)	Inhibition of lymphocyte activation (72)
Sodium aurothiomalate	Griffin & Stevens (1982)	Inhibition of proteolytic enzyme activity (73)
Sodium aurothiomalate Levamisole D-penicillamine	Mowat (1977)	In vitro inhibition of neutrophil chemotaxis by sodium aurothiomalate and stimulation by levamisole. In vivo early stimulation of neutrophil chemotaxis by levamisole and late stimulation by d-penicillamine (74)
Sodium aurothiomalate	Jessop, Vernon-Roberts & Harris (1973)	Inhibition of neutrophil phagocytic activity (75)
Aurothio-glucose D-penicillamine Auranofin	Jessop, Wilkins & Young (1982)	Suppression of phagocytic activity of synovial macrophages (76)
Sodium aurothiomalate	Scheinberg, Santos & Finkelstein (1982)	Inhibition of monocyte chemotaxis and expression of Fc and C3 receptors (77)
D-penicillamine Sodium aurothiomalate	Munthe, Kass & Jellum (1982)	Alteration of free radical scavenging mechanisms (78)
Sodium aurothiomalate	Highton, Panayi & Shepherd et al (1981)	Reduction in immune complex levels (79)

Table II Proposed modes of action of second line agents.

concentrated on alterations of the immune system.

The clinical effect of cytotoxic drugs are broadly similar to those of second line drugs and, in general, they are used when the choice of second line agents is exhausted. For practical purposes cytotoxic drugs are often classified along with second line drugs (Fig. VII).

Section 2

Methods of assessing activity of second line drugs

2.1 Classification of drug response

Rheumatoid arthritis is characterised by inflammation of synovial joints with associated pain, stiffness, swelling and joint destruction which in turn produce loss of function (both reversible and permanent). Any drug used in the treatment of rheumatoid arthritis should help to ease the symptoms of pain and stiffness and reduce local inflammation. In addition second line agents produce improvement in systemic features of inflammation and may also improve long term function and reduce the rate of joint destruction.

Ideally the assessment of a drug for "second line properties" should include observations of its effect on:-

(1) Symptoms of pain and stiffness and evidence of local joint inflammation.

(2) Systemic inflammatory parameters.

(3) Joint destruction.

(4) Patient function.

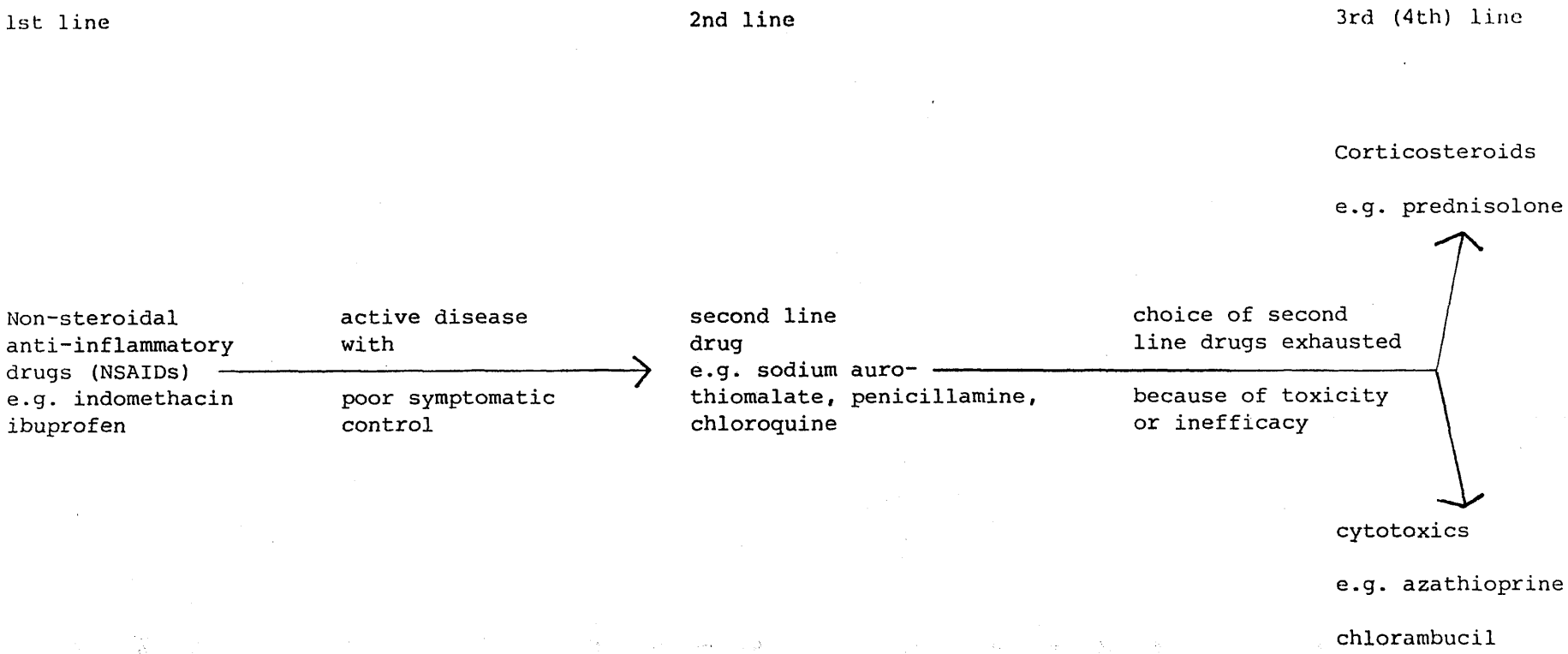


Fig VII Plan of drug therapy for active rheumatoid arthritis.

Categories (1) and (2) are often referred to as "process measurements" (measurements of disease activity) and categories (3) and (4) as "outcome measurements".

Unfortunately there is no single test which will give reliable, reproducible results which are both sensitive and specific with regard to disease activity and outcome. One is therefore forced to use a battery of tests to assess drug effect. Even when results from such a battery of tests are available some may be conflicting. Clinicians often fail to agree on the relative importance of the various results and their use of these results in clinical practice may even differ from their perceived importance of the same information (80, 81).

2.2 Measurements of symptoms and local joint inflammation

Joint tenderness

Numerous methods of assessing joint tenderness have been produced but the two in most widespread use are the American Rheumatism Association Joint Score which is basically a count of inflamed joints (total = 66 joints) (82), and the Ritchie Articular Index (83) which scores joint tenderness, using digital pressure, on a scale of 0-3 (total = 26 joints or groups of joints). These two indices correlate well with each other (83).

Although the Ritchie Index shows good intra-observer reproducibility inter-observer reproducibility is poor and serial measurements must be taken by the same observer. Because of its simplicity, speed and good intra-observer reproducibility, however, the Ritchie Articular Index has proven popular with many researchers (84).

Pain

Perception of pain is notoriously difficult to quantitate and much depends upon the patient's personality, environment and psychological status (85). In clinical trials pain is measured either on a visual analogue pain scale (VAPS) or on a descriptive pain scale. A VAPS is a 10cm line the ends of which define the extremes of pain, eg, "as bad as it could be" and "no pain". This is sometimes modified by the superimposition of descriptive terms, eg, mild, moderate, severe, along the line and is then referred to as a graphic rating scale (86). A number of factors influence both the ability of patients to use such scales to record pain and the sensitivity of the method; these include:- whether the scale is vertical or horizontal, the exact wording of instructions and labelling of the scale, the presence or absence of subdivisions and whether the patient is allowed to see their previous scoring (86, 87). Reproducibility of the score also shows variability along the length of the scale (88). Occasionally patients are unable to understand the concept of a VAPS.

Many descriptive pain scales have been devised using from 4 to 9 divisions (84). There seem to be no advantage in using a large number of divisions and a 5 point scale using the grading of "no pain, mild pain, moderate pain, severe pain and very severe pain" has been shown to be effective in differentiating active treatment from placebo (89).

A failing common to all these methods of pain assessment is the fact that pain relief is an exponential rather than linear fucntion and it is easier to go from very severe to severe pain than from mild to no pain (90). Another approach has been to attempt to quantitate pain relief rather than absolute pain levels. This is only applicable to

certain types of clinical trials (86) and suffers from patients' understandable inability to remember pain severity.

Morning stiffness

This is a common feature of inflammatory arthritis and occurs in 97% of patients with active, untreated rheumatoid arthritis. It has an average duration of three and a half hours (91). Measurement of severity of stiffness requires special appliances (92, 93) and by convention, therefore, duration of morning stiffness (or "limbering up time") is used in most clinical trials of antirheumatic drugs.

Hand grip strength

Hand grip strength is measured using a standard rubber bag or sewn sphygmomanometer cuff inflated to a pre-determined pressure (usually 20 or 30mmHg). This is squeezed three times by each hand and the mean value for each hand calculated. Hand grip strength is dependent upon a number of parameters, both physical (pain, stiffness, muscle power, deformity) and psychological. It can also be affected by patient skill and learning and shows both inter-observer and diurnal variation. Although it does reflect changes in clinical disease activity it is a relatively insensitive test (84, 94). If used in the evaluation of rheumatoid arthritis it should be measured by the same observer at the same time of day.

Digital joint circumference

This can be measured using either jewellers rings or a plastic spring gauge. It changes with anti-inflammatory drug treatment although large inter-observer error has been demonstrated (95). Provided it is

confined to patients with soft tissue joint swelling this measurement can be useful in assessing an anti-inflammatory effect.

Other clinical methods of assessment

Many other less commonly used methods of clinical assessment have been utilised. These include such measurements as the time taken to walk 50 ft, a count of the number of analgesic tablets used and a global assessment which is a measure of either the physician's or the patient's subjective view of the patient's generalcondition without specific reference to any particular symptom or sign. These other methods offer little in terms of sensitivity or specificity.

Thermography

Thermography is a method of recording infra-red emission and displaying this on a video screen in the form of a two-dimensional colour picture with temperature steps being represented by different colours. From this pattern a thermographic index can be calculated and changes in this pattern reflect accurately the reduction in inflammation produced by the injection of local corticosteroids (96) and after the use of NSAIDs, penicillamine and cytotoxic drugs (97). Thermography, however, is a time consuming operation and requires expensive specialised equipment. A technique of microwave radiometery which measures microwave emission rather than heat is presently being assessed and may prove promising (98).

Radio-isotope studies

Joint inflamamtion may be assessed using intravenous or intra-articular injections of radio-isotopes.

The most commonly used intravenous radio-isotope is radio-technetium (^{99m}Tc). Elevated uptake of ^{99m}Tc can be detected over inflamed joints and a number of polyarticular indices have been devised (99). The ^{99m}TC index correlates well with other clinical and laboratory measurements of inflammation and it reflects changes with prednisolone and gold (100).

Measurement of the rate of clearance of intra-articularly administered xenon (^{133}Xe) allows accurate estimation of synovial blood flow (101) and again, this correlates with other parameters of disease activity and changes with the use of anti-inflammatory drugs.

2.3 Systemic inflammatory parameters

Erythrocyte sedimentation rate (ESR)

ESR is a non-specific measure of inflammation which is often, but not always, raised in active rheumatoid arthritis as well as in numerous other inflammatory conditions. The level of ESR in rheumatoid arthritis depends upon the fibrinogen and γ globulin concentrations in the plasma as well as numbers and configuration of the cells (102). It is also related to the age of the patients and the serum cholesterol concentration. The ESR is not influenced by NSAIDs such as indomethacin or aspirin (103) but falls with second line drugs such as gold (104) and penicillamine (105) and also with prednisone (103, 104). ESR correlates well with most other inflammatory indices (100) and, along with C-reactive protein (CRP) levels, relates to radiological progression (106). Although a very non-specific test, ESR was recently voted a "best buy" in measurements at a workshop on assessment of drug efficacy in rheumatoid arthritis (107).

C-reactive protein (CRP)

CRP is an acute phase reactant which is synthesised in hepatocytes. It circulates in the γ globulin fraction of serum proteins and is thought to have some immunoregulatory function. It is present in low concentration in normal serum and is raised in most inflammatory conditions including rheumatoid arthritis (108). Although there is some dissociation between CRP and ESR, these two parameters, in general, correlate well in rheumatoid arthritis (109). Like ESR it reflects disease activity (110), is reduced by treatment with gold, dapsone and prednisone (104) and is related to radiological progression (106). It is not, however, altered by changes in serum immunoglobulins, cholesterol concentration, red cell concentration, size or shape, or age (111).

Other acute phase proteins

These are a heterogeneous group of glycoproteins and include α_1-acid glycoprotein, α_1 antitrypsin, caeruloplasmin, haptoglobin, seromucoid and protein bound hexose and fibrinogen. These show a pattern of response in rheumatoid arthritis similar to that of CRP and ESR but are not as well documented in this respect although seromucoid serum hexose-protein ratio and protein bound hexose concentrations have been suggested to reflect most consistently disease activity (112).

These proteins can be measured "en masse" by their ability to bind to conconavalin A (a plant lectin isolated from the Jack bean). Conconavalin A binding correlates well with levels of individual acute phase reactants and a fall in Conconavalin A binding accompanies

clinical improvement with penicillamine therapy (65). Apart from CRP acute phase reactants are seldom used in clinical practice.

Haemoglobin

Anaemia is a feature of active rheumatoid arthritis. It is said to occur in less than half the cases of active disease (113) although most physicians would probably regard this as an underestimate. Haemoglobin level correlates well with other indices of disease activity (100). Haemoglobin levels rise as the disease becomes less active but its usefulness is obviously limited as it can be affected by, among other things, blood loss, nutritional status, haemoconcentration and haemodilution.

Platelet count

A thrombocytosis occurs in active rheumatoid arthritis and the level of the platelet count correlates with other parameters of disease activity. A similar pattern is also seen in Crohn's disease (66).

Plasma and serum viscosity

In rheumatoid arthritis plasma viscosity is related to the concentration of fibrinogen and other macromolecules while serum viscosity is mainly affected by the concentration of globulins (114). They are both unaffected by such variables as age and haemoglobin levels. Plasma viscosity has been shown to correlate well with articular index and it changes significantly with the use of second line drugs (115). The relationship between plasma viscosity and clinical parameters, however, could not be confirmed in a cross sectional study by Larkin and co-workers (116). They also failed to

show correlation of serum viscosity with clinical parameters and surprisingly could demonstrate no relationship of ESR with clinical parameters. This same study demonstrated an effect of smoking on ESR and for this reason suggested that viscosity measurements might be more reliable.

Rheumatoid factor

IgM rheumatoid factor as measured by the Rose-Waaler titre fails to show significant correlation with most parameters of disease activity (100). Many studies of second line agents do not inclcude assessment of rheumatoid factor titres but titres are reduced by gold salts (117) and by d-penicillamine (118).

Serum sulphydryl levels

Serum sulphydryl (thiol) groups are involved in the scavenging of oxygen derived free radicals and levels correlate inversely with disease activity in rheumatoid arthritis (100). Serum sulphydryl reactivity increases with the second line drugs, gold, penicillamine and levamisole but not with NSAIDs (119).

Combined indices used in the assessment of disease activity in rheumatoid arthritis

Numerous indices combining various clinical and laboratory measurements of disease activity have been devised in an attempt to give an overall description of disease activity. Because of the multiplicity of measurements and the necessarily subjective nature of many of these, none of the indices have met with universal success or acclaim. These indices are summarised in Table III.

Recently in an attempt to define second line drugs a correlation matrix has been developed. This correlates changes in laboratory and clinical parameters of disease activity and is claimed to differentiate between NSAIDs and second line drugs as only the latter produce changes in laboratory indices which correlate with clinical changes (64).

2.4 Radiological progression

The parameters discussed above are all "process measurements", ie, reflect disease activity only at the time of assessment. Changes in the radiological appearances of the joints, however, represent a measurement of outcome, ie, the end effect of the inflammatory process.

There are two main methods of quantitating radiological changes of rheumatoid arthritis in common use. The method described by Larsen et al (123) involves comparison of 5 standard graded radiographs for each joint whereas that of Sharp et al (124) relies upon numerical evaluation of narrowing of the joint space and osseous defects in hand radiographs. These methods rely upon qualitative judgement and are tedious to carry out; joint space narrowing is related to the degree of flexion and erosions of the carpus are often difficult to assess because of overlapping carpal bones. A simplified Sharp's method counting only osseous defects of 11 joints in each hand has recently been described (125). This method displayed good inter and intra-observer variation.

One of the major drawbacks of radiological assessment of drug effect in rheumatoid arthritis is the inability to maintain adequate placebo

Authors	Objective Measurements	Subjective Measurements	Semi-objective Measurements	Advantages	Disadvantages
Boyles & Hall 1943 (120)	ESR Haemoglobin Body weight	Physical activity Joint deformity	Joint involvement		Many parameters affected irreversibly
Duthie et al 1955 (121)	ESR Haemoglobin	Systemic disturbance	Joint involvement	Simple to use	
Lansbury 1956 (91)	ESR	Duration of am stiffness Onset of fatigue	Grip strength Need for analgesics Joint Index	Reproducible Good correlation with patient and physician assessment Easy to use	Time consuming
Mallya & Mace 1981 (122)	ESR Haemoglobin	Duration of am stiffness Pain Score	Articular Index Grip strength	Good balance of objective/ subjective/ semi-objective All indices well documented individually	Groups into only 4 categories of disease activity

Table III Combined indices used in assessing the activity of rheumatoid arthritis.

groups for the time necessary to observe real changes (126) whereas the comparison of the rate of radiological progression prior to and during treatment is not valid as this relies on the fallacious assumption that the rate of progression of erosive disease is linear (127). No study has satisfactorily demonstrated a reduction in the rate of erosive disease with second line drugs. Sigler et al (68) compared 15 gold and 13 placebo treated patients and found significantly more radiographic changes in the placebo group. These authors, however, achieved the remarkable feat of keeping a placebo group for 2 years with no drop-outs which suggests that, perhaps, the disease activity was mild. In an uncontrolled comparison of gold and penicillamine (69), penicillamine but not gold was found to retard the rate of progression. In another two studies of gold salts it was suggested that radiological progression was delayed. In one of these (70), greater radiological progression was found in the low dose group (<500mg total gold) than in the high dose group (>500mg total gold); these groups, however, were not comparable at the start of the study. In the other study (uncontrolled) patients who had a clinical response to gold showed a reduced rate of progression of erosive disease (128). Another study using similar numbers of patients demonstrated that, for the degree of retardation of radiological progression found, between 120 and 170 patients would have to complete 2 years treatment with the active drug and a similar number would need to complete 2 years treatment with placebo before a statistical test with a power of 80% could demonstrate a statistically significant difference between the groups (125).

The dearth of knowledge of the natural history of erosive disease in untreated rheumatoid arthritis and the lack of adequate control groups

in studies of the effect of second line drugs on erosive disease make it seem unlikely that a decisive answer to this question can be produced using present methods. Recent work on microfocal radiography (129) may provide a more sensitive technique with which to answer this question.

2.5 Patient function

The major aims of antirheumatic therapy are to abolish pain, prevent joint destruction and maintain or improve function. The measurement of function can be approached in a number of ways. Measurements of the patient's ability to carry out simple tests such as walking a set distance or squeezing a sphygmomanometer cuff have been dealt with above. Several questionnaires have been produced to assess, overall, the patient's ability to carry out everyday tasks. The earliest of these was the functional classification described by Steinbrocker, Traeger & Batterman (130) which recognised 4 functional classes. Other similar 4 of 5 point scales have been described but classification into such a small number of groups obviously deprives one of sensitive measures of joint function. Numerous longer questionnaires have been devised and one of the best known is that of Lee et al (131) which scores 17 everyday tasks of varying complexity. This is not affected by short term use of anti-inflammatory drugs but is by joint surgery. A recent development in the field of functional assessment (one which can, perhaps, be regarded as a third generation functional index) is the health assessment questionnaire (132) which deals not only with activities of daily living but also with other aspects of the patient's ability to cope with everyday life such as discomfort, drug toxicity and economic impact (as well as death!).

This type of assessment of the patient's overall health status brings us closer to a measure of the World Health Organisation's definition of health as, "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity". Although this questionnaire and a similar assessment, the Arthritis Impact Measurement Scale (133) have been validated as regards reproducibility and relationship to other relevant methods of assessment they have not yet been shown to be of value in the long term evaluation of antirheumatic compounds nor have their relationship to short term treatment or major life events been demonstrated. A recent study examining the effect of joint arthroplasty on these two "health status instruments" and on another three similar instruments has shown a good correlation between the various questionnaires with little to choose between them (134). More work must be done on these various questionnaires before they can achieve an accepted place in the long term assessment of rheumatoid arthritis.

2.6 Conclusion

A multiplicity of methods of assessing the effects of antirheumatic drugs on the process measurements of disease activity in rheumatoid arthritis are available. There is little to choose between most of these and provided one uses a variety of both clinical and laboratory parameters one should be able to detect a second line effect. The final choice of parameters therefore lies with other considerations such as ease of measurement for the patient, the preferences of the physician and of the laboratory staff and the local availability of laboratory tests. It is important, however, that for the measurement of clinical parameters the assessor is kept constant.

The choice of outcome measurement is much more difficult. Radiology is the only way of measuring disease progression but its value is greatly limited by our inability to maintain a proper placebo group for the necessary period. Satisfactory studies of radiological progression of disease is therefore probably an unattainable goal in the assessment of second line drugs.

Measurements of patient function or more general health assessment measurements again suffer from lack of long term control data and have not yet been shown necessarily to represent long term disease outcome. However, until a better measure of outcome is available, health assessment questionnaires appear a useful instrument with which to assess outcome.

Section 3

Toxicity of currently used second line drugs

One of the major problems with the present range of second line drugs is their high incidence of adverse effects. In a recent study 55% of 123 patients commenced on one of three second line drugs (gold, penicillamine or levamisole) experienced definite or possible toxicity necessitating withdrawal of treatment over the first 4 years and a further 22% dropped out for other reasons (Table IV) (1). The other major group of second line drugs, the antimalarials, although they have a different range of toxicity (inhibition of smooth muscle contractility, headaches, corneal and retinal pigmentation) also has a substantial drop out rate and requires close ophthalmological monitoring (135). The cytotoxic drugs such as azathioprine, cyclophosphamide and methotrexate which are used in rheumatoid

Reason for stopping initial treatment	Sodium aurothiomalate n = 49			Penicillamine n = 25			Levamisole n = 49		
	No	%	Month	No	%	Month	No	%	Month
Itch/rash	9	18	1,2,2,3,7,7 12,22,36	4	16	1,2,2,12	7	14	1,2,5,7, 9,13,29
Leucopenia	3	6	1,3,4	-	-	-	8	16	4,4,5,5, 6.8.8.19
Thrombocytopenia	3	6	3,12,47	2	8	9,25	1	2	35
Proteinuria	3	6	4,6,6	2,	8	7,10	2	4	4,5
Haematuria	2	4	8,38	1	4	6	-	-	-
Mouth ulcers	1	2	3	-	-	-	-	-	-

Table IV Reasons for discontinuing therapy in 123 patients on second line therapy followed for 4 years.

Reason for stopping initial treatment	Sodium aurothiomalate n = 49			Penicillamine n = 25			Levamisole n = 49		
	No	%	Month	No	%	Month	No	%	Month
Gastro-intestinal upset	-	-	-	2	8	1,6	5	10	1,2,2, 12,14
'Flu-like symptom	-	-	-	-	-	-	10	20	1,1,1,1, 1,4,6, 13,18,19
Jaundice	1	2	1	-	-	-	1	2	1
Acute joint pain	-	-	-	1	4	1	-	-	-
Lack of effect	-	-	-	3	12	8,8,12	3	6	5,12,12
Loss of effect	4	8	9,20,28,34	1	4	40	6	12	1,2,12, 36,41,46

Table IV Reasons for discontinuing therapy in 123 patients on second line therapy followed for 4 years. (Cont)

Reason for stopping initial treatment	Sodium aurothiomalate n = 49			Penicillamine n = 25			Levamisole n = 49		
	No	%	Month	No	%	Month	No	%	Month
Poor compliance	2	4	7,18	2	8	8,12	2	4	14,15
Industrial action	-	-	-	1	4	14	-	-	-
Lost to follow up	-	-	-	1	4	24	-	-	-
Death	2	4	30,40	-	-	-	-	-	-
TOTAL	30	60		20	80		45	91	

Table IV (Cont) Reasons for discontinuing therapy in 123 patients on second line therapy followed for 4 years.

arthritis are also notorious for their high incidence of (often serious) adverse reactions (67, 136).

Although HLA tissue typing has some value in identifying patients who are susceptible to certain toxic effects with gold salts and penicillamine (137) and sulphoxidation status may also help with the latter drug (138), there is no clinically useful test which will accurately predict the patient who will suffer serious toxicity. The high rate of potentially serious side-effects limits the use of these drugs to the most severe inflammatory disease both because of the high risk:benefit ratio and also because of the logistic problems of close monitoring:- it has been calculated that, in the first 6 months of treatment (even allowing for a high drop out rate), 100 patients (50 gold, 50 d-penicillamine) would require 1,340 clinic visits for blood and urine checks and even if shared care with the general practitioner is carried out this represents 470 hospital visits (139).

A drug which has a low total incidence of side-effects with no need for close blood or urine monitoring, no or very few serious side-effects and an effective method of identifying patients at risk or toxicity would therefore be a useful addition to the second line armamentareum.

Chapter 2

Summary

Second line antirheumatic drugs are characterised by the production of improvement in both clinical and laboratory parameters of inflammation These effects may take weeks or months to develop and may remain for similar periods after treatment is stopped. They may also retard disease progression. Their mode of action is unknown.

Numerous clinical and laboratory measurements are available for the assessment of drug activity but no one single measurement can be used to assess disease activity. Measurements can be classified as process measurements or as outcome measurements. There is a multiplicity of available process measurements. A number of these should be used in conjunction to try to give an overall view of disease activity and a number of cumulative indices have been designed with this aim. Outcome measurements comprise functional indices (the newest generation of which are not yet properly validated) and radiological assessment. The effect of current second line drugs on radiological progression is slight and prolonged follow up of a large number of patients is required. Both methods also suffer from the impossibility of maintaining adequate long term control groups.

Currently available second line agents all have serious drawbacks in terms of toxicity and patient tolerance and the addition of another, perhaps less toxic, agent to this list would be of practical use.

CHAPTER 3

Recent clinical studies of sulphasalazine in rheumatoid arthritis and an outline of the proposed aims of this thesis.

Section 1 Recent studies of sulphasalazine in rheumatoid arthritis

Section 2 Aims and outline of studies

2.1 Proposed aims of thesis

2.2 Outline of individual studies

Summary

Section 1

Recent studies of sulphasalazine in rheumatoid arthritis.

After the initial spate of interest and publications on sulphasalazine in the 1940s there followed a 30 year silence. In 1978, however, interest was renewed when McConkey et al re-investigated sulphasalazine following the observation that it had similarities in its spectrum of clinical activities to dapsone which, although effective in the treatment of rheumatoid arthritis, is of little practical use in this condition because of its toxicity. They described an open study of sulphasalazine in rheumatoid arthritis (8). In this study 32 patients were given sulphasalazine in a dose of up to 3g/day and results at 22 weeks were described. Twenty-two patients completed this period of follow-up. Significant improvement in CRP and clinical score was seen by 6 weeks and in ESR by 12 weeks. These improvements were maintained over the twenty-two weeks. Seven patients had stopped because of side effects (2 dyspepsia, 4 headache and 1 neutropenia). In a second publication 2 years later (9) this series was extended to 74 patients and follow-up was extended to 50 weeks. Patients were started on 0.5g/day and the dose was gradually increased to a usual dose of 2g/day. Thirty-eight patients continued treatment for 50 weeks and again there was significant improvement in clinical score, CRP and ESR. It is difficult to comment upon the pattern of toxicity in this paper because of the inclusion of patients from the first study although it is recorded that 5 patients developed megaloblastic anaemia. Bird et al (140) exposed sulphasalazine to the rigours of their correlation matrix (64) and suggested that it has second line properties.

Simultaneous with the publication of Study 1 described in the next section there appeared a joint study from Leeds and Birmingham which compared, in a double blind fashion, 31 patients allocated randomly to sulphasalazine and 32 to d-penicillamine. Twenty-three patients completed 16 weeks sulphasalazine therapy and after 16 weeks both drugs produced significant improvement in inflammatory indices and no serious toxicity was seen in the sulphasalazine treated patients (141).

Subsequent to completion of the relevant work in this thesis the results of a study comparing 27 sulphasalazine (2g/day) and 29 placebo treated patients were published in abstract form only. This study showed a significant improvement in the sulphasalazine treated patients (142).

More recently 3 uncontrolled longer term follow-up studies of sulphasalazine have been published comparing this drug to sodium aurothiomalate, penicillamine and dapsone (143) to penicillamine (144) and to sodium aurothiomalate (145). All 3 studies confirmed the efficacy of sulphasalazine in rheumatoid arthritis. Because of the chronological relationship of these studies to this present thesis they will be discussed more fully in the appropriate chapters.

Section 2

Aims and outline of studies

2.1 Proposed aims of the thesis

My aims in this thesis are:-

(1) To test, by means of a double blind placebo controlled trial, the hypothesis that sulphasalazine displays second line properties in the treatment of rheumatoid arthritis.

If this hypothesis is true my subsequent aims are:-

(2) To document the toxicity of sulphasalazine in rheumatoid arthritis and to investigate the relationship of a number of variables including dose, serum levels, age, disease duration, previous therapy, acetylator phenotype and disease activity to the efficacy and toxicity of sulphasalazine in rheumatoid arthritis.

(3) To define the single dose pharmacokinetics of sulphasalazine in elderly rheumatoid patients and to relate drug handling to toxicity.

(4) By means of (2) and (3) to attempt to define the optimal clinical use of the drug in rheumatoid arthritis.

(5) To examine, as a possible mode of action, the effect of sulphasalazine (a non thiol containing drug) on the free radical scavenging system. This is of particular importance as many second line drugs affect this system and it is thought that modification of free radical scavenging

mechanisms by a second line drug may be a function of available thiol groups provided by the drug.

(6) To identify the active therapeutic moiety of sulphasalazine in an attempt to separate efficacy from toxicity and, by knowledge of the individual action of the two components, perhaps also to comment further upon the possible aetiopathogenesis of rheumatoid arthritis.

2.2 Outline of individual studies

In an attempt to fulfil the above aims I have designed the following studies:-

(1) A double blind placebo controlled trial of sulphasalazine with the additional use of a sodium aurothiomalate treated group as a "positive control" (30 patients per group).

(2) A comparison of sulphasalazine 1.5g/day with sulphasalazine 3.0g/day (30 patients per group). In this study serum levels of sulphasalazine and its metabolites will also be measured in an attempt to relate these levels to dose and efficacy.

(3) A comparison of a group of slow acetylators allocated to sulphasalazine 1.5g/day and a group of fast acetylators allocated to sulphasalazine 3.0g/day (60 patients in total).

(4) A single dose pharmacokinetic study of 8 elderly patients.

(5) A comparison of 30 patients treated with sulphapyridine alone and 30 patients treated with 5-aminosalicylic acid alone.

(6) Serial measurement of intra- and extra-cellular thiol levels and intracellular superoxide dismutase activity in an unselected subgroup of the above sulphasalazine treated patients.

(7) Documentation of acetylator phenotype in all sulphasalazine treated patients and analysis of its relationship to efficacy and toxicity.

(8) Analysis of the influence of age, sex, disease duration, previous therapy and activity of disease in the above patients on efficacy and toxicity of sulphasalazine.

Summary

Chapter 3

Following a 30 year period of disuse in rheumatology, sulphasalazine was resurrected in 1978 when an open trial suggested it had the characteristics of a second line drug. Further open studies had similar findings but no placebo controlled studies were published. Subsequent to completion of the controlled study described in Chapter 4 a number of further studies were published which confirmed the second line activity of sulphasalazine.

The aims of this thesis are to:-

(1) show by means of a placebo controlled study whether sulphasalazine has a second line effect

(2) to investigate the effect of several variables on efficacy and toxicity of the drug

(3) to investigate the pharmacokinetic profile of sulphasalazine in an elderly rheumatoid population

(4) to use this information to define the optimal clinical use of sulphasalazine

(5) to examine the effect of sulphasalazine on scavengers of oxygen derived free radicals

(6) to identify the active therapeutic moiety of sulphasalazine.

CHAPTER 4

Studies of efficacy and toxicity of sulphasalazine in rheumatoid arthritis.

Section 1

Introduction

To date there has been no placebo controlled trial of sulphasalazine as a second line agent in the treatment of rheumatoid arthritis. In this section I describe the results of such a study. The effect of sulphasalazine is compared to that of sodium aurothiomalate and placebo in the treatment of rheumatoid arthritis over a one year period. In addition the results of a further three studies (Studies 2, 3 and 4) which were designed to investigate the effect of a number of variables such as age, acetylator phenotype and dose are described only in as much as they are relevant to the overall pattern of toxicity and efficacy. The effects of these and other variables on toxicity and efficacy of sulphasalazine are described in subsequent chapters.

Section 2

Patients and Methods

2.1 Selection of patients

Criteria for selection of patients were similar in all studies. All had classical or definite rheumatoid arthritis (146) which remained clinically active (ie, the patient complained of severe pain and/or stiffness and had clinical evidence of synovitis) despite the optimum use of first line drugs and analgesics. All patients remained on first line drugs throughout the studies (except where they stopped them spontaneously because they no longer felt they needed them). In addition patients in Study 4 had to be 65 years of age or over.

Criteria for exclusion from the studies are listed below:-

a) Patients who had previously received sulphasalazine (in study 1, also patients who had previously received gold salts).

b) Patients who were receiving corticosteroids or who had received such drugs in the 3 months preceding entry to the trial (except study 4).

c) Patients who were receiving second line drugs or had received such drugs in the 3 months preceding entry to the trial (except study 4).

d) Pregnant or breast feeding females and patients (males and females) actively attempting to produce a family.

e) Patients with known sulphonamide or aspirin sensitivity.

f) Patients with known malabsorption or liver disease.

All patients in Studies 1, 2 and 3 gave their informed verbal consent and in Study 4 written consent was obtained. The permission of the local Ethics Committee was obtained in all studies.

2.2 Drugs and dosages

In study 1, 90 patients were randomly allocated to sulphasalazine, 3g/day, sodium aurothiomalate or placebo (30 patients per group). In study 2, 60 patients were randomly allocated to sulphasalazine 1.5g/day or sulphasalazine 3g/day (30 patients per group). In study 3, acetylator phenotype was assessed before entry and slow acetylators were given sulphasalazine 1.5g/day while fast acetylators were allocated to sulphasalazine 3.0g/day (40 fast acetylators, 20 slow acetylators). In study 4, 8 patients aged 65 or over were given 2 single oral doses of sulphasalazine 2g or 3g, 1 week apart for

pharmacokinetic measurements. One week after the second dose patients were commenced on a therapeutic dosage regimen aiming at 3g/day.

Patients allocated to sulphasalazine were initially given 0.5g/day enteric coated sulphasalazine orally (Salazopyrin EN 0.5g, Pharmacia) and the dose was increased by weekly increments of 0.5g/day to the allocated dose of 1.5g or 3g in 3 divided doses. Patients were advised to take their medication after food. If dose related toxicity occurred, patients were maintained on the maximum tolerated dose provided this was greater than 1g/day. In studies 2, 3 and 4 patients were allowed prochlorperazine in a dose of up to 10mg t.i.d. for nausea and/or vomiting.

Placebo tablets identical in appearance to sulphasalazine were used. They were given in a dosage regimen equivalent to sulphasalazine 3g/day.

Sodium aurothiomalate (Myocrisin, May & Baker) was given by intramuscular injection. On the first occasion a 10mg test dose was administered and, in the absence of adverse reactions, 50mg was given weekly until a clinical response was achieved. The frequency of injections was then gradually reduced with the eventual aim of maintaining each patient on 50mg each 4-6 weeks. If no clinical response was achieved by the time a total of 1g (20 injections) had been given therapy was discontinued.

2.3 Blinding

Patients and physician in study 1 were unaware whether the tablets contained sulphasalazine or placebo. Placebo tablets were not given to the sodium aurothiomalate group nor were placebo injections given to the tablet treated patients. Patients and physician were, therefore, aware whether the patients were receiving tablets or injections. The metrologist (clinical research nurse) carrying out the subjective and semi-objective measurements and the various laboratories involved with measurement were unaware of the nature of the patient's treatment. In studies 2 and 3 patient and physician were aware of the dose given but again neither the metrologist nor the laboratories were aware of the dose.

The design of the 4 studies is summarised in Table V.

2.4 Toxicity monitoring

A full blood count, including platelet count, was performed at the time of each injection in all patients treated with sodium aurothiomalate and the urine was checked for the presence of blood or protein using a "multistix". In study 1 sulphasalazine and placebo treated patients had these measurements carried out at 6 weekly intervals. In subsequent studies these parameters were measured fortnightly for the first 12 weeks and thereafter 6 weekly. All patients had "liver function tests" [serum alanine transaminase (ALT); aspartate transaminase (AST); alkaline phosphatase and bilirubin] and

Study	Drug	Dose	No of patients	Allocation	Prochlorperazine allowed	Special entry criteria	Steroids/2nd line allowed within previous 3 months
1	Sulphasalazine	3g/day	30				
	Sodium aurothiomalate (GST)	50mg/wk-50mg/6wks	30	Random	No	-	No
	Placebo	6 tabs/day	30				
2	Sulphasalazine	1.5g/day	30	Random	Yes	-	No
	Sulphasalazine	3.0g/day	30				
3	Sulphasalazine	1.5g/day	20	Slow acetylators	Yes	-	No
	Sulphasalazine	3.0g/day	40	Fast acetylators			
4	Sulphasalazine	3.0g/day	8	-	Yes	≥65 yrs old	Yes

Table V Design of the 4 studies of sulphasalazine in rheumatoid arthritis

"urea and electrolytes" measured every 6 weeks for the first 24 weeks and subsequently 12 weekly. In addition at each visit patients were asked to report adverse events and were asked specifically if they had developed skin rash or mouth ulcers.

2.5 Withdrawal from therapy

Table VI shows criteria used for withdrawal from therapy. No hard and fast rules were used to indicate withdrawal of therapy and the final decision was left to "clinical judgement". Patients were strongly encouraged not to stop treatment because of inefficacy before week 24. Patients could, of course, insist on withdrawal at any time.

2.6 Assessment of efficacy

Measurements were carried out before treatment, at 6 weekly intervals for the first 24 weeks and, thereafter 3 monthly. Functional index questionnaire was repeated after 1 year.

Efficacy was assessed in all patients receiving therapy at a particular time point even if those patients discontinued therapy at that visit. Thus the number of patients in whom efficacy was assessed is occasionally greater than would seem apparent from the drop-out tables.

All clinical assessment was carried out "blind" by a single clinical metrologist (a qualified nurse who has been specially trained in measurement techniques).

(1) WBC < 4 x 10^9/l

(2) Platelets < 150 x 10^9/l

(3) Haematuria/proteinuria > trace (sodium aurothiomalate)

(4) Skin rash

(5) Mouth ulceration

(6) Abnormality in serum transaminases

(7) Any other adverse events which were likely to be due to drug therapy and which were either potentially dangerous or too severe to allow continuation of therapy

(8) Failure to respond or loss of response despite optimum dosage (discouraged during the first 24 weeks of all studies)

Table VI Criteria for withdrawal from therapy

Articular index (AI)

Ritchie articular index was used. Each of 26 joints or groups of joints were scored on a scale of 0-3 depending upon the patient's reaction to firm digital pressure. This gives a maximum possible score of 78 (83).

Pain score (PS)

Patients were scored from 0-4 on a 5 point descriptive pain scale. Using this method a pain score is described as follows:- 0 = no pain, 1 = mild pain, 2 = moderate pain, 3 = severe pain, 4 = very severe pain (89).

Duration of morning stiffness/limbering up time (LUT)

Patients were asked to recollect the duration of morning stiffness on the day before assessment.

Hand grip strength

This was measured using a canvas covered rubber bag measuring 9cm by 17 cm attached to an anaeroid manometer and inflated to 20mm Hg. Three measurements were taken for each hand and the mean value calculated.

Functional index (FI)

In studies 1 and 2 functional index as described by Mitchell et al (147) was used. This index has been used in a previous second line

study in the Centre for Rheumatic Diseases and has been found to change with successful second line treatment (Capell H A, Personal Communication). This functional index consists of an administered questionnaire (Appendix I) and is applicable only to females. Correlations between the functional index measurements carried out in these studies and various process measurements are shown in Table VII. A similar pattern was seen when change in functional index was correlated with change in inflammatory parameters.

Laboratory indices

The following laboratory indices of inflammation were measured: Westergren erythrocyte sedimentation rate (ESR), haemoglobin level (Hb), platelet count, Rose Waaler titre (RF), immunoglobulins G, A and M (IgG, IgA, IgM) total serum globulins and total serum albumin. These measurements were all carried out by the routine haematology, biochemistry and immunology laboratories at Glasgow Royal Infirmary, Gartnavel General Hospital and the Western Infirmary, Glasgow.

In study 3 C-reactive protein (CRP) levels were measured by the biochemistry department of Gartnavel General Hospital by an immuno-nephelometric method using a Beckman auto-analyser and reagents. In study 3, serum B12 and folate and red cell folate were measured at 6 weekly intervals and, in an attempt to identify intravascular haemolysis, urine haemosiderin was also measured on these occasions.

	rs	p
Erythrocyte sedimentation rate (ESR)	-0.26	< 0.05
Haemoglobin (Hb)	0.18	> 0.05
Platelet count (Plats)	-0.06	> 0.05
Rose Waaler titre (Rf)	-0.08	> 0.05
Articular index (AI)	-0.43	< 0.001
Pain score (PS)	-0.44	< 0.001
Grip strength	0.58	< 0.001
Limbering up time (LUT)	-0.52	< 0.001
Albumin	0.32	< 0.01
Globulins	-0.14	> 0.05
IgA	-0.35	< 0.005
IgG	-0.19	> 0.05
IgM	-0.22	> 0.05
Disease activity index (DAI)	-0.53	< 0.001

Table VII Correlation (Spearman Rank) of all values for functional index (Studies 1 and 2) with inflammatory indices (n = 88).

Disease activity index

A disease activity index (DAI) based on that of Mallya and Mace (122) was calculated. This was modified from the original in a number of ways.

1) The pain score based on division of a visual analogue scale into 4 equal segments was replaced by gradation of the five point pain score as follows:- No pain/mild pain = 1; moderate pain = 2; severe pain = 3; very severe pain = 4.
2) The score was expressed as a total out of a possible maximum of 24 rather than converted into a mean score out of 4 and then classified into 4 disease activity groups as described by Mallya and Mace.

To validate these alterations, DAI from patients in study 1 was correlated with individual inflammatory parameters and was shown to correlate well with most of these (Table VIII).

2.7 Statistical analysis

This was carried out using the relevant non-parametric statistical tests (148). All tests were two tailed. Where statistical analysis was carried out by computer, an SPSS package was used. Further information on statistical analyses is contained in Appendix 2. The protocols of studies 1, 2 and 3 stipulated that statistical assessment was to be carried out at 24 weeks. Having analysed the 24 week data first and drawn appropriate conclusions from these, earlier and later data were subsequently analysed to define more fully the rate of onset and duration of drug action.

	rs	p
Erythrocyte sedimentation rate (ESR)*	0.646	< 0.001
Haemoglobin (Hb)*	-0.53	< 0.001
Platelet count (Plats)	0.37	< 0.001
Rose-Waaler titre (RF)	0.0358	> 0.05
Articular index (AI)*	0.57	< 0.001
Pain score (PS)*	0.66	< 0.001
Grip strength*	-0.49	< 0.001
Limbering up time (LUT)*	0.63	< 0.001
IgA	0.026	> 0.05
IgG	0.48	< 0.001
IgM	0.33	< 0.001
Total globs	0.4	< 0.001
Albumin	-0.45	< 0.001
Alkaline phosphatase	0.03	> 0.05
Functional index	-0.52	< 0.001

* individual components of DAI

Table VIII Correlation of modified Mallya-Mace Index [Disease activity index (DAI)] with other inflammatory indices at times 0, 6 wks, 12 wks, 24 wks, 48 wks in sulphasalazine, sodium aurithiomalate and placebo groups (n = 307) (Spearman rank correlation)

Section 3

Results

3.1 Study 1

Patient characteristics at the start of study 1 are shown in Table IX. No significant difference could be demonstrated between the treatment groups in respect to demographic or inflammatory indices at the commencement of the study (Kruskal-Wallis one-way analysis of variance $p > 0.05$). There was, however, a trend towards more inflammatory disease in the group allocated to sulphasalazine therapy.

After 24 weeks 18 patients remained both on sulphasalazine and sodium aurothiomalate whereas 14 remained on placebo. By 48 weeks these figures had fallen to 12, 12 and 6 respectively. Toxicity was the most common reason for withdrawal from both sulphasalazine and sodium aurothiomalate whereas most patients who stopped placebo did so because of inefficacy. Table X gives the exact times and reasons for discontinuing the various treatments and Figs VIII and IX display this information graphically. Using life table analysis and log rank test (appendix 2) discontinuation of drug because of inefficacy occurred more frequently in the placebo group ($\chi^2 = 8.71$, $p < 0.01$) than in the sulphasalazine group. Patients on sulphasalazine and patients on sodium aurothiomalate showed significant improvement (Wilcoxon matched-pairs signed-ranks test) in inflammatory indices at 24 weeks In many cases this improvement occurred as early as 6 weeks and this improvement persisted to at least 48 weeks. No such improvements were seen with placebo patients. Tables XI, XII and XIII show median values and ranges for inflammatory parameters and several possible

	Placebo (n = 30)	Sulphasalazine (n = 30)	Sodium aurothiomalate (n = 30)
Age (yrs)	56.5 (18 - 70)	57 (32 - 70)	58 (40 - 74)
Disease duration (yrs)	9.5 (1 - 35)	6 (1 - 23)	8 (1 - 32)
ESR (mm/hr)	47 (4 - 128)	70 (15 - 131)	50 (8 - 119)
Hb (g/dl)	12.7 (8.6 - 14.7)	11.2 (9.4 - 14.2)	11.4 (8.8 - 15)
Platelets (x 10^9/l)	407 (250 - 586)	431 (259 - 786)	398 (276 - 817)
Rose-Waaler titre	1/64 (0 - 1/1024)	1/32 (0 - 1/1024)	1/64 (0 - 1/1024)
Articular index	18 (6 - 40)	22.5 (2 - 54)	18 (5 - 33)
Limbering up time (mins)	60 (0 - all day)	110 (0 - all day)	60 (0 - all day)
Pain score	2.3 (0 - 4)	2.7 (0 - 4)	2.4 (0 - 4)
Grip strength (mmHg)	84 (49 - 225)	78.5 (40 - 160)	89 (52 - 136)
DAI	15 (12 - 22)	17 (14 - 22)	16.4 (10 - 21)

Table IX Study 1. Demographic and inflammatory parameters at commencement of treatment - median (ranges)

	Placebo (n = 30)	Sulphasalazine (n = 30)	Sodium aurothiomalate (n = 30)
Functional index	61.25 (14 - 80)	55.5 (11 - 86)	68.5 (39 - 86)
IgA (g/l)	3.5 (2.1 - 6.4)	3.0 (0.4 - 7.5)	3.2 (1.4 - 7.2)
IgG (g/l)	13.7 (8.7 - 17.0)	13.3 (7.1 - 21.9)	13.0 (7.8 - 21)
IgM (g/l)	2.6 (0.7 - 7.3)	1.2 (0.7 - 3.4)	1.8 (0.2 - 3.1)
Total globs (g/l)	34.25 (25 - 42)	35.0 (21- 45)	35.25 (26 - 50)
Albumin (g/l)	39.3 (32 - 45)	37.25 (30 - 48)	37.25 (30 - 48)
AST (u/l)	15.75 (5 - 25)	14 (5 - 22)	15.25 (6 - 39)
ALT (u/l)	13.5 (5 - 31)	10 (5 - 30)	10.6 (5 - 39)
Alkaline Phosphatase (u/l)	230 (96 - 518)	251 (5 - 580)	226 (74 - 325)
MCV (fl)	82 (66 - 98)	82.5 (71 - 99)	83 (68 - 94)
Creatinine (umol/l)	65.25 (40 - 120)	64 (40 - 135)	70 (35 - 165)

Table IX (Cont) Study 1. Demographic and inflammatory parameters at commencement of treatment - median (ranges)

	PLACEBO		SULPHASALAZINE		SODIUM AUROTHIOMALATE	
	Total	Week Stopped	Total	Week Stopped	Total	Week Stopped
Rash	0	-	2	4, 36	6	16, 18, 22, 24, 30, 31
Mouth Ulcers	0	-	1	2	3	10, 12, 38
Thrombocytopenia	0	-	0	-	2	16, 20
Leucopenia	0	-	1	9	1	12
Nitritoid reaction	0	-	0	-	1	2
Proteinuria	0	-	0	-	1	10
Nausea/vomiting	3	6, 8, 26	7	5, 6, 6, 12, 18, 20, 30	0	-
Lack/loss of effect	19	6, 12, 12, 16 18, 18, 18, 18, 24, 24, 24, 24, 30, 30, 30, 30, 36, 42	5	18, 18, 26, 42, 48	3	24, 28, 48

Table X Study 1. Reason for stopping therapy and week stopped - 1 year follow up

	PLACEBO		SULPHASALAZINE		SODIUM AUROTHIOMALATE	
	Total	Week Stopped	Total	Week Stopped	Total	Week Stopped
Lost to follow up	0	-	1	12	0	-
Depression	1	12	0	-	0	-
Intercurrent medical problems	1	28	0	-	0	-
Death	0	-	1	32	1	36
Total	24		18		18	

Table X (Cont) Study 1. Reason for stopping therapy and week stopped - 1 year follow up

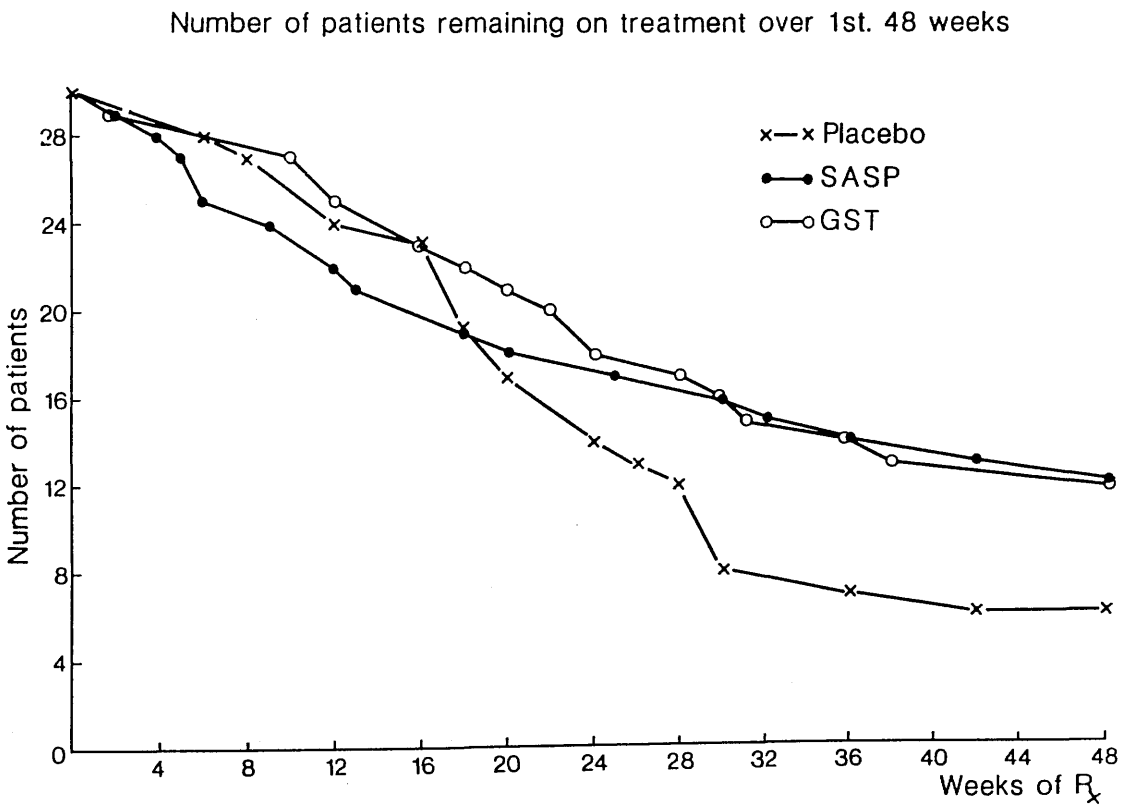


Fig. VIII Pattern of drop out from the three treatment groups in Study 1.

REASONS FOR DISCONTINUING SULPHASALAZINE OVER 48 WEEKS OF TREATMENT.

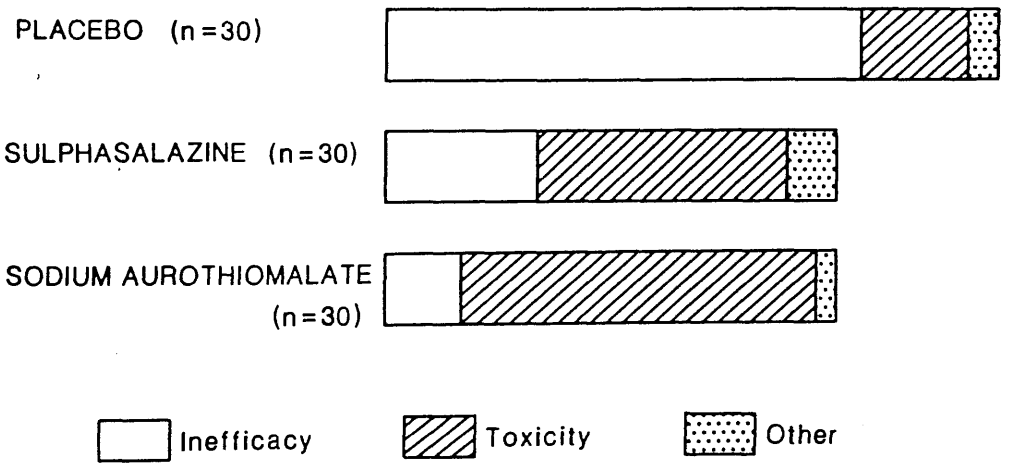


Fig. IX Reasons for discontinuing therapy in Study 1.

Week No	0	6	12	24	48
n	30	30	27	19	6
ESR (mm/hr)	47 (4-128)	50 (17-140)	52 (9-114)	43 (10-85)	26.5 (15-65)
Hb (g/dl)	12.7 (8.6-14.7)	13.1 (7.9-15.9)	13.2 (9.5-15.3)	13.4 (9.1-15.5)	13.9 (9.7-14.9)
Plats (x 10^9/l)	407 (250-286)	373 (191-716)	375 (279-584)	335 (279-584)	242 (180-401)
RF (Rose Waaler Titre)	1/64 (0-1/1024)	1/128 (0-1/512)	0 (0-1/2048)	1/256 (0-1/1024)	1/512 (0-1/1024)
Articular index	18 (6-40)	14.5 (2-38)	11.75** (13-29)	12.5 (2-35)	9 (2-13)
LUT (mins)	60 (0-all day)	62 (0-all day)	63 (15-all day)	59 (10-all day)	82.5 (30-300)
Pain score	2.3 (0-4)	2.5 (1-4)	2.4 (1-4)	2.2 (1-4)	2.25 (2-4)
Grip (mmHg)	84 (49-225)	91.5 (49-175)	89 (46-260)	90.5 (43-275)	103 (54-175)
DAI	15 (12-22)	15.9 (11-20)	15.75 (12-22)	14.7 (11-20)	14.3* (12-16)
FI	61.25 (14-80)	-	-	-	-

Table XI Study 1 Clinical, haematological and biochemical data for placebo treated patients over the 1st year of treatment - medians (ranges). Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week No	0	6	12	24	48
n	30	30	27	19	6
IgA (g/l)	3.5 (2.1-6.4)	-	-	3.5 (2.0-6.0)	-
IgG (g/l)	13.7 (8.7-17.0)	-	-	13.5 (9.0-15.5)	-
IgM (g/l)	2.6 (0.7-7.3)	-	-	1.5 (0.6-5.3)	-
Total globs (g/l)	43.25 (21-42)	34.75 (25-42)	32.8 (24-42)	33.3 (25-46)	-
Alb (g/l)	39.3 (32-45)	39.0 (29-45)	38.2 (32-43)	39 (33-45)	-
AST (u/l)	15.75 (5-25)	17 (5-26)	13.5 (5-24)	17.25 (5-36)	-
ALT (u/l)	13.5 (5-31)	13.75 (5-17)	12.0 (5-30)	15.75 (5-35)	-
Alkaline Phophatase (u/l)	230 (96-518)	254 (106-400)	227 (87-475)	270 (106-570)	-
MCV (fl)	82 (66-98)	-	-	83 (61-90)	88.5 (65-89)
Creatinine (umols/l)	65.25 (40-120)	63.5 (45-125)	69 (45-130)	66 (55-130)	-

Table XI (Cont) Study 1 Clinical, haematological and biochemical data for placebo treated patients over the 1st year of treatment - medians (ranges). Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week No	0	6	12	24	48
n	30	27	24	18	13
ESR (mm/hr)	70 (15-131)	56* (17-140)	36.5* (7-118)	25*** (6-69)	17** (9-81)
Hb (g/dl)	12.2 (9.4-14.2)	11.4 (7.9-15.9)	11.8 (8.4-15.1)	11.8 (9.7-15.0)	12.5* (10.5-14.8)
Plats (x 10^9/l)	431 (259-786)	355** (191-716)	308*** (195-511)	354**** (134-479)	301*** (172-454)
RF (Rose Waaler Titre)	1/32 (0-1/1024)	2 (0-1/512)	0 (0-1/2048)	0 (0-1/1024)	1/16 (0-1/512)
Articular index	22.5 (2-54)	16.2* (2-38)	11.75* (0-41)	3.5**** (0-33)	3*** (0-34)
LUT (mins)	110 (0-all day)	60 (0-all day)	31.25 (0-all day)	15 (0-all day)	4**** (0-75)
Pain score	2.7 (0-4)	2.4 (1-4)	2.3 (1-4)	1.9 (0-4)	1.4 (0-4)
Grip (mmHg)	78.5 (40-160)	93.5** (49-175)	84.5** (46-260)	84.5*** (9-185)	102* (41-230)
DAI	17 (14-22)	16.5*** (11-20)	15*** (9-19)	13.5*** (10-20)	11**** (8-18)
FI	55.5 (11-86)	-	-	-	82** (58-90)
IgA (g/l)	3.0 (0.4-7.5)	-	-	2.1 (0.3-3.9)	2.3 (0.5-4.4)
IgG (g/l)	13.3 (7.1-21.9)	-	-	10.5 (6.4-14.2)	11.4 (7.2-21.8)

Table XII Study 1. Clinical, haematological and biochemical indices in sulphasalazine treated patients during 1st year of study - medians (ranges). Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week No	0	6	12	24	48
n	30	27	24	18	13
IgM (g/l)	1.2 (0.7-3.4)	-	-	0.8* (0.4-3.4)	0.95* (0.4-1.7)
Total globs (g/l)	35.0 (21-45)	31.5* (25-42)	31.8**** (21-40)	27.5** (19-34)	-
Alb (g/l)	37.25 (30-48)	39.25 (29-45)	39.75* (32-45)	40*** (36-48)	-
AST (u/l)	14 (5-22)	15.5 (5-26)	16.25* (9-23)	16.5 (10-34)	-
ALT (u/l)	10 (5-30)	9.3 (5-17)	10.5 (5-21)	13.5 (5-41)	-
Alkaline Phosphatase (u/l)	251 (50-580)	235 (106-400)	225* (94-435)	245* (96-365)	-
MCV (fl)	82.5 (71-99)	-	99 (78-115)	88.5* (74-114)	92.5*** (80-105)
Creatinine (umols/l)	64 (40-135)	61 (40-135)	70 (40-145)	66 (50-155)	78 (61-95)

Table XII (Cont) Study 1. Clinical, haematological and biochemical indices in sulphasalazine treated patients during 1st year of study - medians (ranges). Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week No	0	6	12	24	48
n	30	29	27	20	13
ESR (mm/hr)	50 (8-119)	62.5 (3-129)	45.5** (1-97)	31*** (1-110)	36** (1-75)
Hb (g/dl)	11.4 (8.8-15)	11.0 (8.4-15.1)	11.7 (9.3-15.9)	11.2 (8.7-16.5)	12.1*** (5.8-17.0)
Plats (x 10^9/l)	398 (276-817)	390 (161-677)	364* (183-588)	313 (201-751)	317* (251-458)
RF (Rose Waaler Titre)	1/64 (0-1/1024)	0 (0-1/1024)	0**** (0-1/1024)	0* (0-1/1024)	0 (0-1/512)
Articular index	18 (5-33)	17* (2-35)	12**** (1-24)	10**** (0-19)	4*** (0-18)
LUT (mins)	60 (0-all day)	61 (0-all day)	45 (0-all day)	20 (0-all day)	2* (0-60)
Pain score	2.4 (0-4)	2.3 (1-4)	2.1 (1-4)	1.5*** (0-3)	0.9* (0-3)
Grip (mmHg)	89 (52-136)	85 (57-160)	90 (46-225)	81 (50-260)	112* (68-300)
DAI	16.4 (10-21)	16.4 (9-20)	15.25*** (7-19)	13.3*** (6-18)	11** (6-18)
FI	68.5 (39-86)	-	-	-	77.5 (72-88)

Table XIII Study 1. Clinical, haematological and biochemical data for sodium aurothiomalate treated patients over the 1st year of treatment - medians (ranges). Wilcoxon v Wk 0 - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week No	0	6	12	24	48
n	30	29	27	20	13
IgA (g/l)	3.2 (1.4-7.2)	-	-	2.5 (0.8-5.4)	1.7 (1.1-2.9)
IgG (g/l)	13.0 (7.8-21)	-	-	11.4 (7.8-14.1)	0.91 (7.4-12.8)
IgM (g/l)	1.8 (0.2-3.1)	-	-	1.1 (0.3*-1.6)	0.95 (0.3*-1.5)
Total globs (g/l)	35.25 (26-50)	31.75*** (24-44)	29.5** (22-41)	27* (19-43)	23.5* (22-29)
Alb (g/l)	37.25 (30-48)	37.7 (30-47)	39.1 (34-43)	39.5 (33-46)	44 (40-46)
AST (u/l)	15.25 (6-39)	17 (6-33)	14.5 (5-36)	17 (9-53)	16 (13-21)
ALT (u/l)	10.6 (5-39)	11.25 (5-25)	12.0 (8-39)	19* (11-57)	11 (6-28)
Alkaline Phosphatase (umols/l)	226 (74-325)	225 (77-335)	197 (69-300)	184 (55-335)	168 (64-245)
MCV (fl)	83 (68-94)	-	-	83.5 (67-94)	86.5 (66-91)
Creatinine	70 (35-165)	65.2 (40-210)	67.5 (55-140)	72 (35-99)	71 (40-80)

Table XIII (Cont) Study 1. Clinical, haematological and biochemical data for sodium aurothiomalate treated patients over the 1st year of treatment) - medians (ranges). Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

	SULPHASALAZINE V PLACEBO	SODIUM AUROTHIOMALATE V PLACEBO	SULPHASALAZINE V SODIUM AUROTHIOMALATE
ESR	<0.01	<0.005	NS
Hb	NS	NS	NS
Plats	<0.05	NS	NS
RF	NS	NS	NS
AI	<0.01	<0.05	NS
LUT	<0.001	<0.05	NS
Pain score	NS	<0.05	NS
Grip strength	NS	NS	NS
DAI	<0.05	<0.001	NS
FI	-	-	-
IgA	NS	NS	NS
IgG	NS	NS	NS
IgM	<0.05	<0.05	NS
Total globs	<0.05	NS	NS
Alb	<0.05	NS	NS
AST	NS	NS	NS
ALT	NS	NS	NS
Alkaline Phosphatase	<0.05	NS	NS
MCV	NS	NS	NS
Creatinine	NS	NS	NS

Table XV Study 1. Percent change in indices (wk 0 - 24) Mann-Whitney U test - p values (NS = not significant; $p > 0.05$).

	SULPHASALAZINE V PLACEBO	SODIUM AUROTHIOMALATE V PLACEBO	SULPHASALAZINE V SODIUM AUROTHIOMALATE
ESR	<0.05	<0.05	NS
Hb	NS	NS	NS
Plats	NS	NS	NS
RF	NS	NS	NS
AI	<0.05	NS	NS
LUT	<0.001	<0.05	NS
Pain score	NS	<0.05	NS
Grip strength	NS	NS	NS
DAI	<0.005	<0.05	NS
FI	*	*	NS
IgA	NS	NS	NS
IgG	NS	NS	NS
IgM	NS	NS	NS
Total globs	NS	NS	NS
Alb	NS	NS	NS
AST	NS	NS	NS
ALT	NS	NS	NS
Alkaline Phosphatase	NS	NS	NS
MCV	<0.01	NS	<0.05
Creatinine	NS	NS	NS

Table XVI Study 1. Percent change in indices (Wk 0 - 48) Mann-Whitney U test - p values (NS = not significant; $p > 0.05$).

* = analysis could not be carried out because of inadequate numbers remaining in placebo group

	ALLOCATED DOSE	
	1.5g/day	3g/day
Age (yrs)	57 (30-69)	54.5 (28-71)
Disease Duration (yrs)	7 (1-30)	12 (1-22)
ESR (mm/hr)	54 (12-130)	68 (17-140)
Hb (g/dl)	11.9 (8.9-15.6)	11.3 (8.6-17.0)
Platelets (x 10^9/l)	353 (203-798)	425 (227-888)
Rheumatoid factor titre (Rose Waaler)	1/64 (0-1/1024)	1/64 (0-1/1024)
Articular index	23 (2-57)	19 (3-61)
Limbering up time (mins)	112 (0-all day)	67 (0-all day)
Pain score	3 (1-4)	3 (1-4)
Grip strength (mmHg)	80 (45-190)	83 (44-140)
DAI	17 (12-21)	17.5 (12-23)
Functional index	49 (21-79)	55 (8-82)
IgA (g/l)	3.8 (1.8-5.7)	3.2 (0.8-7.1)
IgG (g/l)	12.8 (9.0-19.2)	13.9 (6.6-40.2)
IgM (g/l)	1.2 (0.5-3.0)	1.3 (0.7-7.0)
Total globs (g/l)	32 (23-42)	35 (24-56)
Albumin (g/l)	38 (32-44)	37 (25-44)
AST (u/l)	17 (6-28)	16 (8-28)
ALT (u/l)	11 (4-25)	13 (5-30)
Alkaline Phosphatase (u/l)	232 (92-530)	219 (24-880)
MCV (fl)	82 (68-98)	82 (67-94)
Creatinine (umol/l)	65 (40-108)	70 (50-217)

Table XVII Study 2. Demographic and inflammatory indices at commencement of study.

to 18 and 17 respectively. Table XVIII shows the reason for and the week of discontinuing therapy. Of the 18 patients initially allocated to 1.5g/day who remained on treatment at 48 weeks, 13 were receiving their allocated dose and 5 were receiving a higher dose (two 2.0g; three 3.0g). Of the 17 patients still on treatment at 48 weeks who were initially allocated to 3.0g/day, 12 were receiving this dose, whereas 3 were receiving lower doses (one 1.5g/day, one 2.0g/day and one 2.5g/day) and 2 were receiving a higher dose (both 4.0g/day).

Patients who were receiving doses in excess of the allocated dose were doing so because the allocated dose failed to adequately control their disease. Those patients who were receiving a dose lower than the allocated dose were doing so because of dose related toxicity.

Tables XIX and XX show median values and ranges for inflammatory indices and some indicators of toxicity over the 48 week follow-up period. Similar changes were found irrespective of whether the results were analysed by actual dose or by allocated dose and the results are therefore presented as allocated dose. Once more there was an improvement in some indices as early as 6 weeks and this was expanded and consolidated at later assessments. The relationship of dose to efficacy and toxicity will be explored in Chapter 5.

3.3 Study 3

In this study the 40 fast acetylators were allocated to 3.0g/day and the 20 slow acetylators to 1.5g/day. The role of acetylator phenotype will be discussed in Chapter 6 and only efficacy and toxicity aspects of this study will be further described at present. The follow-up period in this study was confined to 24 weeks. Table XXI shows the

	ALLOCATED DOSE			
	1.5g/day		3g/day	
n	30		30	
	Total	Week Stopped	Total	Week Stopped
Rash	2	1, 18	1	11
Mouth Ulcers	0	-	1	1
Leucopenia	1	8	0	-
Nausea/vomiting	2	1, 27	5	4, 5, 12, 22, 32
Dyspnoea	0	-	1	3
Drowsiness	1	8	0	-
Lack/loss of effect	5	36, 36, 36 48, 48	3	24, 30, 42
Poor Compliance	0	-	1	11
Intercurrent medical problems	1	10	1	6
Total	12		13	

Table XVIII Study 2. Week of stopping therapy and reasons for stopping therapy - 1 yr follow up

Week	0	6	12	24	48
n	30	28	25	24	20
ESR (mm/hr)	54 (12-130)	48* (15-127)	42* (12-108)	45**** (5-129)	40*** (2-96)
Hb (g/dl)	11.9 (8.9-15.6)	11.6 (8.7-16.5)	11.8 (9.5-15.6)	12.5* (9.3-15.6)	12.8* (9.6-15.0)
Plats (x 10^9/l)	353 (203-798)	336 (211-530)	337 (131-527)	312 (161-475)	362 (152-486)
RF	1/64 (0-1/1024)	1/256 (0-1/1024)	1/256 (0-1/1024)	1/512 (0-1/1024)	1/128 (0-1/1024)
AI	23 (2-57)	16* (5-37)	12**** (0-28)	8.5**** (0-30)	9.75*** (2-25)
LUT (mins)	112 (0-all day)	64 (0-all day)	61 (0-all day)	60* (0-all day)	44 (0-all day)
Pain score	3 (1-4)	3 (1-4)	2.5 (1-4)	2*** (0-4)	2.5 (0-4)
Grip (mmHg)	80 (45-190)	94* (49-254)	96* (59-290)	95*** (57-215)	86 (37-240)

Table XIX Study 2. Clinical, haematological & biochemical indices for patients allocated to 1.5g/day - medians (ranges) Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week	0	6	12	24	48
n	30	28	25	24	20
DAI	17 (12-21)	17 (10-20)	16.7* (9-21)	14.6**** (9-21)	15.6** (10-19)
FI	49 (21-79)	- -	- -	- -	58 (20-90)
IgA (g/l)	3.8 (1.8-5.7)	3.2 (1.3-6.0)	3.2 (1.3-6.8)	3.0 (1.2-7.9)	3.2* (1.5-4.8)
IgG (g/l)	12.8 (9.0-19.2)	13.0 (7.9-19.3)	12.9 (7.4-21.5)	12.5 (7.0-22.4)	11.3* (8.1-19.5)
IgM (g/l)	1.2 (0.5-3.0)	1.3 (0.6-3.0)	1.2 (0.6-3.6)	1.0 (0.6-2.7)	1.0*** (0.4-3.0)
Alb (g/l)	38 (32-44)	39*** (32-46)	40*** (34-44)	41**** (36-47)	40** (35-44)
Globs (g/l)	32 (23-42)	35 (26-41)	32 (21-46)	31* (23-43)	31* (22-38)
AST (u/l)	17 (6-28)	17 (13-24)	21*** (17-36)	22* (12-32)	16.5 (8-38)

Table XIX (Cont) Study 2. Clinical, haematological & biochemical indices for patients allocated to 1.5g/day - medians (ranges) Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week	0	6	12	24	48
n	30	28	25	24	20
ALT (u/l)	11 (4-25)	14 (7-25)	17* (7-25)	17* (6-38)	13 (7-38)
Alkaline Phosphatase (u/l)	232 (92-530)	241 (125-460)	235 (128-720)	236 (125-780)	219 (85-370)
MCV (fl)	82 (68-98)	73 (68-77)	82 (68-101)	87*** (72-102)	81 (68-104)
Creatinine (umol/l)	65 (40-108)	80 (43-113)	70 (42-110)	75 (50-118)	91 (50-120)

Table XIX (Cont) Study 2. Clinical, haematological & biochemical indices for patients allocated to 1.5g/day - medians (ranges) Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week	0	6	12	24	48
n	30	26	23	21	17
ESR (mm/hr)	68 (17-140)	51*** (16-137)	40**** (8-90)	24**** (7-118)	25**** (3-103)
Hb (g/dl)	11.3 (8.6-17.0)	11.7 (8.6-16.2)	11.4 (8.5-15.8)	12.0*** (10.2-17.7)	12.2** (7.7-16.9)
Plats (x 10^9/l)	425 (227-888)	389** (170-845)	409*** (182-941)	341**** (224-809)	337**** (190-865)
RF	1/64 (0-1/1024)	1/512 (0-1/1024)	1/32 (0-1/1024)	1/512 (0-1/1024)	1/16 (0-1/1024)
AI	19 (3-61)	10**** (0-27)	8**** (1-33)	7**** (0-26)	5**** (0-18)
LUT (mins)	67 (0-all day)	60* (0-all day)	30**** (0-all day)	15*** (0-all day)	11** (0-all day)
Pain score	3 (1-4)	2.5 (1-4)	2*** (1-3)	1.5*** (0-4)	1.75** (0-4)
Grip (mmHg)	83 (44-140)	90* (82-170)	92**** (55-205)	95**** (57-235)	92**** (58-215)

Table XX Study 2. Clinical, haematological & biochemical indices for patients allocated to 3.0g/day - medians (ranges) Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week	0	6	12	24	48
n	30	26	23	21	17
DAI	17.5 (12-23)	15.5*** (8-20)	14.9**** (7-18)	12.2**** (7-18)	12.2**** (8-20)
FI	55 (8-82)	- -	- -	- -	63.5* (26-87)
IgA (g/l)	3.2 (0.8-7.1)	3.5 (1.6-5.7)	2.9*** (0.9-5.4)	3.7*** (1.2-5.9)	2.1**** (0.7-5.0)
IgG (g/l)	13.9 (6.6-40.2)	13.9 (8.7-27.8)	13.1*** (7.6-29.3)	12.1*** (6.5-28.8)	10.5*** (4.2-22.4)
IgM (g/l)	1.3 (0.7-7.0)	1.4 (0.7-2.4)	1.2 (0.5-3.0)	1.17* (0.6-2.1)	1.3 (0.6-3.0)
Alb (g/l)	37 (25-44)	38 (31-44)	38**** (28-46)	40**** (36-45)	40.5*** (28-45)
Globs (g/l)	35 (24-56)	32* (25-47)	32*** (25-51)	32.5** (22-45)	28.5**** (20-44)
AST (u/l)	16 (8-28)	18* (8-49)	18*** (11-39)	18*** (7-80)	18* (10-29)

Table XX (Cont) Study 2. Clinical, haematological & biochemical indices for patients allocated to 3.0g/day - medians (ranges) Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week	0	6	12	24	48
n	30	26	23	21	17
ALT (u/l)	13 (5-30)	14.5 (7-39)	14 (4-42)	16 (7-69)	17.5 (5-26)
Alkaline Phosphatase (u/l)	219 (24-880)	230 (90-1210)	195 (95-690)	195 (10-250)	186 (101-510)
MCV (fl)	82 (67-94)	89* (72-95)	86* (69-99)	89**** (80-112)	89 (75-100)
Creatinine (umol/l)	70 (50-217)	72 (50-145)	74 (40-202)	75 (49-135)	75 (55-130)

Table XX (Cont) Study 2. Clinical, haematological & biochemical indices for patients allocated to 3.0g/day - medians (ranges) Wilcoxon v Wk 0; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

	1.5g/day (n = 20)	3g/day (n = 40)
Age (yrs)	50.5 (40-73)	52.5 (35-73)
Disease Duration (yrs)	10.0 (2-25)	8.5 (1-33)
ESR (mm/hr)	37.5 (2-125)**	73 (10-150)
Hb (g/dl)	12.3 (7.4-16.1)**	11.4 (7.8-16.6)
Platelets (x 10^9/l)	355 (118-607)	421 (126-802)
Rheumatoid factor titre (Rose Waaler)	1/256 (0-1/1024)	1/512 (0-1/1024)
Articular index	17.5 (2-39)	16 (0-39)
Limbering up time (mins)	120 (0-all day)	76 (0-all day)
Pain score	2.4 (1-4)	2.7 (1-4)
Grip strength (mmHg)	67.5 (39-167)	83.5 (38-190)
CRP (ug/l)	25.5 (<6.0-40.1)*	40.5 (<6.0-100)
DAI	15 (13-21)	17 (13-22)
IgA (g/l)	3.1 (1.2-6.3)	3.2 (0.3-6.5)
IgG (g/l)	12.3 (9.3-23.6)	14.4 (6.2-24.4)
IgM (g/l)	1.1 (0.6-3.7)	1.4 (0.4-9.9)
Total globs (g/l)	32 (21-48)	35 (26-52)
Albumin (g/l)	38 (35-43)	38 (24-48)
AST (u/l)	13 (6-21)**	18 (10-39)
ALT (u/l)	12 (4-31)	15 (3-40)
Alkaline Phosphatase (u/l)	212 (102-380)	255 (89-830)

Table XXI Study 3. Demographic and inflammatory paramaters at commencement of treatment - median (ranges)
*Wilcoxon $p<0.05$; **Wilcoxon $p<0.01$ - 1.5g v 3.0g

	1.5g/day (n = 20)	3g/day (n = 40)
MCV (fl)	85 (71-102)	81 (66-93)
Serum B_{12} (pg/ml)	274 (115-720)	303 (82-836)
Serum folate (ng/ml)	2.3 (1.5-4.9)	2.5 (1.1-6.0)
RBC folate (ng/ml)	181 (86-254)	163 (5-247)
Creatinine (umol/l)	70 (45-130)	70 (40-18

Table XXI (Cont) Study 3. Demographic and inflammatory paramaters at commencement of treatment - median (ranges)
*Wilcoxon $p<0.05$; **Wilcoxon $p<0.01$ - 1.5g v 3.0g

starting characteristics of the patients. Unfortunately the slow acetylator/low dose patients entered the study with significantly lower ESR (Mann-Whitney $p < 0.01$) and CRP (Mann-Whitney $p < 0.05$) and a higher haemoglobin level (Mann-Whitney $p < 0.01$). Other inflammatory parameters showed no statistical difference.

Reasons for and time of drop out are shown in Table XXII. After 24 weeks 15 (75%) of those allocated to low dose and 27 (68%) of those allocated to high dose remained on treatment. Of those allocated to low dose, one was temporarily off treatment, 9 were receiving 1.5g/day, 3 were receiving 2g/day and 2 were receiving 3g/day at 24 weeks. Of the 27 patients allocated to 3g/day, who continued therapy to week 24, 14 were receiving this dose, 5 were receiving 4g/day and 8 were receiving lower doses (one 1g/day, three 1.5g/day, two 2g/day, two 2.5g/day).

Tables XXIII and XXIV show the changes in disease activity and some indicators of toxicity. A general pattern of improvement is again seen in the 3g/day group but in this instance this is not as apparent in the lower dose group. The relevance of this will be discussed in Chapter 6. Urine was positive for haemosiderin in only 3 patients and in no instance was its presence associated with frank clinical haemolysis. A further 2 patients had positive test for haemosiderin before but not during treatment.

3.4 Study 4

Following the initial pharmacokinetic part of the study the 8 elderly patients were commenced on a therapeutic regimen of sulphasalazine (3g/day). Four patients had to stop early because of upper

	Allocated dose			
	1.5g/day (n = 20)		3.0g/day (n = 40)	
	Total	Week Stopped	Total	Week Stopped
Rash	-	-	1	9
Mouth ulcers	-	-	-	-
Leucopenia	2	6, 10	3	4, 8, 10
Nausea/vomiting	3	3, 3, 4	3*	6, 6, 12*
Abnormal LFTs	-	-	1	12
Dizziness/lightheadedness	-	-	2*	6, 12*
Poor compliance	-	-	-	-
Lack/loss of effect	-	-	1	24
Other	-	-	3	4, 6, 6
Total	5 (25%)		13 (32%)	

Table XXII Study 3. Week of stopping therapy and reason for stopping therapy - 24 week follow-up (* same patient)

Week No	0	6	12	24
n	20	17	15	15
ESR (mm/hr)	37.5 (2-125)	35 (3-136))	28 (2-110)	39.5 (2-115)
Hb (g/dl)	12.3 (7.4-16.1)	12.6 (9.0-15.5)	11.8 (9.1-15.1)	12.4 (7.1-15.2)
Platelets (x 10^9/l)	355 (118-607)	335 (119-535)	346 (156-439)	322 (143-502)
Rose Waaler titre	1/256 (0-1/1024)	1/256 (0-1/1024)	1/256 (0-1/1024)	1/16 (0-1/512)
Articular index	17.5 (2-39)	-	6.5** (0-46)	8.5*** (0-23)
Limbering up time (mins)	120 (10-all day)	-	61 (0-all day)	75 (0-all day)
Pain score	2.4 (1-4)	-	2.1 (1-4)	1.9 (1-4)
Grip strength (mmHg)	67.5 (39-167)	-	98.5* (45-260)	70.5 (36-243)
CRP (ug/ml)	25.5 (<6.0-40.1)	-	-	13.9 (<6.0-62.1)
DAI	15 (13-21)	-	14* (8-20)	13.5 (7-21)
IgA (g/l)	3.1 (1.2-6.3)	2.6*** (0.9-6.0)	2.4 (1.1-6.4)	2.3 (1.1-6.2)
IgG (g/l)	12.3 (9.3-23.6)	12.7 (7.4-22)	10.3* (7.5-19.8)	10.9 (4.7-18)

Table XXIII Study 3. Clinical, haematological & biochemical indices for patients allocated to 1.5g/day - medians (ranges) - n = 20. Wilcoxon v Wk 0 - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week No	0	6	12	24
n	20	17	15	15
IgM (g/l)	1.1 (0.6-3.7)	1.1 (0.4-2.4)	1.0* (0.4-2.3)	1.1 (0.3-3.0)
Total globs (g/l)	32 (21-48)	31 (21-48)	28 (22-42)	28 (22-45)
Albumin (g/l)	38 (35-43)	38 (31-48)	40 (30-45)	39 (34-45)
AST (u/l)	13 (6-21)	17 (5-26)	18 (5-25)	15.5 (8-301)
ALT (u/l)	12 (4-31)	14 (6-33)	18 (2-31)	12.5 (5-282)
Alkaline Phosphatase (u/l)	212 (102-380)	205 (102-425)	182 (101-360)	170 (119-355)
MCV (fl)	85 (71-102)	84 (73-101)	84 (75-323)	87 (69-100)
Serum B12 (pg/ml)	275 (115-720)	226 (111-407)	238 (172-347)	256 (185-332)
Serum folate (ng/ml)	2.3 (1.5-4.9)	2.1 (1.5-4.4)	2.2 (0.9-3.9)	2.2 (0.8-2.8)
RBC folate (ng/ml)	181 (86-254)	185 (84-258)	175 (70-251)	153 (95-216)
Creatinine (umols/l)	70 (45-130)	69 (42-105)	72 (45-110)	75 (43-120)

Table XXIII (Cont) Study 3. Clinical, haematological & biochemical indices for patients allocated to 1.5g/day - medians (ranges) - n = 20. Wilcoxon v Wk 0 - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week No	0	6	12	24
n	40	38	29	28
ESR (mm/hr)	73 (10-150))	63*** (13-135)	42**** (8-125)	40**** (5-105)
Hb (g/dl)	11.4 (7.8-16.6)	10.6 (8.7-15.8)	11.4 (9.1-16.5)	11.5 (8.6-17.1)
Platelets (x 10^9/l)	421 (126-802)	355 (113-998)	375 (78-761)	295**** (202-754)
Rheumatoid factor titre	1/512 (0-1/1024)	1/256** (0-1/1024)	1/512 (0-1/1024)	1/128* (0-1/1024)
Articular index	16 (0-39)	-	7.5**** (0-29)	6**** (0-21)
Limbering up time (mins)	76 (0-all day)	-	59 (0-all day)	30**** (0-all day)
Pain score	2.7 (1-4)	-	2.0*** (1-4)	1.7**** (0-4)
Grip strength (mmHg)	83.5 (38-190)	-	91.5 (47-205)	91.5* (45-240)
CRP (ug/ml)	40.5 (<6.0-100)	-	-	10.7** (<6.0-42.1)
DAI	17 (13-22)	-	15**** (10-19)	13.5**** (8-20)
IgA (g/l)	3.2 (0.3-6.5)	3.1 (0.4-7.0)	2.5** (0.4-5.5)	2.7**** (0.2-5.3)
IgG (g/l)	14.4 (6.2-24.4)	13.1**** (4.8-21.6)	12.3**** (5.1-21.9)	10.4**** (4.3-20.9)

Table XXIV Study 3. Clinical, haematological & biochemical indices for patients allocated to 3.0g/day - medians (ranges) - n = 40. Wilcoxon v Wk o - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

Week No	0	6	12	24
n	40	38	29	28
IgM (g/l)	1.4 (0.4-9.9)	1.4 (0.5-5.0)	1.2 (0.5-2.2))	1.2*** (0.2-3.3)
Total globs (g/l)	35 (26-52)	35 (22-49)	31*** (22-46)	31**** (19-39)
Albumin (g/l)	38 (24-48)	38 (28-43)	38 (25-44)	41**** (34-45)
AST (u/l)	18 (10-39)	17 (6-33)	17 (4-500)	21* (10-104)
ALT (u/l)	15 (3-40)	16 (3-37)	15.5 (5-998)	16 (7-42)
Alkaline Phosphatase (u/l)	255 (89-830)	220* (96-495)	225 (93-360)	225 (95-365)
MCV (fl)	81 (66-93)	85** (68-97)	86*** (69-95)	85**** (75-98)
Serum B12 (pg/ml)	303 (82-836)	281 (143-802)	310 (213-585)	327 (162-585)
Serum folate (ng/ml)	2.5 (1.1-6.0)	2.4 (1.0-5.3)	2.2 (1.7-3.1)	2.7 (1.3-6.9)
RBC folate (ng/ml)	163 (5-247)	209 (68-278)	190 (101-582)	168 (112-227)
Creatinine (umols/l)	70 (40-184)	73 (45-110)	72 (43-103)	70 (42-110)

Table XXIV (Cont) Study 3. Clinical, haematological & biochemical indices for patients allocated to 3.0g/day - medians (ranges) - n = 40. Wilcoxon v Wk o - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

gastrointestinal symptoms. Formal clinical indices of disease activity were not measured. Patient characteristics are shown in Table XXV.

3.5 Total experience with sulphasalazine in rheumatoid arthritis with reference to efficacy and toxicity

The studies so far described contain information on one hundred and fifty-eight patients with rheumatoid arthritis treated with sulphasalazine with a follow-up period of 24 weeks. In an attempt to give an overview of this experience Fig X shows the overall drop out rate over the first 24 weeks and Fig XI shows the reasons for discontinuation of therapy.

One hundred and eight (68%) of patients continued sulphasalazine past 24 weeks. Of the 108 patients who continued, 19 were receiving a lower than allocated dose because of dose related toxicity at their allocated dose. Ninety of the 158 patients studied were followed for at least 48 weeks and of these 47 (53%) remained on treatment, of whom 6 were receiving a lower than allocated dose because of dose related toxicity.

In total 21 (14%) patients stopped over the first 24 weeks because of nausea and/or vomiting, a further 54 experienced the side effect without discontinuing therapy and of these 38 managed to achieve their allocated dose. In these patients symptoms tended to be transient and in general occurred early. Three patients developed a marked rise in hepatic enzymes while receiving sulphasalazine; one of these stopped the drug simultaneously because of upper gastrointestinal symptoms; one was stopped because of the hepatic abnormalities and biopsy showed

Patient	Age yrs	Reason for Discontinuing therapy	Week of Discontinuing therapy
1	69	Nausea	3
2	75	Vomiting	2
3	73	Vomiting	4
4	79	Nausea and vomiting	6
5	69	)	
		)	
		)	
6	80	)	
		)	Continued for at least 24 weeks
		)	
7	78	)	
		)	
		)	
8	65	)	

Table XXV Toxicity data on patients in Study 4

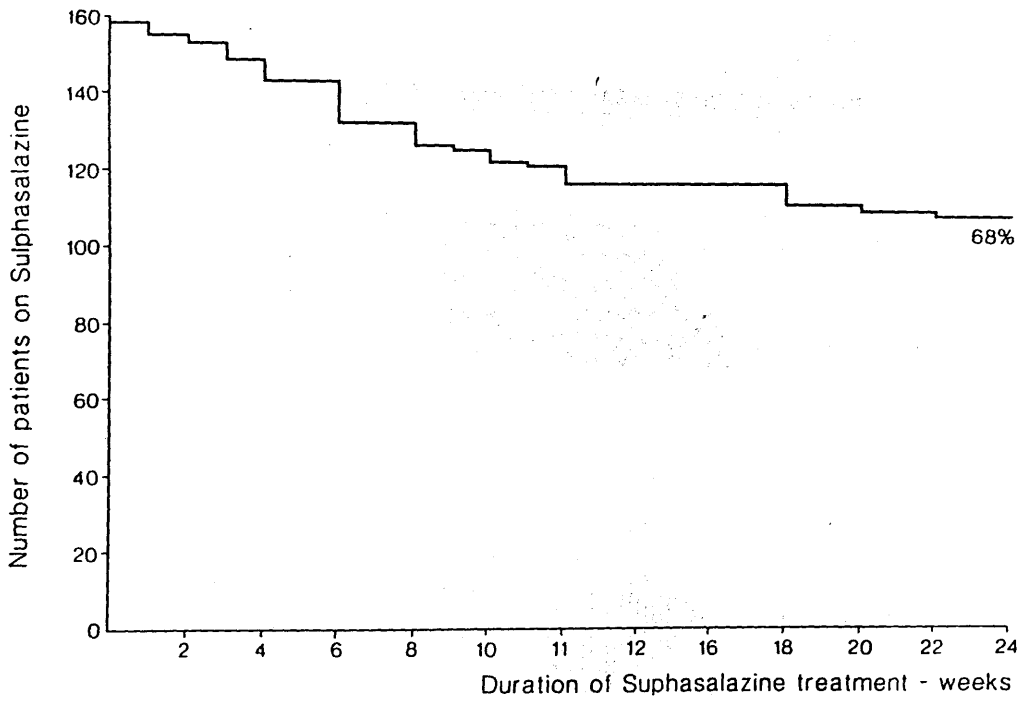


Fig. X Pattern of discontinuation of sulphasalazine therapy over the first 24 weeks (n = 158).

REASONS FOR DISCONTINUING SULPHASALAZINE DURING FIRST 24 WEEKS OF THERAPY. (n =158)

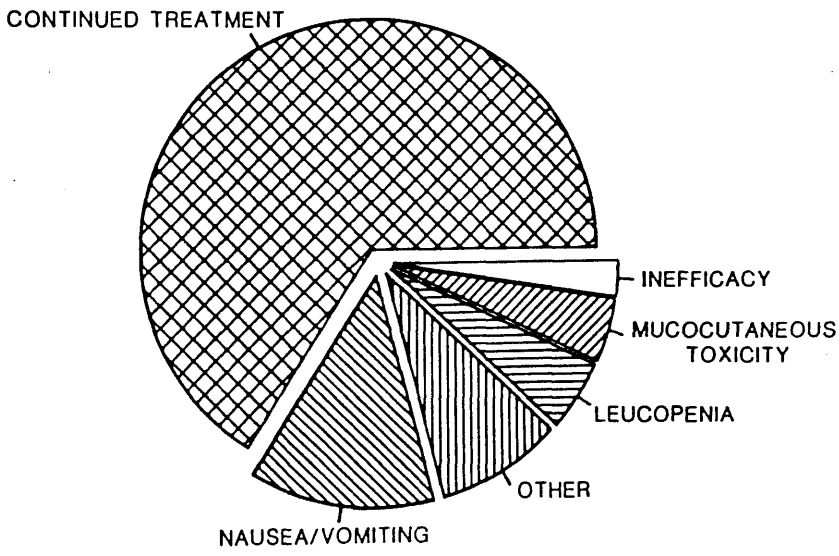


Fig. XI Reasons for discontinuing sulphasalazine therapy over the first 24 weeks (n = 158).

drug induced changes (Appendix 3) and the third patient's hepatic enzymes were normal when re-checked and in this case transient infection or laboratory error would seem the most likely explanations. In addition 5 patients showed a mild rise in ALT (maximum 69u/l) and 3 mild rises in AST (maximum 80u/l) outwith the normal range but showed no progressive changes and medication was continued. Overall a generalised rise in hepatic transaminases was seen during the study (Table XXVI). Although both acute hepatotoxicity (41, 42, 43, 44) and hepatotoxicity after many years (45) has been described, no such generalised rise in transaminases has previously been reported.

A significant rise was seen in the mean cell volume over the study period, however, no patient developed a frank haemolytic anaemia and in study 3 (Tables XXIII and XXIV), B12 and folate levels did not alter significantly either in the group as a whole, in the sub-group of patients who developed an MCV > 96fl or in any individual patient. Even in those 28 patients whose MCV rose to > 96fl (the upper limit of normal) a significant rise in haemoglobin level was seen by week 24 (Wilcoxon matched-pairs signed-rank test $p < 0.05$).

Seven patients (4%) had treatment discontinued because of leucopenia. In 5 of these cases total WBC did not fall below 2.0×10^9/l but in 2 cases a profound neutropenia of $< 0.5 \times 10^9$ polymorphs/l was found; all patients recovered with conservative management. All seven cases of leucopenia occurred within the first 12 weeks of treatment and in some of these patients, including one of the patients with a profound leucopenia, there was a progressive reduction in WBC. Another one patient developed thrombocytopenia shortly after stopping sulphasalazine because of upper gastrointestinal symptoms.

Mouth ulcers occurred within 2 weeks of starting treatment in 2 cases and in both cases resolved with conservative management after therapy was stopped. One of these patients had severe Sjogren's syndrome noted before starting sulphasalazine. A further 5 patients stopped in the first 24 weeks and one at week 36 because of skin rash. In most cases this was maculopapular in type but in one case the patient developed large urticarial lesions on her back. All cleared up with discontinuation of therapy. No evidence of persistent proteinuria or haematuria attributable to sulphasalazine therapy was apparent.

Descriptions of individual patients who developed serious toxicity are given in Appendix 3.

Fig XII shows the reasons for discontinuing sulphasalazine therapy between weeks 24 and 48. It is apparent that at this stage inefficacy is the most common reason for stopping treatment.

Table XXVI shows changes in various indices during 24 weeks treatment in 150 patients (this excludes the 8 patients in study 4 for whom complete efficacy data were not available). Again, as in individual groups a general improvement in most indices is seen. In 8 patients the DAI fell to 8 or less which corresponds to Mallya and Mace's "inactive" group (122).

Section 4

Discussion

4.1 Efficacy

A second line drug in the treatment of rheumatoid arthritis is

n = 61 (68%) n = 47 (53%)

wk 24 wk 48

nausea/vomiting	2
rash	1
inefficacy	10
other	1

Fig. XII Pattern of drop out in Studies 1 and 2 (n = 90) over the second 24 weeks of treatment.

	Wk 0	Wk 24
Age (yrs)	55.7 (28-77)	
Disease Duration (yrs)	8.2 (1-57)	
ESR (mm/hr)	65 (2-150))	35*** (2-129)
Hb (g/dl)	11.4 (7.4-17.0)	11.9**** (7.1-17.7)
Platelets (x 10^9/l)	395 (118-888)	320**** (134-809)
Rheumatoid factor titre (Rose Waaler)	1/128 (0-1/1024)	1/64 (0-1/1024)
Articular index	19.3 (0-61)	7.2**** (0-33)
Limbering up time (mins)	91 (0-all day))	30**** (0-all day)
Pain score	2.7 (1-4)	1.8**** (0-4)
Grip strength (mmHg)	80 (35-190)	91**** (9-245)
DAI	16.9 (12-23)	13.6**** (7-21)
IgA (g/l)	3.2 (0.3-7.5)	2.8**** (0.2-7.9)
IgG (g/l)	13.4 (6.2-40.2)	12.1**** (4.3-28.8)
IgM (g/l)	1.25 (0.4-8.7)	1.06**** (0.2-3.4)
Total globs (g/l)	34.5 (21-56)	29.5**** (19-45)
Albumin (g/l)	37.6 (24-53)	40.5**** (34-48)
AST (u/l)	16 (5-39)	19**** (7-301)
ALT (u/l)	12 (3-40)	16**** (5-282)
Alkaline Phosphatase (u/l)	235 (24-880)	215* (10-850)
MCV (fl)	82 (66-102)	87**** (69-114)
Creatinine (umol/l)	70 (40-217)	75 (42-155)

Table XXVI Clinical, haematological and biochemical indices for all sulphasalazine treated patients in Study 1, 2 and 3 who completed 24 weeks' therapy - median (range) Wilcoxon v Wk 0 - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001

characterised by its ability to improve both clinical and laboratory indices of disease activity.

In study 1 these criteria were fulfilled by sulphasalazine and by sodium aurothiomalate but not by placebo. Both sulphasalazine and sodium aurothiomalate produced a similar pattern of improvement in these indices and, in the studies where functional index was measured, sulphasalazine in a dose of 3g/day also caused significant improvement in this outcome measure. Neither sulphasalazine nor sodium aurothiomalate (a well proven and widely accepted second line drug) produced absolute 24 week values which were significantly different from placebo and even at 48 weeks such differences were minimal. This apparent discrepancy between intra- and inter-group comparisons is discussed below.

In most cases sulphasalazine or sodium aurothiomalate was stopped because of side effects whereas significantly more patients stopped placebo because of lack of effect. It is likely, therefore, that the placebo treated patients who continued therapy were a biased group selected on the basis of milder disease activity and therefore able to continue on inactive treatment. This is confirmed statistically in that the 6 placebo patients who continued treatment to 48 weeks had a significantly lower starting ESR and DAI than those who stopped (Mann-Whitney U Test - $p < 0.05$), whereas there were no statistically significant differences in initial inflammatory parameters between those patients on sodium aurothiomalate or sulphasalazine who stopped therapy and those who continued therapy (Mann-Whitney $p > 0.05$). In addition, those placebo patients who continued had a significantly lower ESR and DAI at week 0 than patients allocated to sulphasalazine

who continued treatment. This self selection of the patients with milder initial disease activity to remain on placebo therapy renders the comparison of absolute values for inflammatory parameters between the groups of little value and most probably explains the apparent paradox of highly significant within group improvements in the active treatment groups but an inability to demonstrate, at the time of assessment, marked differences between the absolute values for inflammatory parameters in the active and placebo treated patients. This pattern which consists of a high drop out rate because of inefficacy in placebo or inactive groups combined with a failure to improve statistically over 24 or 48 weeks in those placebo patients who remain on treatment and and inability to demonstrate a significant difference between placebo/inactive and active drug groups using 24 or 48 week values has been seen in a number of other similar studies of second line drugs published over the past few years from the Centre for Rheumatic Diseases (149, 150, 151) (Tables XXVII and XXVIII). As patients who discontinue placebo therapy almost invariably commence an active drug an "intention to treat" analysis would offer no further advantage.

It is quite apparent on examining the pattern of p values in table XXVIII which agents are active second line drugs and which are not. One could conceivably argue, however, that the reason we do not see any major statistical improvement in patients who remain on placebo is that, as this group shows a bias towards lower disease activity at the outset, there is perhaps little scope for improvement. To test this hypothesis I have therefore selected from the 12 sulphasalazine patients who achieved 48 weeks treatment in study 1, the 6 patients with the lowest ESR, at week 0 (median = 47mm/hour, range = 18-

	Auranofin	GST	Placebo	Ketotifen	Placebo	GST	Penicillamine	Levamisole 450mg/wk	GST	Levamisole 150mg/wk
No entered	30	30	30	30	30	25	25	25	24	24
No on treatment at 24 weeks	26	23	17	19	11	16	18	9	20	17
No on treatment at 48 weeks	17	22	0	2	1	15	11	7	16	12
Drop outs over 48 weeks because of lack of effect	6	0	27	27	29	0	2	1	1	3

Table XXVII Pattern of drop out over first 48 weeks in previous studies carried out at the Centre for Rheumatic Diseases, 1977-1982 (GST = sodium aurothiomalate)

	n=30	n=30	n=30	n=30	n=30	n=25	n=25	n=25	n=24	n=24
Wk 0 v Wk 24	Auranofin	GST	Placebo	Ketotifen	Placebo	GST	Penicillamine	Levamisole 450mg/wk	GST	Levamisole 150mg/wk
n	26	23	17	19	11	16	18	19	20	17
ESR	***	NS	NS	NS	NS	**	**	NS	****	NS
Hb	NS	NS	NS	NS	NS	**	**	**	NS	NS
Plats	***	****	NS	NS	NS	*	*	NS	*	NS
RF	***	****	NS	NS	NS	*	*	**	***	NS
AI	NS	**	NS	***	***	**	**	**	****	NS
Pain score	**	****	NS	NS	NS	**	**	-	**	NS
Grip	NS	NS	NS	NS	NS	NS	NS	***	-	NS
LUT	NS	*	NS	NS	NS	_	_	_	NS	NS

Table XXVIII Pattern of change in inflammatory indices in previous studies carried out at the Centre for Rheumatic Diseases, 1977-82. p values Wilcoxon matched-pairs signed rank test, Wk 0 v Wk 24 and Wk 0 v Wk 48. *p<0.05; **p<0.01; ***p<0.005; ****p<0.001; NS p > 0.05.

NB - n values are numbers analysed and include some patients who discontinued therapy at the time of analysis

	n=30	n=30	n=30	n=30	n=30	n=25	n=25	n=25	n=24	n=24
Wk 0 v Wk 48	Auranofin	GST	Placebo	Ketotifen	Placebo	GST	Penicillamine	Levamisole 450mg/wk	GST	Levamisole 150mg/wk
n	17	12	0	3	1	-	-	-	16	12
ESR	****	****							**	**
Hb	NS	*							-	*
Plats	-	-							*	NS
RF	-	-							***	NS
AI	*	**							-	-
Pain score	*	***							**	NS
Grip	**	**							-	-
LUT	***	**							-	-

Table XXVIII (Cont) Pattern of change in inflammatory indices in previous studies carried out at the Centre for Rheumatic Diseases, 1977-82. p values Wilcoxon matched-pairs signed rank test, Wk 0 v Wk 24 and Wk 0 v Wk 48. *p<0.05; **p<0.01; ***p<0.005; ****p<0.001; NS p > 0.05.
NB - n values are numbers analysed and include some patients who discontinued therapy at the time of analysis

55mm/hour). Table XXIX shows the pattern of improvement in inflammatory indices in these patients and it can be seen that although changes in the ESR are not seen, many other inflammatory indices do show improvement. Data presented in Chapter 8 from a larger number of patients with an initial ESR $\leq$ 30mmHg confirm this finding.

The percent change in the various indices in the 3 treatment groups in study 1 has also been analysed (Tables XV and XVI) and again significant differences are seen between sulphasalazine and placebo groups and between sodium aurothiomalate and placebo groups but not between sodium aurothiomalate and sulphasalazine groups. Changes in absolute values showed significance but because of possible bias in the pattern of drop out this may not be as meaningful. It is also perhaps interesting to note that although the only improvement seen in the placebo group is in the DAI both the percentage change and final value is significantly better in the sulphasalazine and sodium aurothiomalate groups. Thus, overall, the evidence is strongly in favour of sulphasalazine being a second line agent.

The above findings may also suggest that, provided one uses the occasional placebo group and known active drug group as a "quality control" in one's assessment system, rigidly designed placebo controlled trials may not be necessary and, if the differences in the pattern of drop out in the placebo group are not appreciated, may be frankly misleading.

Comparison of sodium aurothiomalate and sulphasalazine groups shows no major differences other than perhaps a slightly higher drop out rate due to inefficacy and a slightly earlier onset of activity in the

latter. This does not mean, however, that no difference in the efficacy of these drugs exists but merely that we would need much larger numbers to show this. The power of study 1 to demonstrate a significant difference in the change in ESR between sulphaslazine and placebo group is 89% (assuming, for the moment, a normal distribution for ESR) but only 12.8% to demonstrate a real difference between ESR changes for sodium aurothiomalate and sulphasalazine (Appendix 2). Such a difference, however, is probably not of practical importance as most patients with rheumatoid arthritis, once commenced on second line drugs, will require more than one such agent (1) and, as only about half a dozen of these drugs exist, all are needed.

In addition to a multitude of "process measurements", functional index (an outcome measure) was also assessed. Again this showed a statistically significant improvement in the sulphasalazine treated groups over 1 year of treatment. The functional index used here correlated well with inflammatory indices both in terms of absolute values (Table VII) and change over the one year treatment period. Such a relationship is important to demonstrate with drug treatment as it shows that the usual parameters measured (process measurements) do in fact have some bearing upon outcome at least in the short (1 year) term.

The other commonly used outcome measurement, namely radiological progression, was not assessed because my previous experience suggests that large numbers of patients need to be assessed over long periods and even then, as the effect of second line drugs is to slow deterioration rather than halt or reverse these changes, a large, representative, long term placebo group (almost an impossibility) is

required (125).

4.2 Toxicity

The results shown here suggest that sulphasalazine is not the relatively non-toxic second line drug suggested from experience of its use in inflammatory bowel disease (6 and Truelove S.C. personal communication). At least 3 patients had serious life threatening side effects (2 had severe leucopenia and one developed thrombocytopenia after discontinuation of therapy). Several more showed leucopenia and raised transaminase levels which may have proven equally serious if not detected early. Sulphasalazine does, however, hold an advantage over other second line drugs in that the serious haematological and hepatic toxic effects were all apparent during the first twelve weeks of treatment and, therefore, the most intensive monitoring can be concentrated over this period. In addition minor changes occurred in MCV and transaminases which, although real, appeared to be of little clinical significance at 1 year follow up.

No evidence of nephrotoxicity, as is found with other second line drugs such as gold salts and penicillamine, occurred with sulphasalazine. This would suggest that sulphasalazine may be of use in patients who have evidence of proteinuria or haematuria prior to treatment but who do require second line drugs, as monitoring would prove easier than with most other second line agents.

The most frequent side effect of sulphasalazine was found to be the relatively mild one of nausea/vomiting. Methods of dealing with this will be discussed in Chapter 8.

The range of haematological side effects was interesting in that it tended to differ from previously published reports in producing a high incidence of leucopenia but no evidence of frank haemolysis or of megaloblastic anaemia.

The three long term studies which were published after completion of the work in this chapter (143, 144, 145) followed a total of 201 sulphasalazine treated patients (2g/day) over periods ranging from 12 to 42 months. Only 2 of these patients developed leucopenia, one of whom was later diagnosed as having Felty's syndrome and it seems likely that the other patient with leucopenia has already been described (8, 9). Again in these studies the most common side effect necessitating treatment withdrawal was nausea/vomiting (15%). Two of these studies, however, showed a high late drop out rate because of inefficacy (143, 145) whereas the third (shorter term) study failed to confirm this (144). Certainly the finding in my work that 10 (11%) patients discontinued therapy between weeks 24 and 48 because of inefficacy, would support the finding of a high late drop out rate.

In contrast, of 200 patients commenced on sulphasalazine for ulcerative colitis and followed for up to 20 years, 184 remained on therapy. Of the 13 who stopped because of side effects 9 stopped because of cutaneous toxicity (8 rash, 1 alopecia), 2 because of gastrointestinal symptoms, 1 because of dyspnoea and one because of a leucopenia with a drop in WBC count from 6.5 x 10^9/l to 2.5 x 10^9/l. The other 3 patients stopped of their own volition as they felt well (Truelove S.C. personal communication).

Section 5

Conclusions

In conclusion data presented in this chapter allow the following conclusions to be drawn.

1. Sulphasalazine is an effective second line drug in the treatment of rheumatoid arthritis.

2. Serious toxicity can occur but tends to occur early. I would recommend fortnightly monitoring of full blood count and platelets over the first twelve weeks and, thereafter, 6 weekly monitoring. In addition liver function tests should be checked at weeks 0, 6 and 12 and 12 weekly thereafter. This is considerably less monitoring over the longer term than is required with either gold salts or d-penicillamine.

3. The range of side effects of sulphasalazine is different from other second line drugs particularly in respect to the lack of evidence of nephrotoxicity.

4. Late drop out because of inefficacy may be a problem.

5. In view of 1), 2) and 3) sulphasalazine is a useful addition to the second line armamentareum.

6. Although desirable for the assessment of a drug as a second line agent, placebo groups are impractical in the medium term, impossible in the long term and may not be strictly necessary.

SUMMARY

Chapter 4

Ninety patients with active, definite or classical rheumatoid arthritis were randomly allocated to sulphasalazine 3g/day, placebo tablets or sodium aurothiomalate. Comparison of sulphasalazine and placebo was double blind. At the 24 week assessment using a Wilcoxon matched-pairs signed-rank test, sulphasalazine and sodium aurothiomalate but not placebo treated patients showed significant improvement in laboratory and clinical indices of inflammation. The failure to show such marked differences between groups using a Mann-Whitney test is probably explained by the significantly higher drop-out because of inefficacy in the placebo group which resulted in the remaining placebo treated patients suffering initially less active disease. Improvement could be seen as early as 6 weeks with sulphasalazine but with sodium aurothiomalate similar improvement was not seen until the 12 week assessment. Improvement was maintained to 48 weeks.

Analysis of drug efficacy from subsequent studies primarily designed to test other hypotheses confirmed the above findings. Sixty-eight percent of patients continued sulphasalazine for at least 6 months and 53% continued for 1 year. The most common toxic events related to sulphasalazine consisted of nausea and/or vomiting but more serious haematological and hepatic side effects also occurred.

CHAPTER 5

Relationship of dose and serum levels of sulphasalazine to its efficacy in rheumatoid arthritis

Section 1

Introduction

Chapter 4 has shown sulphasalazine to be an effective second line drug in the treatment of rheumatoid arthritis. The rate of drop out because of side effects was high. As with ulcerative colitis (23) this may be related to the administered dose.

The dose of sulphasalazine used to treat rheumatoid arthritis has varied considerably in the various published studies. In her studies Nana Svarz used 4-6g/day initially and then reduced to a maintenance dose of 1.5-3g/day (2). A similar initial regimen was employed by Sinclair and Duthie who settled for a starting dose of 5g/day but reduced to a maintenance dose of 1g/day (4).

Recent studies have tended to use either 2g/day (9, 141, 142, 143, 144, 145) or 3g/day (8, 140) built up gradually over a number of weeks and then retained as the maintenance dose.

In study 1, I chose a dose of 3g/day as the aim of this study was to investigate sulphasalazine for efficacy and thus the highest of the currently used doses seemed most appropriate.

In the treatment of ulcerative colitis it has been shown that the higher the dose of sulphasalazine the greater the therapeutic effect. The price of the higher dose, however, is a greater incidence of adverse effects (23). No direct comparison of differing doses of sulphasalazine in the treatment of rheumatoid arthritis has been made and there has been no attempt to demonstrate a relationship between dose and efficacy or to justify any of the currently used doses.

To date no data are available on the relationship of blood levels of sulphasalazine or its various metabolites to its therapeutic efficacy in rheumatoid arthritis. Even in the ulcerative colitis literature there are contradictions. Das and co-workers (17) have shown that non-responders have lower total sulphapyridine levels than responders and that, on increasing the dose in these patients, clinical improvement is seen and this is accompanied by rising blood levels of total sulphapyridine. On the basis of this observation they suggest that the low blood levels of total sulphapyridine allow relapse of the disease whereas the higher blood levels produced by an increase in dose produce improvement in the disease. An alternative explanation of these data has been offered, however, suggesting that lower disease activity allows greater absorption of sulphapyridine and thus the higher blood levels found in patients with quiescent disease and the rise in levels with improvement are an effect rather than a cause of reduced disease activity (21). This claim is backed by longitudinal data which show no relationship between circulating blood levels of any metabolite and the liability to relapse in ulcerative colitis and a fall in serum levels of sulphapyridine during a spontaneous relapse which is maintained until remission (24).

In view of the possible dose related effects in ulcerative colitis it is important to investigate the relationship of dose to efficacy in rheumatoid arthritis. Is is also important to investigate the relationship of serum levels to efficacy as this may prove a useful method in choosing the optimum dose and, in addition, may give some indication as to the active metabolite of sulphasalazine.

In the first part of this chapter I describe the results of a study comparing 1.5g/day and 3.0g/day (Study 2).

The second part of the results section describes a subgroup of patients in Study 2 in whom blood levels of sulphasalazine and its metabolites were measured and related to disease activity.

Section 2

Patients and methods

Sixty patients with active classical or definite rheumatoid arthritis (146) not controlled by first line drugs alone were studied. Patients were randomly allocated (30 per group) to enteric coated sulphasalazine 0.5g/day rising by weekly increments of 0.5g/day to either 1.5g/day (low dose) or 3.0g/day (high dose). All patients continued to receive NSAIDs and none received corticosteroids or other second line drugs during or in the 3 months prior to the study.

These patients have already been described (Chapter 4 - Study 2) and exclusion criteria and methods of assessment were as described earlier.

Clinical assesment was carried out "blind" by a single metrologist and laboratory assessment was carried out"blind" in the routine laboratories. Percent change in inflammatory indices was calculated as described in Chapter 4. Patients were seen for monitoring of full blood count and platelet count fortnightly for the first 12 weeks and 6 weekly thereafter. Liver function tests were checked 6 weekly. During the study the patients were given free access to prochlorperazine in a maximum dose of 10mg t.i.d. for symptoms of

nausea or vomiting.

In addition to the above measurements (the results of which will be described in the first part of the results section) 44 patients (No 201-244 inclusive) had serum levels of sulphasalazine and metabolites (sulphapyridine and acetylsulphapyridine) measured at 12, 18 and 24 weeks at which times they could be expected to be in a steady state. All samples were taken in the forenoon following an early morning dose of sulphasalazine. Samples were allowed to clot at room temperature and then centrifuged at 3000rpm for 15 minutes and the supernatant stored at -20^{o}C until analysis. The measurements were carried out by Pharmacia GB Ltd using high performance liquid chromatography (152, 153). The mean serum levels of sulphasalazine and its metabolites were calculated for each patient from the 12, 18 and 24 week samples as it was felt that by "ironing out" other variables this would give a more accurate assessment of steady state levels than would a single level and would give the most easily handled representation of the serum levels. A crude assessment of "total sulphapyridine" was made by summating the sulphapyridine and acetyl sulphapyridine levels. Acetylator phenotype was calculated from:-

$$\% \text{ acetylated} = \frac{[\text{acetylsulphapyridine}]}{[\text{total sulphapyridine}]} \times 100\%$$

(19).

These values were related to the two most representative of the inflammatory indices, the ESR and the disease activity index (DAI).

Section 3

Results

3.1 Effect of dose

Table XVII shows the initial indices for the two groups (1.5g/day and 3g/day). No difference between the groups could be demonstrated with respect to age, body weight, disease duration or inflammatory indices at week 0(Mann-Whitney U test $p > 0.05$). After 24 weeks, 24 (80%) of patients allocated to 1.5g/day and 20 (66%) of those allocated to 3.0g/day remained on sulphasalazine. Table XXX shows the reason for and time of discontinuation of therapy over the first 24 weeks.

Prochlorperazine was used by 12 patients (6 allocated to 1.5g/day and 6 allocated to 3.0g/day). All but 2 of these were able to continue sulphasalazine to 24 weeks and 8 of those who continued achieved their allocated dose (3 - 1.5g/day, 5 - 3.0g/day).

Two patients allocated to 1.5g/day received a smaller dose because of dose related toxicity and 3 had their doses increased because of inefficacy. Two patients allocated to 3.0g/day could not achieve this dose because of dose related toxicity (Table XXXI).

Statistical analysis comparing 1.5g/day and 3.0g/day was subsequently carried out using both allocated dose and actual 24 week dose but as the results are very similar only analysis by allocated dose is described here.

Tables IXX and XX show the medians and ranges of inflammatory indices at each assessment and also the p value compared to the wk 0 results (Wilcoxon matched-pairs signed-rank test). Table XXXII compares both

	1.5g/day (n = 30)		3.0g/day (n = 30)	
	Total	Week Stopped	Total	Week Stopped
Rash	2	1, 18	1	11
Mouth ulcers	0	-	1	1
Leucopenia	1	8	0	-
Nausea/vomiting	1	1	4	4, 5, 12, 22
Lack of effect	0	-	1	24
Acute dyspnoea	0	-	1	3
Other	2	8, 10	2	6, 11
	6		10	

Table XXX Study 2. Reason for stopping sulphasalazine therapy - 24 week follow up.

	1.5g/day (n = 30)		3.0g/day (n = 30)	
	Total	Week Stopped	Total	Week Stopped
Rash	2	1, 18	1	11
Mouth ulcers	0	-	1	1
Leucopenia	1	8	0	-
Nausea/vomiting	1	1	4	4, 5, 12, 22
Lack of effect	0	-	1	24
Acute dyspnoea	0	-	1	3
Other	2	8, 10	2	6, 11
	6		10	

Table XXX Study 2. Reason for stopping sulphasalazine therapy - 24 week follow up.

Actual 24 week dose (g/day)	Patients allocated to 1.5g/day No of patients (%)	Patients allocated to 3g/day No of patients (%)
1.0g/day	2 (8%)	0
1.5g.day	19 (80%)	1 (5%)
2.0g/day	2 (8%)	1 (5%)
2.5g/day	0	0
3.0g/day	1 (4%)	19* (90%)

Table XXXI Study 2. Actual dose of sulphasalazine at 24 weeks for patients allocated to 1.5g/day and 3.0g/day.

*1 patient in this group discontinued treatment at the 24 week visit.

	1.5g/day	3g/day	
n	24	21	p
ESR (mm/hr)	45 (5-129)	24 (7-118)	NS
Haemoglobin (g/dl)	12.5 (9.3-15.6)	12.0 (10.2-17.7)	NS
Platelets (x 10^9/l)	312 (161-475)	341 (224-809)	NS
Rose Waaler Titre	1/512 (0-1/1024)	1/512 (0-1/1024)	NS
Articular Index	8.5 (0-30)	7 (0-24)	NS
Pain score	2 (0-4)	1.5 (0-4)	NS
Grip strength (mmHg)	95 (57-215)	95 (57-235)	NS
LUT	60 (0-all day)	15 (0-all day)	NS
Albumin (g/l)	41 (36-47)	40 (36-45)	NS
Globulin (g/l)	31 (23-43)	32.5 (22-45)	NS
Alk phos (u/l)	236 (125-780)	195 (10-250)	NS
SGOT (u/l)	22 (12-32)	18 (7-80)	NS
SGPT (u/l)	17 (6-38)	16 (7-69)	NS
IgA (g/l)	3.0 (1.2-7.9)	3.7 (1.2-5.9)	NS
IgG (g/l)	12.5 (7.0-22.4)	12.1 (6.5-28.8)	NS
IgM (g/l)	1.0 (0.6-2.7)	1.17 (0.6-2.1)	NS
Creatinine (umol/l)	75 (50-118)	75 (49-135)	NS
Mean cell colume (fl)	87 (72-102)	89 (80-112)	NS
DAI	14.6 (9-21)	12.2 (7-18)	NS

Table XXXII Study 2. Comparison of inflammatory indices at week 24 - medians (ranges)- in patients allocated to 1.5g/day and those allocated to 3g.day (Mann-Whitney U test). NS = $p > 0.05$.

groups in terms of the 24 wk values for inflammatory indices. However, platelet count, serum albumin, serum globulins, IgG, IgM and DAI did show a significantly greater percentage improvement in the 3g/day group.

The range of body weights in the patients studied, however, varied from 40-91kg. In view of this it is probably more useful to compare dose expressed as mg/kg body weight. This figure (using the actual dose at 24 weeks) was correlated with both percent change in ESR and percent change in DAI over 24 weeks (Figs. XIII, XIV). In each case a significant negative correlation was found, ie, the greater the dose the greater the fall in ESR and DAI (Spearman Rank correlation rs = -0.428 and -0.516 respectively; $p < 0.01$ for both measurements).

Previous second line studies from this centre have suggested that the average expected improvement is around 50% improvement in ESR and 33% improvement in disease activity index (Personal communication H A Capell). Using these levels of improvement as our standard, patients receiving in excess of 40mg/kg body weight showed such improvements more commonly than those receiving a lower dose ($\chi^2 = 4.02$; $p < 0.05$ and $\chi^2 = 8.188$; $p < 0.01$ repsectively) (Figs. XV, XVI).

3.2 Effect of serum levels

Of the 44 patients who had serum drug levels measured, complete data was available for 29 (10 had stopped treatment before 24 weeks and in 5 ESR or serum levels were not available for the appropriate assessment either because of clotted or missing specimens). Table XXXIII shows the medians and ranges for measurements of sulphasalazine and its metabolites and their relationship to dose and acetylator

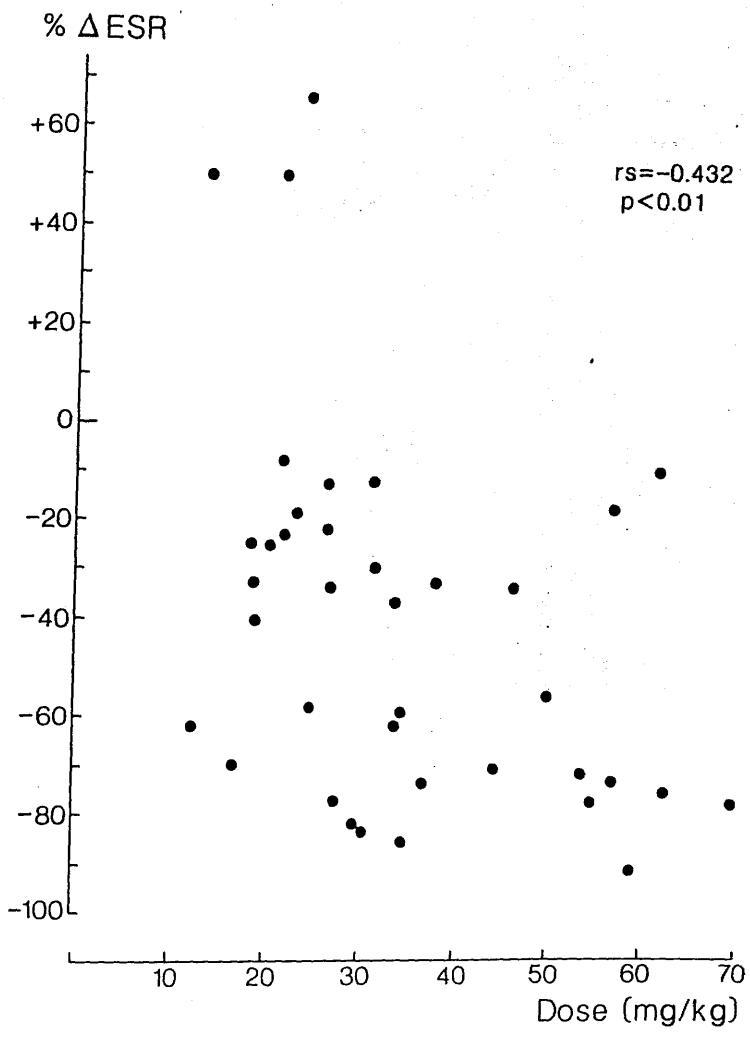


Fig. XIII Correlation between actual 24 week dose expressed as mg/kg body weight and percent change in ESR over 24 week period (Spearman Rank).

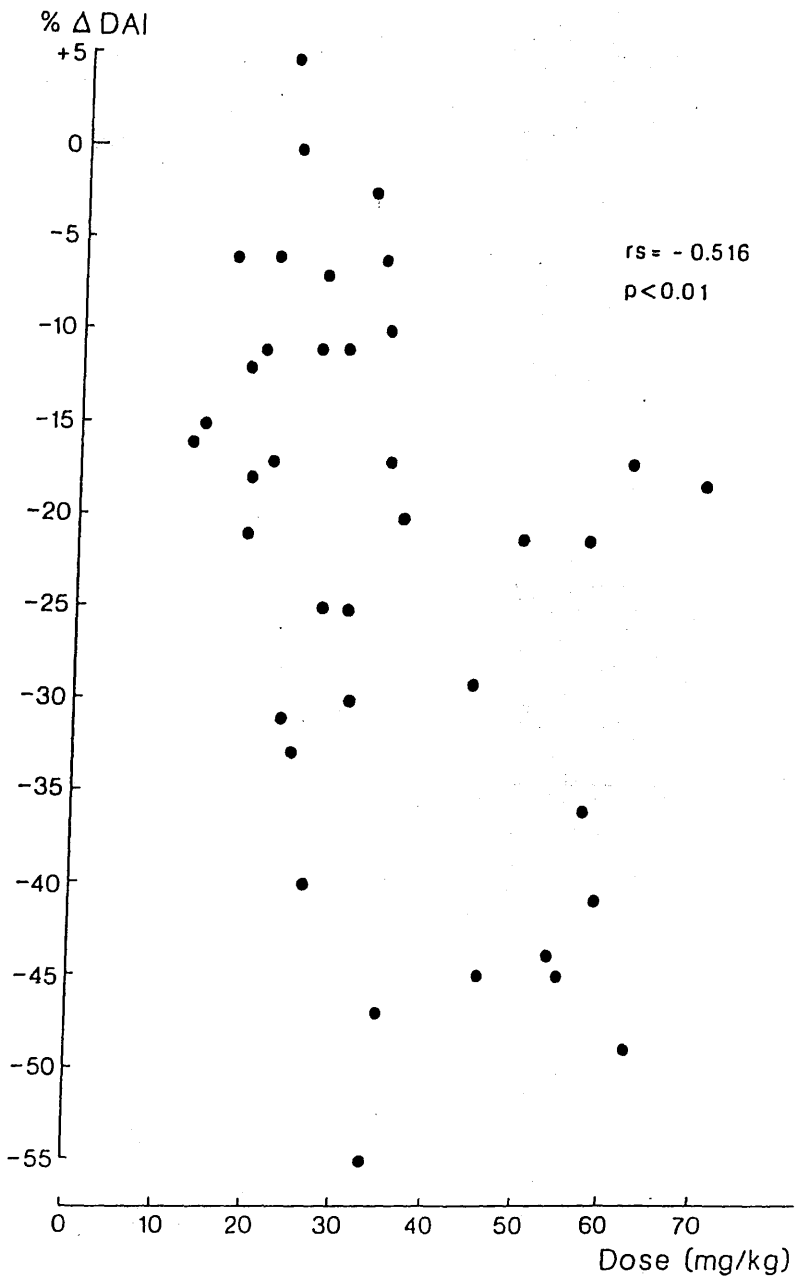


Fig. XIV Correlation between actual 24 week dose expressed as mg/kg body weight and the percent change in DAI over 24 week period (Spearman Rank).

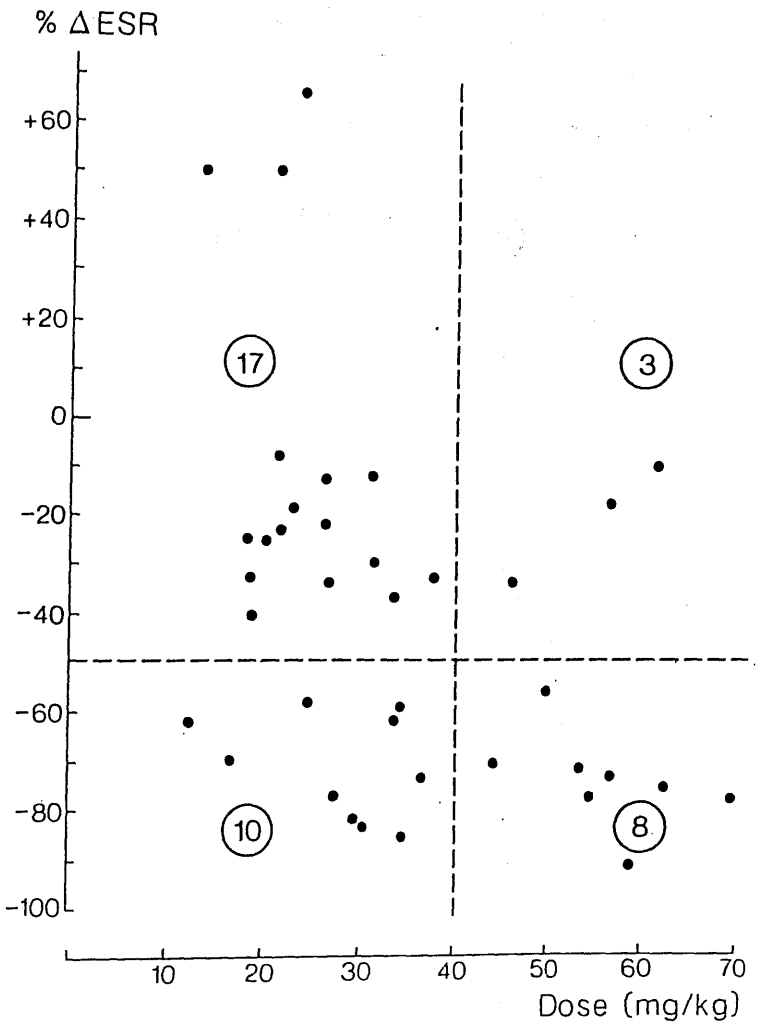


Fig. XV Number of patients achieving an improvement in ESR >50% related to the actual 24 week dose of sulphasalazine expressed as mg/kg body weight ($X^2 = 4.02$, $p < 0.05$).

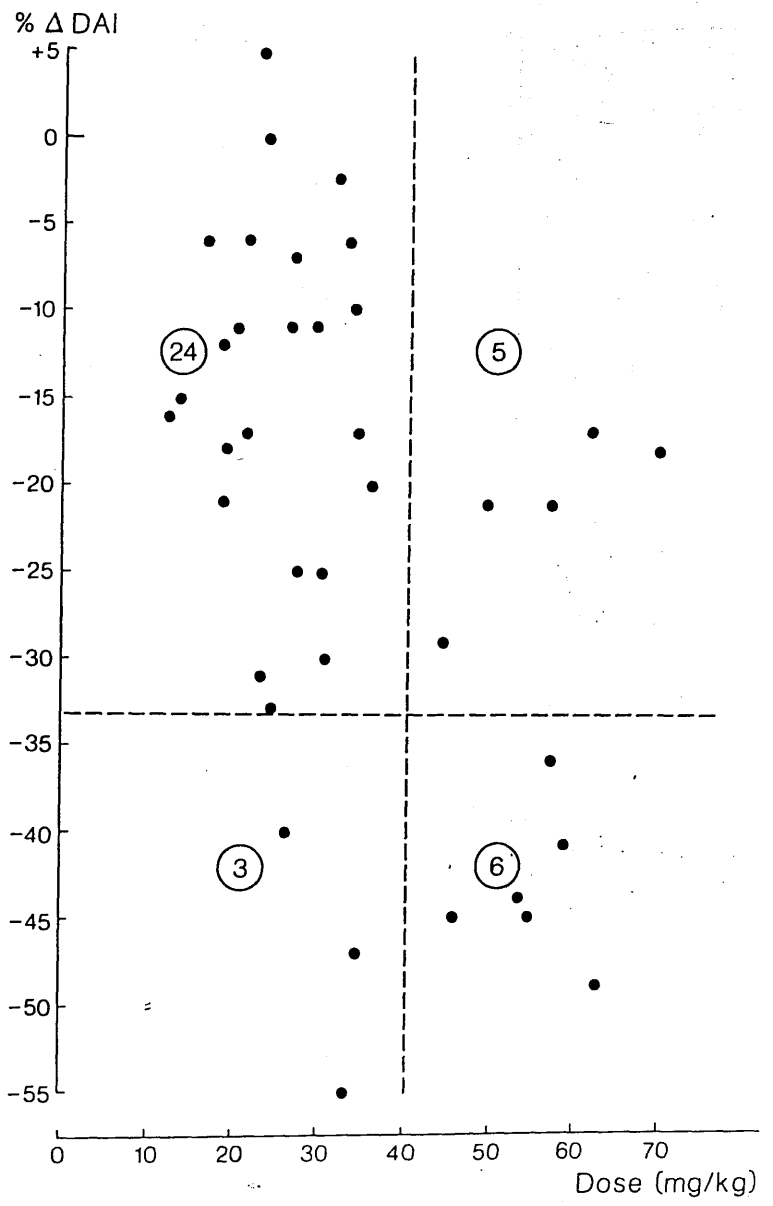


Fig. XVI Number of patients achieving an improvement in DAI >33% related to the actual 24 week dose of sulphasalazine expressed as mg/kg body weight ($X^2 = 8.188$; $p < 0.01$).

	Serum levels Median (ug/ml) (range)		Mann-Whitney U Test
Total Sulphapyridine			
Total Whole group (SP + ASP) (n=29)	32.5 (5.5 - 83)		
High dose/slow acetylator (n=6)	35 (27 - 68.5)	)	NS
High dose/fast acetylator (n=5)	49.5 (30.5 - 83)	)	
Low dose/slow acetylator (n=12)	33 (5.5 - 52)	)	NS
Low dose/fast acetylator (n=6)	25.5 (17.5 - 38)	)	

Table XXXIII (Cont) Study 2. Serum levels of sulphasalazine and its metabolites following therapeutic dosing. NS = $p > 0.05$.

	Serum levels Median (ug/ml) (range)	Mann-Whitney U Test
Sulphasalazine		
Whole group (n=29)	5.7 (0.5 - 25.4)	-
High dose (n=11)	12 (1.4 - 25.4)	$p < 0.02$
Low dose (n=18)	5 (0.5 - 12.5)	

Table XXXIII Study 2. Serum levels of sulphasalazine and its metabolites following therapeutic dosing. NS = $p > 0.05$.

	Serum levels Median (ug/ml) (range)	Mann-Whitney U Test
Sulphapyridine (SP)		
Whole group (n=29)	22 (4.5 - 47)	
High dose/slow acetylator (n=6)	27.5 (18 - 47)	$p < 0.01$
High dose/fast acetylator (n=5)	18.5 (13 - 27.5)	
Low dose/slow acetylator (n=12)	23 (4.5 - 35)	NS
Low dose/fast acetylator (n=6)	8.7 (6.5 - 20)	

Table XXXIII (Cont) Study 2. Serum levels of sulphasalazine and its metabolites following therapeutic dosing. NS = $p > 0.05$.

	Serum levels Median (ug/ml) (range)		Mann-Whitney U Test
Acetylsulphapyridine (ASP)			
Whole group (n=29)	11.2 (1.2 - 56.12)		
High dose/slow acetylator (n=6)	9.8 (1.2 - 22.0)	)	$p < 0.01$
High dose/fast acetylator (n=5)	30.5 (16.2 - 56.2)	)	
Low dose/slow acetylator (n=12)	10 (1.2 - 17.5)	)	NS
Low dose/fast acetylator (n=6)	13 (9.5 - 22)	)	

Table XXXIII (Cont) Study 2. Serum levels of sulphasalazine and its metabolites following therapeutic dosing. NS = $p > 0.05$.

	Serum levels Median (ug/ml) (range)		Mann-Whitney U Test
Total Sulphapyridine			
Total Whole group (SP + ASP) (n=29)	32.5 (5.5 - 83)		
High dose/slow acetylator (n=6)	35 (27 - 68.5)	)	NS
High dose/fast acetylator (n=5)	49.5 (30.5 - 83)	)	
Low dose/slow acetylator (n=12)	33 (5.5 - 52)	)	NS
Low dose/fast acetylator (n=6)	25.5 (17.5 - 38)	)	

Table XXXIII (Cont) Study 2. Serum levels of sulphasalazine and its metabolites following therapeutic dosing. NS = $p > 0.05$.

phenotype. As might be expected there was a close relationship between dose and serum levels (Table XXXIV, Figs. XVII, XVIII, XIX). In addition slow acetylators on high dose had significantly higher sulphapyridine and lower acetylsulphapyridine levels than fast acetylators on high dose but no difference was seen in the total sulphapyridine levels in fast and slow acetylators (Figs. XX, XXI, XXII). As in the whole group dose expressed as mg/kg body weight correlated with the improvement in ESR and disease activity index. No such correlation, however, was seen between serum levels of sulphasalazine or its metabolites and percentage change in ESR or disease activity index (Table XXXV) ($p > 0.05$).

Section 4

Dicsussion

These data would suggest that patients who are allocated to 3g/day show a trend towards greater improvement than those allocated to 1.5g/day. Although this difference only reached statistical significance for a few parameters, other measurements showed a similar trend; much larger numbers in each group would be required to show a definite difference in all indices. Comparison of fixed dose regimens, however, is a very crude method of assessing the relationship between dose and efficacy especially in rheumatoid arthritis where some patients might have abnormally low body weight due to muscle wasting and others might be overweight due to immobility. When dose is expressed as mg/kg body weight the relationship between dose and efficacy becomes clearer: only two measures of disease activity (ESR and DAI) were used in this

Dose mg/kg with [sulphasalazine]	rs = 0.473 ; p <0.01
Dose mg/kg with [unmetabolised sulphapyridine]	rs = 0.399 ; p <0.05
Dose mg/kg with [acetylsulphapyridine]	rs = 0.469 ; p <0.01
Dose mg/kg with [total sulphapyridine]	rs = 0.552 ; p <0.01

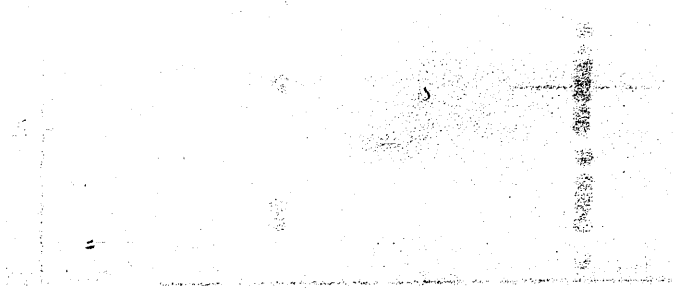


Table XXXIV Study 2. Correlations of dose in mg/kg with serum levels of sulphasalazine and its metabolites (Spearman Rank) (n = 29).

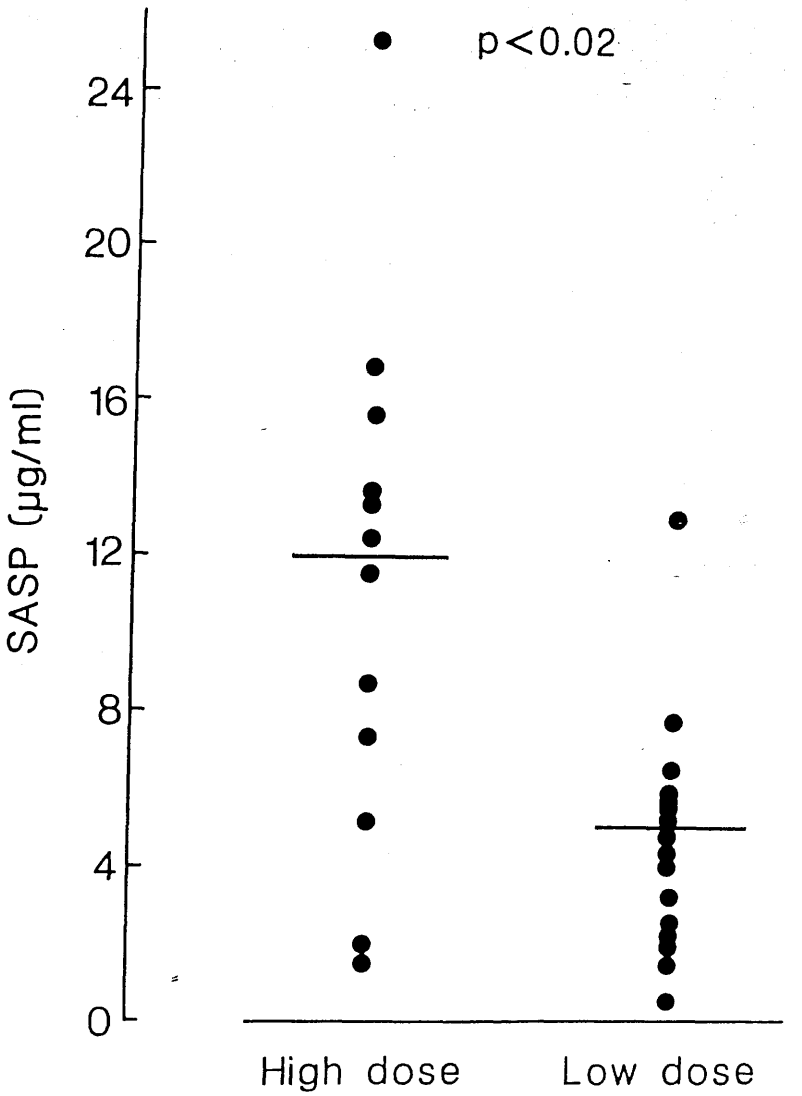


Fig. XVII Serum sulphasalazine (SASP) levels in patients allocated to high dose (3g/day) and low dose (1.5mg/day), $p < 0.02$.

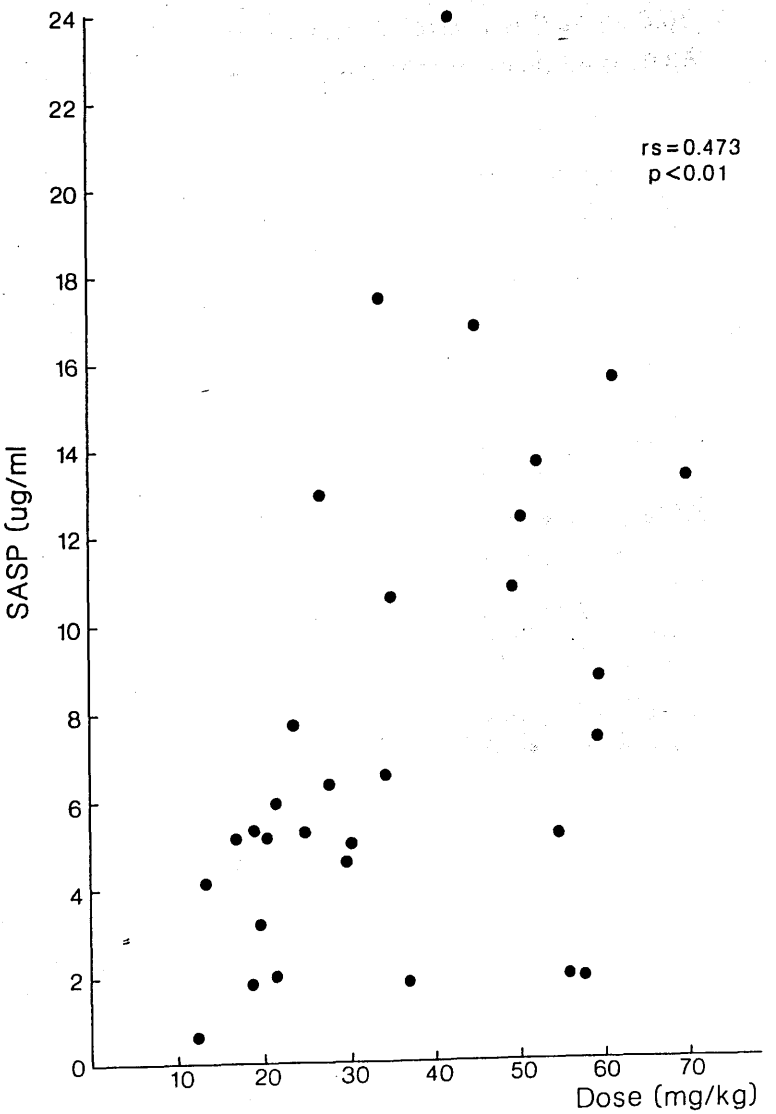


Fig. XVIII Correlation between actual 24 week dose of sulphasalazine expressed as mg/kg body weight and serum sulphasalazine (SASP) levels ($r_s = 0.473$; $p < 0.01$).

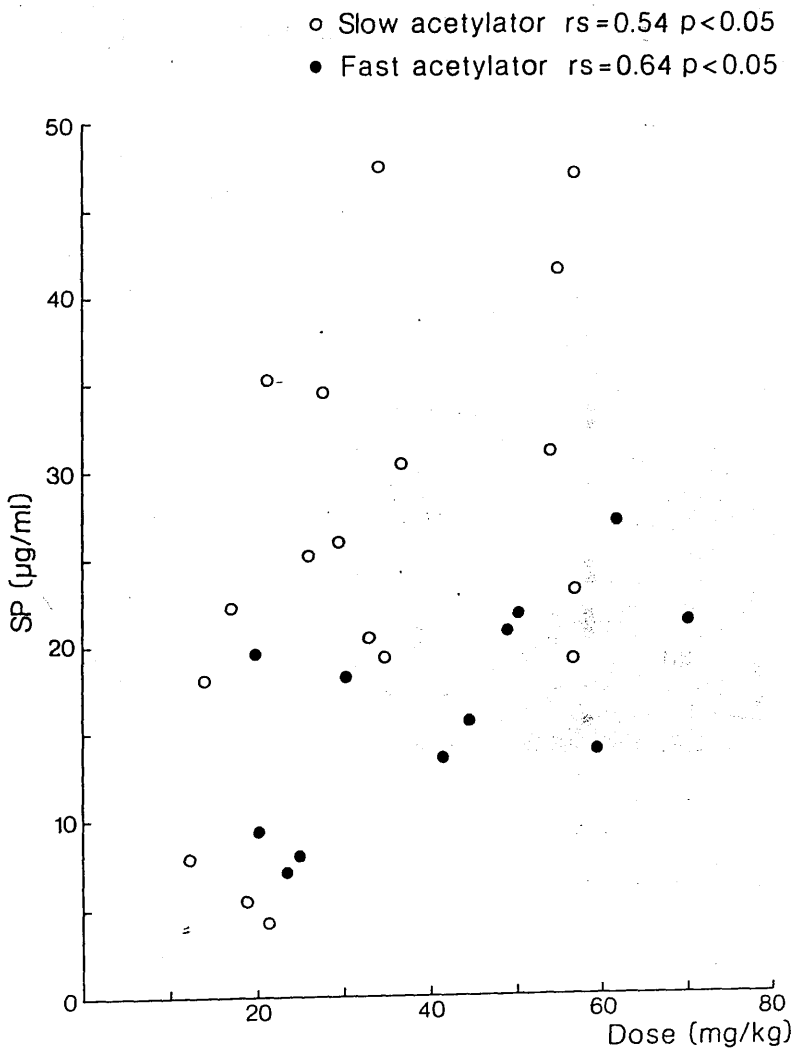


Fig. XIX Correlation between actual 24 week sulphasalazine (SASP) dose expressed as mg/kg body weight and serum levels of unmetabolised sulphapyridine (SP); rs = 0.54; $p < 0.05$ (slow acetylators); rs = 0.64; $p < 0.05$ (fast acetylators).

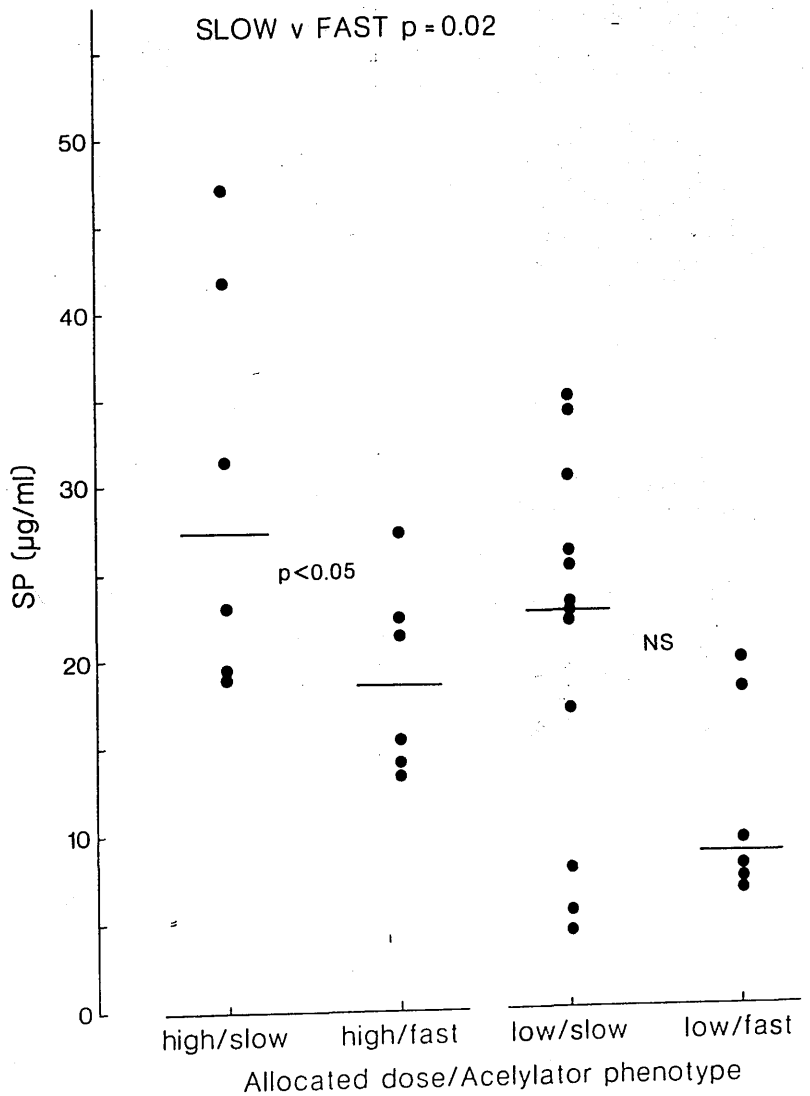


Fig. XX Relationship of serum levels of unmetabolised sulphapyridine (SP) to acetylator phenotype in high and low dose groups.

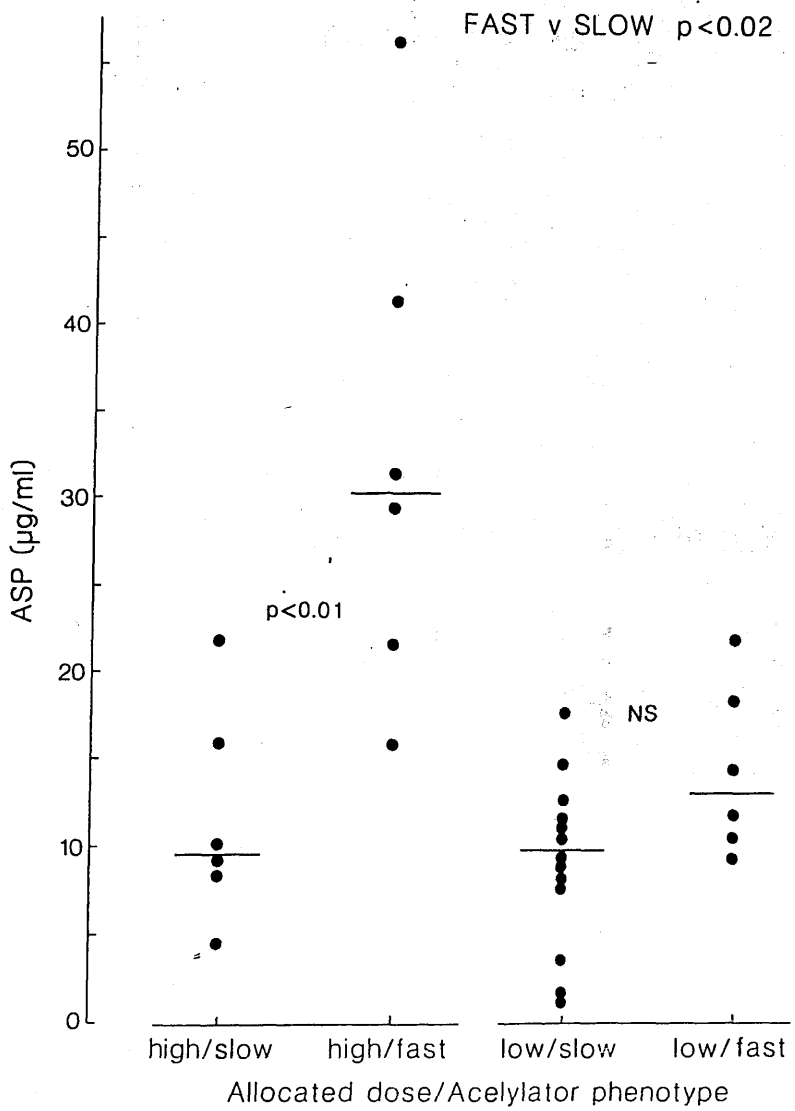


Fig. XXI Relationship of serum levels of acetylated sulphapyridine (ASP) to acetylator phenotype in high and low dose groups.

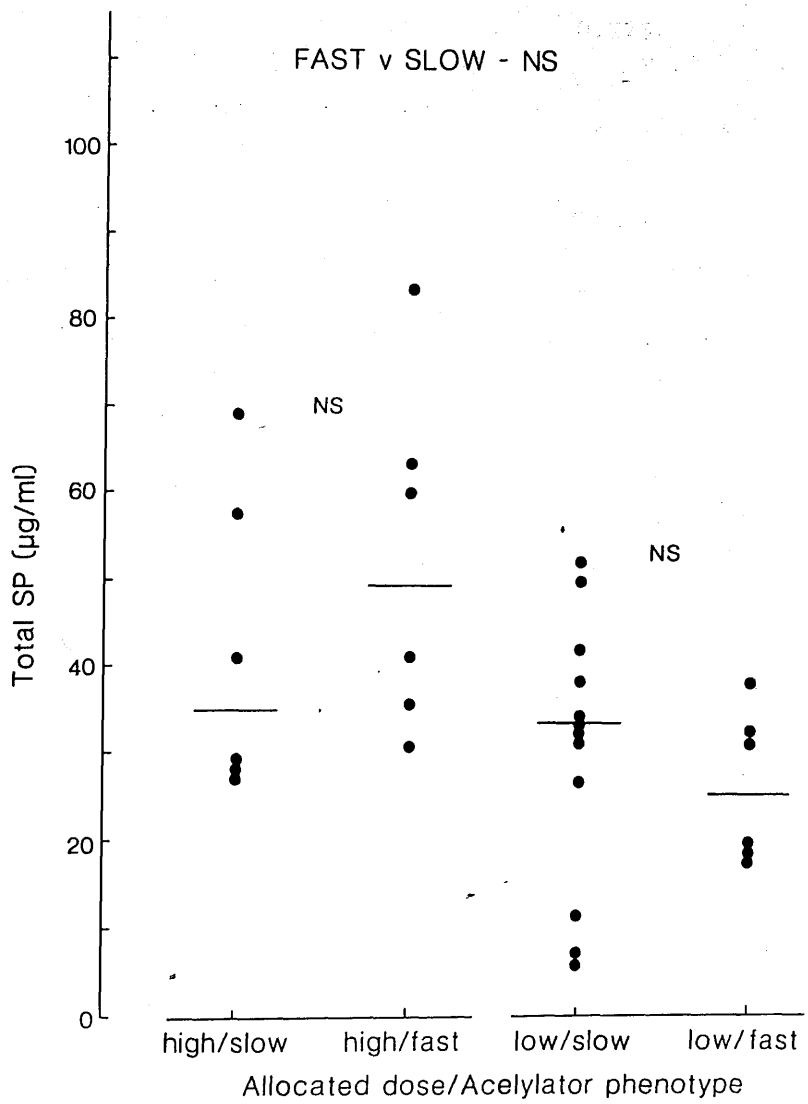


Fig. XXII Relationship of serum levels of total sulphapyridine (total SP) to acetylator phenotype in high and low dose groups.

	rs	p
% change in ESR with SASP levels	0.234	> 0.05
% change in ESR with SP levels	0.275	> 0.05
% change in ESR with ASP levels	0.223	> 0.05
% change in ESR with total SP levels	0.285	> 0.05
% change in DAI with SASP levels	0.261	> 0.05
% change in DAI with SP levels	0.176	> 0.05
% change in DAI with ASP levels	0.116	> 0.05
% change in DAI with total SP levels	0.106	> 0.05

Table XXXV Study 2. Correlation of serum levels of sulphasalazine (SASP) and its metabolites sulphapyridine (SP), acetylsulphapyridine (ASP), total sulphapyridine (total SP) with percent changes in ESR and DAI. (Spearman Rank correlation) (n = 29)

correlation but these have previously been shown to be sensitive markers of overall disease activity (100, 110, 122). Patients who received 40mg/kg or more of sulphasalazine per day had a greater likelihood of clinically significant improvement in ESR and DAI. It is noteworthy that one of the three patients who received more than 40mg/kg/day and who failed to lower the ESR by more than 50% had the lowest sulphasalazine levels measured in this study. This finding suggests poor compliance in that individual.

The demonstration of a relationship between dose and efficacy of sulphasalazine in rheumatoid arthritis is of the utmost importance as otherwise one might be content with a slight improvement in disease activity, whereas, armed with this knowledge one could now use a higher dose which might produce more marked beneficial effect.

In general, the concept of a direct relationship between dose and response to a second line agent is not well recognised in the practice of rheumatology and the tendency with second line agents is to expect an "all or none" response. Thus, both sodium aurothiomalate and d-penicillamine tend to be used in the minimum dose which produces an anti-inflammatory effect. The awareness of the possibility of a dose response phenomenon with second line agents could therefore be of major practical importance and should be more fully investigated with other second line drugs.

There was good correlation between dose and serum levels of sulphasalazine and its metabolites although such a relationship is not necessarily seen in ulcerative colitis (21). The effect of acetylator phenotype on serum levels of sulphapyridine and acetyl sulphapyridine in the 3.0g/day patients was as previously reported (17, 22, 23, 24)

and the results in the 1.5g/day patients, although they did not reach statistical significance, showed a similar trend. Effect of acetylator phenotype on total sulphapyridine levels is more controversial and, although my results agree with Schroder et al (22), in other studies total sulphapyridine has been lower in fast acetylators (17, 23, 24). Despite the good correlation between dose of sulphasalazine and levels of sulphasalazine, sulphapyridine, acetylsulphapyridine and total sulphapyridine it was not possible to show a relationship between circulating levels of these substances and efficacy of sulphasalazine in rheumatoid arthritis. This would suggest that perhaps another metabolite of sulphasalazine which has not been measured (eg, 5-ASA or hydroxysulphapyridine) is the active metabolite or alternatively that the concentration of the active moiety in the compartment in which it produces antirheumatic activity may not be in direct equilibrium with the serum. It has been suggested that rheumatoid arthritis is an enteropathic arthropathy (154) and thus in addition to more obvious sites such as synovial tissue it may be that sulphasalazine or its metabolites exert their antirheumatic properties within the gut lumen, the intestinal mucosa, the portal circulation, or the liver.

This situation is analogous to the situation in ulcerative colitis where circulating levels of sulphasalazine bear no relationship to its beneficial effect (23) and although there is a relationship between total sulphapyridine levels and activity of ulcerative colitis, this is probably due to decreased absorption in the active phase of the disease as there is no relationship between total sulphapyridine levels and relapse rate (24).

A similar situation occurs in rheumatoid arthritis with sodium aurothiomalate where neither serum gold nor serum thiomalate levels relate to the efficacy of the drug (155, 156, 157) and with d-penicillamine where, again, blood levels correlate with neither efficacy nor toxicity (158).

In the present study, patients with nausea and/or vomiting dropped out early, and therefore it is not possible from this study to comment upon the relationship between circulating levels or acetylator phenotype and this side effect. Patients who experienced this symptom, however, and who could continue treatment did not show higher levels of any measured metabolites although obviously this is a group with less severe gastrointestinal symptoms. In study 1, 20% of patients stopped sulphasalazine because of nausea and vomiting, while in this study only 5 (7%) (4 (13%) on 3g.day and 1 (3%) on 1.5g/day) stopped for this reason. Increased experience with the drug or the use of prochlorperazine may have contributed to a decline in the number of patients who stopped therapy because of nausea and vomiting.

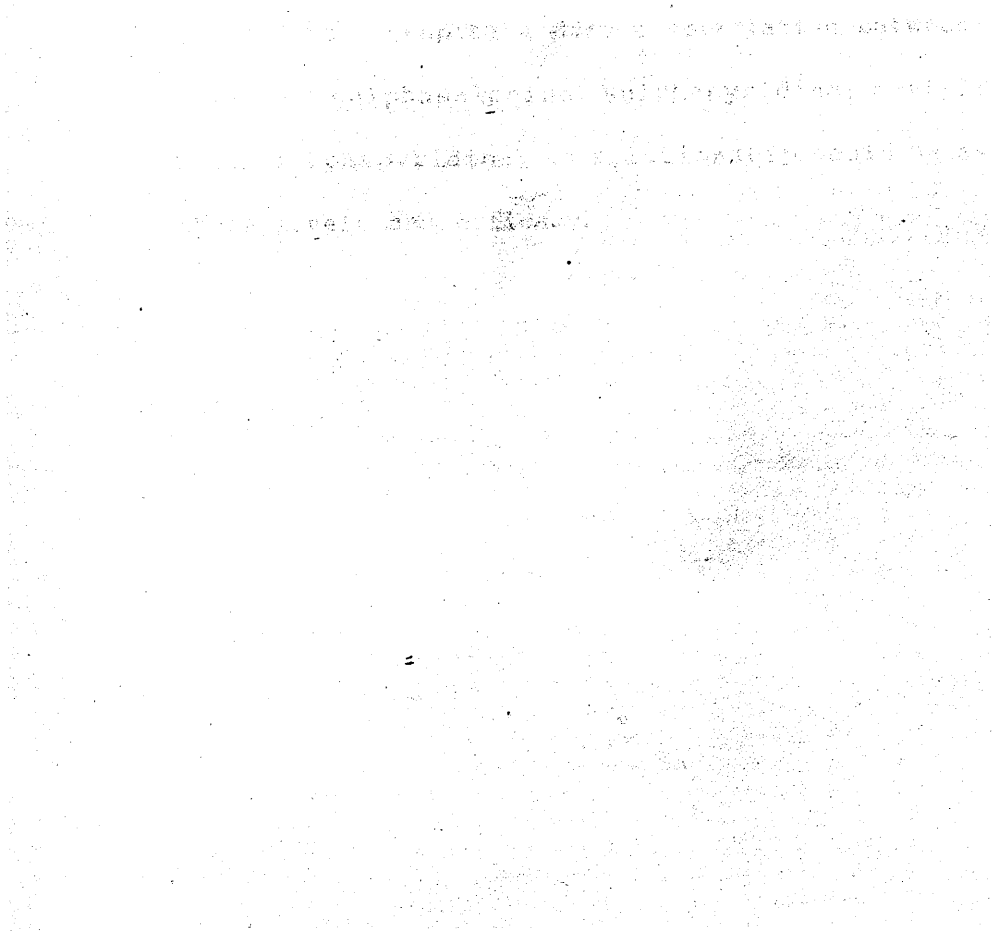
Although numbers are too small for a meaningful statistical comparison there was a trend towards greater toxicity in the high dose group.

Section 5

Conclusions

In conclusion the efficacy of sulphasalazine in rheumatoid arthritis appears to vary directly with dose but not with circulating levels of sulphasalazine or its measured metabolites. However, the response to this drug does not follow an "all or none" pattern. The best way of

using this drug clinically may be to aim at a high dose (>40mg/kg) and to attempt to treat nausea and vomiting symptomatically with prochlorperazine and if this fails to reduce the dose of sulphasalazine to one which is tolerated. In addition the dissociation between serum levels and efficacy suggests a site of action which is not in equilibrium with the serum; alternatively one of the lesser metabolites, such as 5-ASA, may be active.



Chapter 5

Sixty patients with active, definite or classical rheumatoid arthritis were randomly allocated to sulphasalazine 1.5g/day or sulphasalazine 3g/day. Significant improvement was seen in inflammatory parameters in both groups and no significant difference between groups could be demonstrated. When these patients were analysed in terms of dose expressed as mg/kg body weight a direct relationship between dose and reduction in disease activity was apparent. Patients who received in excess of 40mg/kg did significantly better than those who received a lower dose. Despite a direct correlation between the dose and serum levels of sulphasalazine, sulphapyridine, acetylsulphapyridine and total sulphapyridine, no relationship could be established between these levels and efficacy.

CHAPTER 6

Influence of acetylator phenotype on the efficacy and toxicity of sulphasalazine in rheumatoid arthritis

Section 1

Introduction

Once sulphasalazine is split in the large bowel, the major metabolic pathway followed by the absorbed sulphapyridine involves N-acetylation. This is carried out in the colonic mucosa and liver by the enzyme N-acetyl transferase (17). The rate of N-acetylation is genetically determined and follows a bimodal distribution. Individuals can thus be classed as slow (autosomal recessive) or fast (autosomal dominant) acetylators (20). The ratio of slow to fast acetylators varies between different populations but in the United Kingdom approximately 60% are slow acetylators and 40% are fast acetylators (159).

In addition to sulphapyridine a number of other drugs which possess an aromatic ring and an amino group exhibit acetylation polymorphism. These include isoniazid, hydralazine, procainamide, sulphadimidine, dapsone, phenelzine and nitrazepam. With many of these agents acetylator phenotype affects toxicity and/or efficacy of the drug. In general slow acetylators suffer from a higher incidence of side effects and fast acetylators derive less benefit although in the case of isoniazid hepatitis is commoner in fast acetylators as it is the acetylated form which is hepatotoxic (160).

Sulphapyridine metabolites are excreted more rapidly in the urine than is the unmetabolised form (10). A number of studies have shown a shorter elimination half-life and lower steady state levels of unmetabolised sulphapyridine (17, 21, 22, 24) in fast acetylators. Most studies have also shown a lower total sulphapyridine

concentration in the serum of fast acetylators (17, 24) although this relationship is not as clear cut (22) and I have been unable to show it in Study 2. There is evidence in normal individuals and patients with inflammatory bowel disease that toxicity is seen more commonly in slow acetylators (22, 23, 25, 161).

It is thus possible that the rate of acetylation of sulphapyridine may influence the toxicity and/or efficacy of sulphasalazine in rheumatoid arthritis and, if this is the case, it may be possible to identify a subgroup who would benefit from a different approach as regards dosage or monitoring.

Acetylator phenotype is determined from calculating the ratio of acetylated to total drug in urine or serum either after a single dose or in the steady state. A number of protocols have been devised (Table XXXVI) and their results appear to correlate well with each other (19, 162).

In this chapter I have assessed the influence of acetylator phenotype on efficacy in both a retrospective study and, using different doses in fast and slow acetylators, in a prospective study. I have also compared the toxicity rate between fast and slow acetylators in the entire patient group who have been treated with sulphasalazine.

Section 2

Patients and methods

2.1 Retrospective study

Sixty patients were studied retrospectively with respect to acetylator

phenotype. All patients had been allocated to enteric coated sulphasalazine 3g/day and comprised the 30 sulphasalazine treated patients from study 1 and the 30 patients from study 2 who had been allocated to 3g/day. Fifty-four patients were available for determination of acetylator phenotype. Patients who remained on sulphasalazine at the time of assessment of acetylator phenotype had this calculated from the urinary ratio of acetylated:total sulphapyridine (162) (Study 1) or the serum ratio of acetylated:total sulphapyridine (19) (Study 2). Those who had discontinued sulphasalazine but who had not exhibited sulphonamide hypersensitivity were typed from a urine sample collected between the 5th and 6th hour following a single oral dose of sulphadimidine (159, 162) whereas in those patients who exhibited possible hypersensitivity to sulphonamide isoniazid was used (163) and the urinary ratio calculated from a sample taken between the 6th and 8th hour (Table XXXVI). Assessment of drug efficacy was carried out at weeks 0 and 24.

2.2 Prospective study

In this study (Study 3) 60 patients had acetylator phenotype determined using a single dose of sulphadimidine (162) before commencement of therapy.

Slow acetylators (20 patients) were subsequently allocated to 1.5g/day enteric coated sulphasalazine and fast acetylators (40 patients) to 3g/day enteric coated sulphasalazine. Assessment was, again, carried out at weeks 0 and 24. In both studies patients' actual dose corresponded well to the allocated dose (Table XXXVII).

Test drug	No of patients	Dose	Sample	Ratio	Fast acetylator	Slow acetylator	Reference
Sulphasalazine	44	steady state	serum	acetylated sulphapyridine: total sulphapyridine	> 42.5%	< 42.5%	Das & Eastwood 1975 (19)
Sulphasalazine	31	steady state	urine	acetylated sulphapyridine: total sulphapyridine	> 65%	< 65%	Schroder 1972 (162)
Sulphadimidine	70	10mg/kg	urine 5-6hrs	acetylated sulphadimidine: total sulphadimidine	> 70%	< 70%	Evans 1969 (159) Schroder 1972 (162)
Isoniazid	4	10mg/kg	urine 6-8hrs	acetylisoniazid: total hydrazines	> 70%	< 70%	Eidus & Hodgkin 1973 (163)

Table XXXVI Various methods used to ascertain aceylator phenotype (see text).

Group	Allocated dose	0g	0.5g	1.0g	1.5g	2.0g	2.5g	3.0g	3.5g	4.0g
Retrospective study (total)	3g	-	-	1	-	4	-	30	-	-
slow acetylators n = 16	3g	-	-	-	-	3	-	13	-	-
fast acetylators n = 19	3g	-	-	1	-	1	-	17	-	-
Prospective study (total)		1	-	1	12	5	2	16	-	5
slow acetylator/low dose n = 15	1.5g	1*	-	-	9	3	-	2	-	-
fast acetylator/high dose n = 27	3g	-	-	1	3	2	2	14	-	5

Table XXXVII Actual 24 week dose in various patient groups (acetylator studies).

* temporarily off treatment.

2.3 Toxicity study

In total, acetylator phenotype was determined in 149 patients (the 114 described above, the 8 patients from study 4 and 27 patients from study 2 allocated to 1.5g/day in whom acetylator phenotype was available). Of the 9 patients in whom acetylator phenotype was not available, one had died a non drug related death, one had left the area, 4 had refused to cooperate and 3 had equivocal results for acetylator phenotype on two occasions making classification impossible.

Section 3

Results

3.1 Retrospective group

Thirty one (57%) of the 54 patients were fast and 23 (43%) slow acetylators. At week 24, 19 fast and 16 slow acetylators remained on therapy. Table XXXVIII shows patient data for each group at weeks 0 and 24. No significant difference could be demonstrated between the groups at week 0 for any parameter (Mann-Whitney U test $p > 0.05$). Both groups showed a statistically significant improvement in most parameters over the 24 weeks (Table XXXVIII). No significant difference could be seen between the groups either in the percent improvement in any of the parameters over the 24 weeks (Mann-Whitney U test $p > 0.05$), in the absolute week 24 values ($p > 0.05$) or in the number of patients who achieved an improvement of > 50% in ESR or of > 33% in disease activity index ($\chi^2 = 0.93$ and 1.21; $p > 0.05$ in both instances).

	Slow acetylators		Fast acetylators	
Wk	0	24	0	24
n	23	16	31	19
Haemoglobin (g/dl)	11.4 (9.4-14.2)	12.0 (10.8-15.0)	11.2 (8.6-17.0)	11.9** (10.2-17.9)
ESR (mm/hr)	71 (7-131)	25.5*** (6-77)	63.5 (18-140)	20.5*** (7-118)
Plats ($x10^9/l$)	437 (277-781)	341*** (134-448)	416 (239-888)	353*** (206-809)
Rose Waaler titre	1/32 (0-1/1024)	1/16 (0-1/1024)	1/128 (0-1/1024)	1/64 (0-1/1024)
Ritchie articular index	20 (2-54)	7.25**** (0-33)	20 (4-61)	5.25**** (0-30)
5 point pain score	3.0 (2-4)	1.7 (0-4)	2.7 (1-4)	1.7* (1-4)
Mean grip strength (mmHg)	85 (46-134)	119* (9-118)	80 (40-140)	90**** (46-230)

Table XXXVIII Median values (ranges) for various indices at wks 0 and 24 for patients allocated to Sulphasalazine 3g/day (retrospective acetylator study).
*p<0.05; **p<0.01; ***p<0.005; ****p<0.001 - Wilcoxon matched pairs signed rank test wk 0 v 24.

	Slow acetylators		Fast acetylators	
Wk	0	24	0	24
n	23	16	31	19
Duration of morning stiffness (mins)	66 (0-all day)	10 (0-all day)	188 (0-all day)	30* (0-all day)
IgA (g/l)	2.7 (0.4-5.1)	2.25* (1.2-3.9)	3.25 (0.7-7.5)	3.0** (0.3-5.9)
IgG (g/l)	13.4 (7.1-22.2)	12.4* (6.4-20.5)	13.25 (8.5-40.2)	11.15** (6.7-28.0)
IgM (g/l)	1.4 (0.8-3.1)	1.1* (0.7-3.4)	1.2 (0.7-7.0)	0.95* (0.4-2.1)
MCV (fl)	81 (70-95)	90** (76-114)	82.5 (69-94)	88.5** (74-112)
AST (u/l)	15.75 (6-24)	21* (12-38)	15.5 (8-28)	16.75* (7-80)
ALT (u/l)	8.5 (5-24)	16.25* (5-41)	14.5 (5-30)	17.75 (7-69)

Table XXXVIII (Cont) Median values (ranges) for various indices at wks 0 and 24 for patients allocated to Sulphasalazine 3g (retrospective acetylator study).
*p<0.05; **p<0.01; ***p<0.005; ****p<0.001 - Wilcoxon matched pairs signed rank test wk 0 v 24.

3.2 Prospective study

Table XXI, XXIII and XXIV show the values of various inflammatory indices in these patients. Unfortunately the slow acetylator/low dose group had significantly lower ESR and CRP and higher haemoglobin at week 0 than the fast acetylator/high dose group (Mann-Whitney U test $p < 0.01$ for ESR and Hb and $p < 0.05$ for CRP) although other parameters were comparable.

By week 24, however, this difference in ESR and haemoglobin was lost. Tables XXIII and XXIV show the pattern on improvement in the two groups over the 24 week period. The fast acetylator/high dose group showed a significant improvement in most indices but only the articular index in the slow acetylator/low dose group showed significant improvement at 24 weeks. In addition the percent change in ESR was significantly greater in the fast acetylator/high dose group when compared to the slow acetylator/low dose group (Mann-Whitney U test $p < 0.05$) (Fig. XXIII).

3.3 Toxicity study

Acetylator phenotype was available in 149 patients [83 (56%) fast and 66 (44%) slow acetylators]. Table XXXIX shows the reasons for and time of discontinuing therapy over the first 24 weeks. Twenty-one of 66 slow and 23 of 83 fast acetylators stopped treatment over this period ($\chi^2 = 0.33$; $p > 0.05$). In total 18 stopped because of nausea and/or vomiting, 13 slow acetylators and 5 fast acetylators ($\chi^2 = 6.23$; $p < 0.02$) (Fig. XXIV).

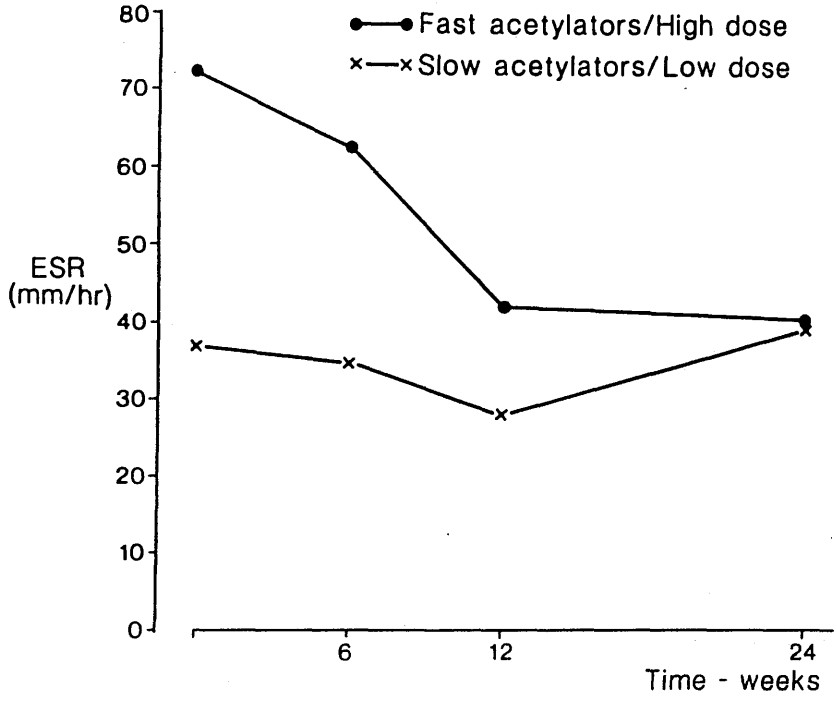


Fig. XXIII Study 3. Median ESR wks 0 and 24 in fast acetylator/ high dose group and slow acetylator/low dose group.

	Slow acetylators		Fast acetylators	
n	66		83	
Reason stopped	No stopped	Week stopped	No stopped	Week stopped
Nausea/vomiting	13 (20%)	3, 3, 3, 4, 4, 4, 6, 6, 6, 8, 12, 12, 18	5* (6%)	3, 6, 6, 12*, 24
Leucopenia	2 (3%)	6, 10	4 (5%)	1, 4, 8, 10
Rash	2 (3%)	1, 18	2 (2%)	9, 11
Mouth ulcers	0		2 (1.5%)	1, 2
Drowsiness	1 (1.5%)	8	0	
Dizziness	0		2* (1.5%)	6, 12*
Hepatitis	0	-	1 (1%)	18
Lack of efficacy	0	-	5 (6%)	18, 18, 24, 24, 24

Table XXXIX Reasons for and week of discontinuing therapy over first 24 weeks treatment - slow and fast acetylators
* same patient.

	Slow acetylators		Fast acetylators	
n	66		83	
Reason stopped	No stopped	Week stopped	No stopped	Week stopped
Poor compliance	2 (3%)	6, 12	0	-
Intercurrent illness	1 (1.5%)	10	0	-
Other	0	-	3 (3.5%)	4, 6, 6
Total	21 (32%)		23 (28%)	

Table XXXIX (Cont) Reasons for and week of discontinuing therapy over first 24 weeks treatment - slow and fast acetylators

SLOW ACETYLATORS n=66

NAUSEA/VOMITING 20%*	MUCOCUTANEOUS 3%	LEUCOPENIA 3%	OTHER 7%	33%

FAST ACETYLATORS n=83

NAUSEA/VOMITING 6%*	MUCOCUTANEOUS 2.5%	LEUCOPENIA 5%	OTHER 7.5%	INEFFICACY 6%	27%

*χ^2 $p < 0.02$

Fig. XXIV Reasons for discontinuing therapy in the fast and slow acetylator groups.

No difference was seen in the percent rise in serum hepatic transaminase concentration or mean cell volume between the fast and slow acetylators (Mann-Whitney U test $p > 0.05$).

Section 4

Discussion

In this study the proportions of slow and fast acetylators (44% and 56% respectively) are a reversal of those previously described in a British population (159). A previous study of acetylator phenotype in rheumatoid patients in the West of Scotland showed 72% of the rheumatoid patients and 64% of the control group to be slow acetylators (164). It is therefore unlikely that the finding of a reversed acetylator phenotype ratio in the present study is related to geography or the presence of rheumatoid arthritis per se. It is possible that there may be some relationship with severity of rheumatoid arthritis as patients in a second line drug study group will tend to have more severe rheumatoid disease. More likely, however, it is merely a chance finding.

The questions asked in this chapter are:

1. does acetylator phenotypeinfluence the toxicity of sulphasalazine in rheumatoid arthritis?
2. does acetylator phenotypeinfluence the efficacy of sulphasalazine in rheumatoid arthritis?

The answer to the first question appears to be that acetylator phenotype does affect the pattern of toxicity of this drug in rheumatoid arthritis with slow acetylators displaying a greater

incidence of upper gastrointestinal side effects. This is despite the fact that, in the total group of 149 patients, because of the design of study 3, there was a preponderance of slow acetylators receiving a lower dose which might be expected to reduce the incidence of toxicity among the slow acetylators. Although this difference is real it does not necessarily imply that the dose of sulphasalazine should be limited in slow acetylators as 13 out of 23 (57%) slow acetylators allocated to 3g/day, (over 80% of those continuing treatment) achieved this dose. The finding of a higher incidence of upper gastrointestinal side effects in slow acetylators is in keeping with previous studies in non-rheumatoid patients (25, 161). It is important to document this finding in rheumatoid arthritis, however, as in many instances rheumatoid patients are thought to react differently to drugs and, in fact, contrary to what one might have expected from the non rheumatoid literature (165), rheumatoid patients who are slow acetylators react no differently than fast acetylators to dapsone (166).

The present study fails to demonstrate any strong relationship between acetylator phenotype and potentially serious toxic effects such as agranulocytosis.

The answer regarding the relationship of efficacy to acetylator phenotype is less clear cut. The retrospective analysis shows no clear statistically significant difference between fast and slow acetylators and, in fact, not even a trend is apparent. This may represent the fact that there is no difference or it may be that a dose of 3g/day is too high to allow any subtle difference to be demonstrated. It is possible that a difference would show up at a

lower dose.

In the prospective study the 2 dose regimens were chosen empirically on the hypothesis that slow acetylators should require a lower dose than fast acetylators to produce similar tissue levels of unmetabolised sulphapyridine. The initial difference in objective inflammatory indices between the two groups obscures the issue somewhat. The fact that this is lost by 24 weeks, however, suggests that the fast acetylator/high dose patients have achieved greater improvement even though this change from a significant difference between groups at week 0 to no significant difference at week 24 over the study period may not be as meaningful as a change from no significant difference to a significant difference. In addition the percent change in ESR was significantly greater in the fast acetylator/high dose group. Finally most parameters improved significantly at 24 weeks in the fast acetylator/high dose group although only articular index showed significant improvement in the slow acetylator/low dose group. All of these findings taken together would strongly suggest that fast acetylators who receive 3g/day show a greater improvement than slow acetylators who receive 1.5g/day despite the fact that the elimination half-life of sulphapyridine would be expected to be longer in slow acetylators. This finding is balanced, in part at least, however, by the finding that all 5 patients who stopped sulphasalazine in the first 24 weeks because of inefficacy were fast acetylators.

Another possible confounding factor is that although there is a genetic basis for classifying individuals as fast or slow acetylators there is a wide spectrum of rate of acetylation within each group and

it may thus be better to regard acetylator phenotype as a continuous variable. Such an approach would be impossible here, however, as a number of different methods were used to assess acetylator phenotype.

Overall these findings would suggest that acetylator phenotype does not play a major part in determining the efficacy of sulphasalazine in rheumatoid arthritis although the fact that patients who stopped therapy because of inefficacy were all fast acetylators makes this conclusion less certain than it might otherwise have been. It would appear, however, that if any difference does exist between slow and fast acetylators it is small compared to the influence of dose.

Section 5

Conclusions

The data described in this chapter suggest that acetylator phenotype is important in determining the incidence of nausea and/or vomiting in rheumatoid patients receiving sulphasalazine and that slow acetylators display a higher incidence of such side effects. No difference is demonstrated between fast and slow acetylators in terms of efficacy and, in fact, fast acetylators who receive a high dose appear to do better than slow acetylators who receive a low dose. If any difference in efficacy does exist between slow and fast acetylators this is of minor importance when compared to the effect of dose.

In practical terms, therefore, despite the higher incidence of upper gastrointestinal symptoms (a relatively mild side effect) in slow acetylators, the absence of a major influence of acetylator phenotype on therapeutic efficacy means that even in slow acetylators we should

aim at the previously recommended dose of 40mg/kg. In the average 70kg person this is (to the nearest tablet) 3g/day, a dose which over 80% of slow acetylators who remain on therapy can achieve.

Summary

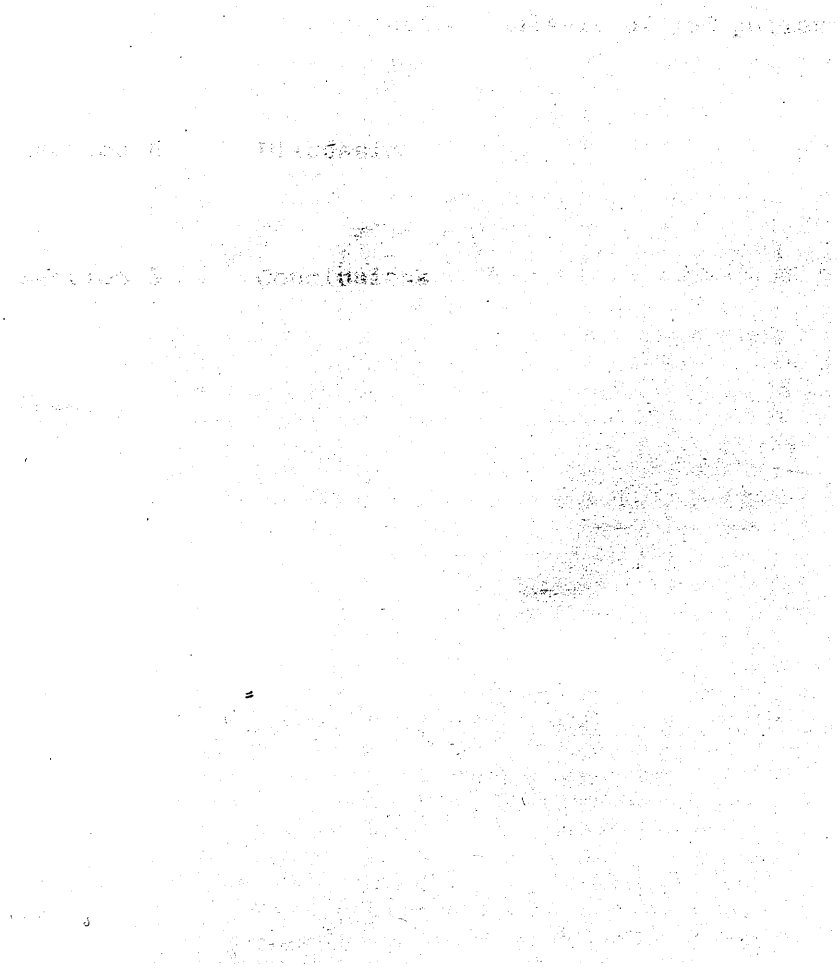
Chapter 6

After absorption the sulphapyridine component of sulphasalazine undergoes hepatic metabolism. The major metabolite of sulphapyridine is N-acetyl sulphapyridine and the rate of acetylation is genetically determined. From experience of other drugs which exhibit genetic polymorphism and from experience of sulphasalazine in ulcerative colitis it is likely that acetylator phenotype will influence toxicity and perhaps efficacy of sulphasalazine in rheumatoid arthritis.

Of the 60 patients in studies 1 and 2 who were randomly allocated to sulphasalazine 3.0g/day acetylator phenotype was available in 54. Of these 16 slow and 19 fast acetylators completed 24 week treatment. Both slow and fast acetylators showed similar improvement in inflammatory parameters. A further group of 60 patients had acetylator phenotype assessed before treatment; the 20 slow acetylators were allocated to sulphasalazine 1.5g/day while the 40 fast acetylators were allocated to 3.0g/day sulphasalazine. Unfortunately at the outset the slow acetylators had milder disease. At 24 weeks, however, improvement in inflammatory indices were confined to the fast acetylator/3.0g/day group and their disease had reached the level of activity of the slow acetylator/1.5g/day group.

Of the total group of 158 patients acetylator phenotype was available in 149 and of these 66 were slow acetylators and 83 fast acetylators. These patients were followed for 24 weeks. Thirteen (20%) of the slow acetylators but only 5 (6%) of the fast acetylators stopped treatment because of nausea and/or vomiting (χ^2 $p < 0.02$). Overall 21 (32%)

slow and 23 (28%) fast acetylators discontinued therapy (χ^2 $p > 0.05$). Acetylator phenotype was, however, unable to predict efficacy or serious toxicity. It is therefore not necessary routinely to assess acetylator phenotype before commencing sulphasalazine for the treatment of rheumatoid arthritis.



CHAPTER 7

Sulphasalazine in elderly rheumatoid patients

Section 1

Introduction

The onset of rheumatoid arthritis commonly occurs in middle age but nevertheless may occur in later life. In addition, because of its chronic nature, many people continue to suffer active disease for many years. Thus, many elderly patients show evidence of active rheumatoid arthritis and require the addition of second line drugs. From previous experience it has become apparent that approximately 10% of patients receiving second line drugs at the Centre for Rheumatic Diseases are over 65 years old.

The process of ageing is accompanied by changes in many aspects of physiological function. This, in turn, may have profound effects on the pharmacodynamic and pharmacokinetic profiles of drugs. There is little evidence for significant age related changes in drug absorption and, although changes in plasma protein binding, volume of distribution and hepatic metabolism have been described no generalisation can be made about these functions in the elderly and their clinical significance is unknown. The most profound effect of age appears to be on the renal handling of drugs which are excreted in the urine (167).

For many years it has been recognised that digoxin and the aminoglycosides pose greater dangers in the elderly and that lower doses are required. Such differences relate to reduced renal function in elderly patients. More recently this aspect of treatment with antirheumatic drugs aroused interest when it was found that benoxaprofen (a drug which may have exhibited other than purely first

line properties) (168) displayed a slower rate of clearance and a longer plasma elimination half-life in elderly patients (169) and was subsequently found to produce serious toxicity in the elderly (170, 171, 172).

Sulphapyridine, the major metabolite of sulphasalazine is excreted almost entirely by the kidneys either unchanged or following hepatic metabolism. It is possible, therefore, that the handling of sulphasalazine in the elderly may differ from that found in younger patients and that this may produce differences in the toxicity profile of the drug.

In this chapter I have therefore investigated the pharmacokinetics of sulphasalazine in an elderly rheumatoid population and related this to toxicity. I have also reviewed the pattern of toxicity and efficacy found in older patients as compared to that found in a younger age group.

Section 2

Patients and methods

Eight patients with definite or classical (146) rheumatoid arthritis were studied. All patients were at least 65 years old and required the addition of a second line agent to their treatment regimen to control their disease activity. Patients were given a single dose of enteric coated sulphasalazine (5 patients were given 2g and 3 patients were given 3g - this difference was because of a change in protocol to allow comparison with some previously documented young volunteers) washed down with 150ml water following a 10 hour overnight fast and

followed by a further 2 hour fast. Venous blood was sampled before dosing and at 1.5, 3, 4.5, 6, 9, 12, 18, 24, 48 and 72 hours. In addition 3 x 24 hour urine collections were made over this period. The dose and timing of sampling were chosen to make this study compatible with previous studies carried out by Pharmacia AB. Seven days later the protocol was repeated and after the last sample was collected patients were commenced on a therapeutic dose of the drug (0.5g/day rising by weekly increments of 0.5g/day to 3g/day or until the dose was limited by dose related toxicity).

Blood samples were stored at room temperature for 30 minutes and following centrifugation at 3000 rpm the supernatant was stored at -20°C. Urine volumes were measured and a 20ml aliquot stored at -20°C. Analysis for sulphasalazine, sulphapyridine, acetylsulphapyridine, sulphapyridine glucuronate and acetylsulphapyridine glucuronate was carried out by Pharmacia AB using high performance liquid chromatography with UV detection (173). "Total serum sulphapyridine" was calculated as the sum of the sulphapyridine equivalents of the above substances expressed in ug/ml. Urinary excretion of metabolites was also expressed as molar equivalents of sulphasalazine to facilitate comparison of ingested and excreted quantities.

Plasma concentration-time curves were plotted for each patient using the mean values for the same time point measured one week apart. From these curves peak serum concentrations (C max) and area under the curve from time 0 to infinity (AUC) could be calculated. The AUC was calculated using the trapezoid rule. Serum elimination half life ($t^1/_2$) was calculated from the terminal portion of a semi-log plot of

concentration against time. Acetylator phenotype was assessed from the serum ratio of acetylated:total sulphapyridine (19). Because of the absence of an intravenous dose and the incomplete absorption of sulphapyridine no realistic estimation of clearance could be made. In the second part of the results section the efficacy and toxicity of sulphasalazine in the total 158 patients described in Chapter 4 are related to age.

Section 3

3.1 Pharmacokinetic study

Pre-treatment data on the 8 patients are shown in Table XL. Derived pharmacokinetic indices are shown in Table XLI. Figs XXV, XXVI and XXVII show the plasma concentration-time curves in patient No 6 for the various metabolites on the 2 occasions of measurement separated by 1 week. Similar close relationships between the two single dose studies were seen for all patients except patients No 2 and 5 whose 2nd studies showed lower values compared to the first.

Four patients (Nos 1, 2, 3 and 4) stopped their therapeutic dosing regimens because of upper gastrointestinal symptoms. One of these patients (patient No 3) who stopped had received 3g doses in the single dose studies and achieved a much higher peak concentration (C max) and greater AUC for all metabolites than the other patients. This patient's results are, therefore, excluded from further analysis as they may have produced bias. Two patients (patients No 7 and 8) who continued therapy also received 3g doses in the single dose study. Despite this, however, they still produced low C max's and AUC's and,

Patient No	Age (yrs)	Dose Administered (g)	Acetylator Phenotype	Tolerated Therapeutic Dosing	Reason Stopped	Week Stopped	Creatinine Clearance (ml/min/ 1.74m^2 body surface)	Concomitant Drugs	Smoker
1	69	2	Slow	No	Nausea	3	42	Indomethacin Quinine Sulphate Nitrazepam Paracetamol	No
2	75	2	Fast	No	Nausea/ Vomiting	3	43	Flucloxacillin Nifedipine Isosobide Prednisolone Naproxen Frusemide Gaviscon	No
3	73	3	Slow	No	Nausea/ Vomiting	6	74	Indomethacin Naproxen Temazepam	No
4	79	2	Slow	No	Nausea/ Vomiting	7	37	Piroxicam Gastrocote Aludrox Paracetamol Temazepam	No

Table XL Starting data for patients in pharmacokinetic study (Study 4).

Patient No	Age (yrs)	Dose Administered (g)	Acetylator Phenotype	Tolerated Therapeutic Dosing	Reason Stopped	Week Stopped	Creatinine Clearance (ml/min/ $1.74m^2$ body surface)	Concomitant Drugs	Smoker
5	69	2	Fast	Yes	-	-	103	Nifedipine Propranolol Solpadeine Navidrex K Diazepam	No
6	80	2	Fast	Yes	-	-	125	Fenclofenac Fesovit Piroxicam	No
7	78	3	Fast	Yes	-	-	41	Nadolol Bumetanide Prednisolone Ketoprofen	No
8	65	3	Slow	Yes	-	-	99	Temazepam Indomethacin Distalgesic	No

Table XL (Cont) Starting data for patients in pharmacokinetic study (Study 4).

Patient No	Sulphasalazine			Unmetabolised Sulphapyridine			Total Sulphapyridine			% administered dose excreted in urine (sulphasalazine equivalents)
	Cmax (ug/ml)	$t^1/_2$ (hrs)	AUC (ughrs/ml)	Cmax (ug/ml)	$t^1/_2$ (hrs)	AUC (ughrs/ml)	Cmax (ug/ml)	$t^1/_2$ (hrs)	AUC (ughrs/ml)	
*1	18	7.5	169	25.5	18	879	36	12	1324	66
*2	13	7	161	12	15	328	32	9	1363	80
*3	23	7.5	186	19	17	997	32	-	-	74
*4	16.5	6	220	14	16	556	34	19	1445	64
5	12	5.5	134	5.5	12	155	18	12	386	83
6	7	2	52	13	9.5	234	27	11	696	92
7	8.5	5	92	13	9.5	256	58	9	1798	95
8	2.7	4	31	12.5	21	448	22	18	935	46

Table XLI Pharmacokinetic parameters for the 8 patients in Study 4.

* stopped treatment because of upper gastrointestinal side effects.

Patient No	Sulphasalazine			Unmetabolised Sulphapyridine			Total Sulphapyridine			% administered dose excreted in urine (sulphasalazine equivalents)
	Cmax (ug/ml)	$t^1/_2$ (hrs)	AUC (ughrs/ml)	Cmax (ug/ml)	$t^1/_2$ (hrs)	AUC (ughrs/ml)	Cmax (ug/ml)	$t^1/_2$ (hrs)	AUC (ughrs/ml)	
Mean for patients who discontinued because of side-effects (excluding patient 3 for dose dependent parameters)	15.8	7	183	17.2	16.5	588	34	13.3	1377	71
Mean for patients who continued therapy	7.6	4.1	77	11	13	273	31	12.5	954	79

Table XLI (Cont) Pharmacokinetic parameters for the 8 patients in Study 4.

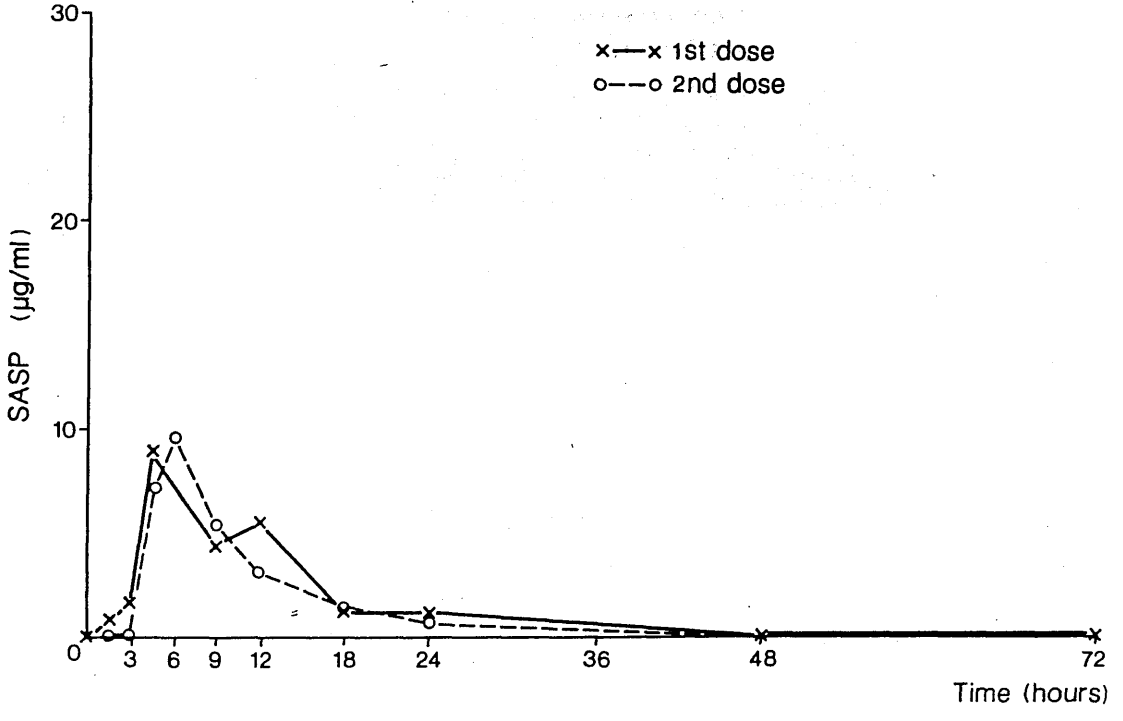


Fig. XXV Sulphasalazine concentration-time curves on two separate occasions for patient No 6 (Study 4).

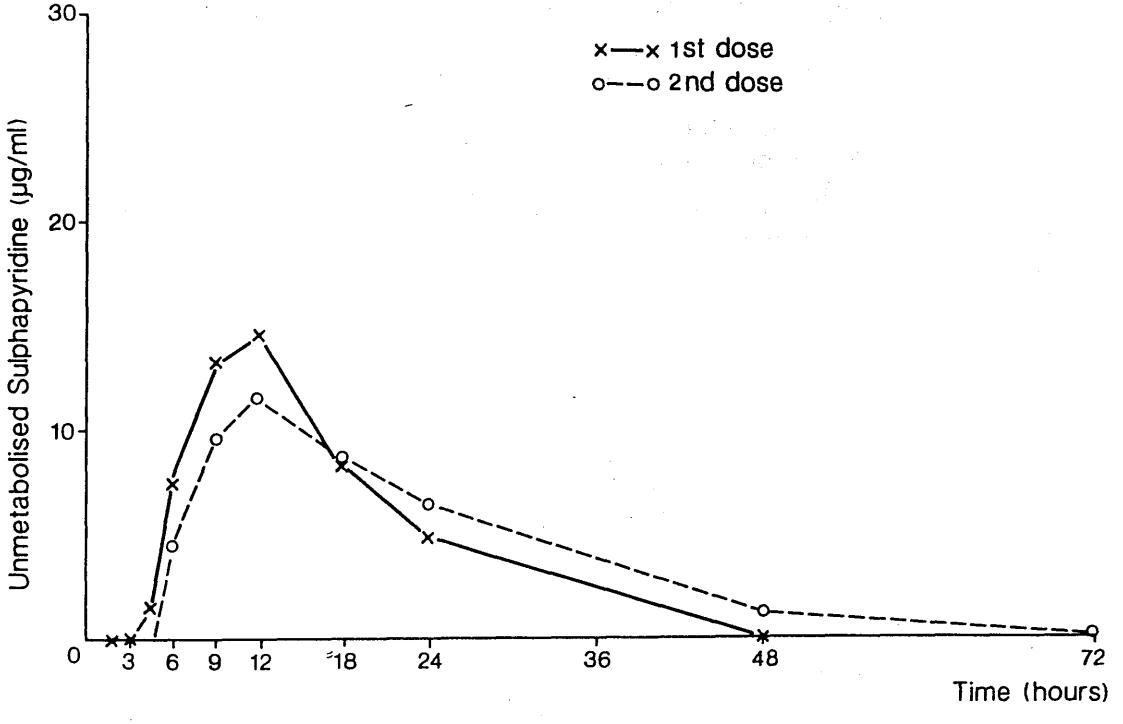


Fig. XXVI Unmetabolised sulphapyridine concentration-time curves on two separate occasions for patient No 6 (Study 4).

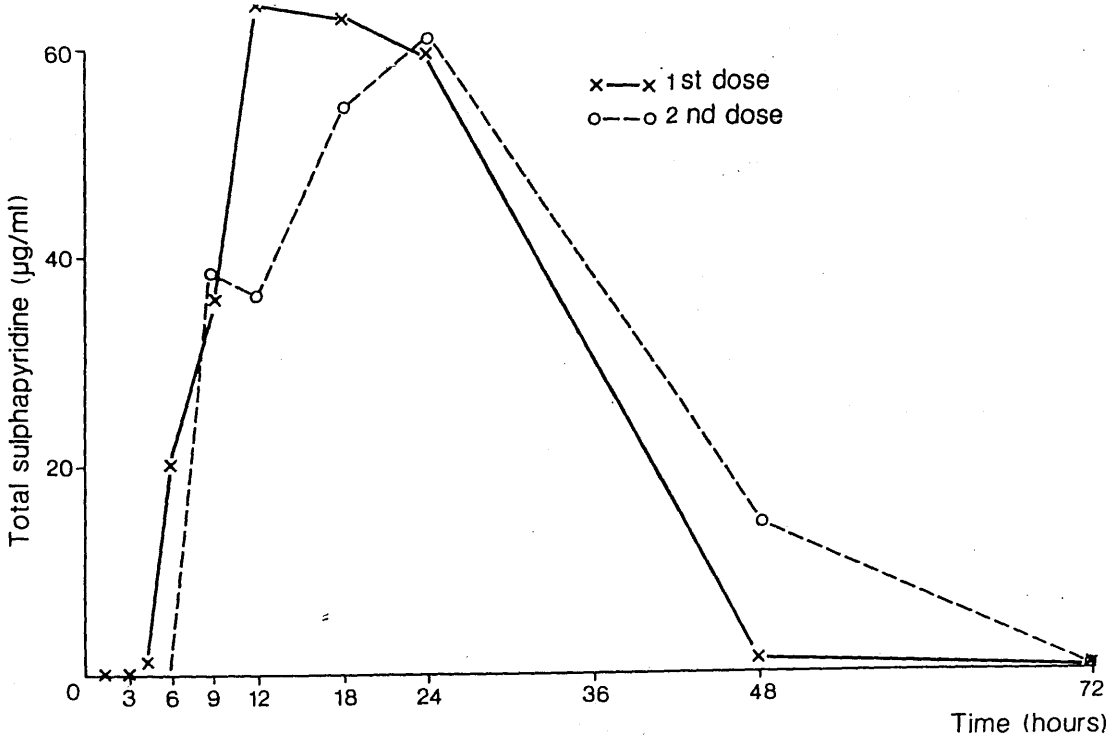


Fig. XXVII Total sulphapyridine concentration-time curves on two separate occasions for patient No 6 (Study 4).

assuming that these values would be no higher with a 2g dose, these patients are included as any bias produced by the higher doses would be towards no demonstrable difference between patients who continued and those who did not.

Figs XXVIII, XXIX and XXX show the mean concentration-time curves for the two single dose studies for the group who were able to tolerate extended treatment and those who could not. Despite the fact that two patients in the group who eventually continued treatment received higher doses in the single dose studies it can be seen that these patients who stopped because of nausea/vomiting achieved a greater C max and to a lesser extent a longer $t^1/_2$ and AUC for sulphasalazine and unmetabolised sulphapyridine (Table XLI).

3.2 Retrospective analysis of 158 patients

In the group of 158 patients 31 were $\geq$65 years old. There was no significant difference between the groups for any of the starting parameters (except, of course, age) in the 150 patients for whom full clinical details were available (Table XLII). The patients analysed comprised a mixture of doses and acetylator phenotypes but there was no major difference in the distribution of these variables (Table XLIII). The pattern of drop out from treatment for the two groups is shown in Table XLIV. Significantly more people in the elderly group stopped treatment in the first 6 months (χ^2 = 4.01; $p < 0.05$) for all reasons and also because of side effects (χ^2 = 4.42; $p < 0.05$) (Fig XXXI). Table XLV shows the pattern of change in the various parameters of efficacy over the 24 week follow up. No difference could be demonstrated between the two groups with reference to the percent

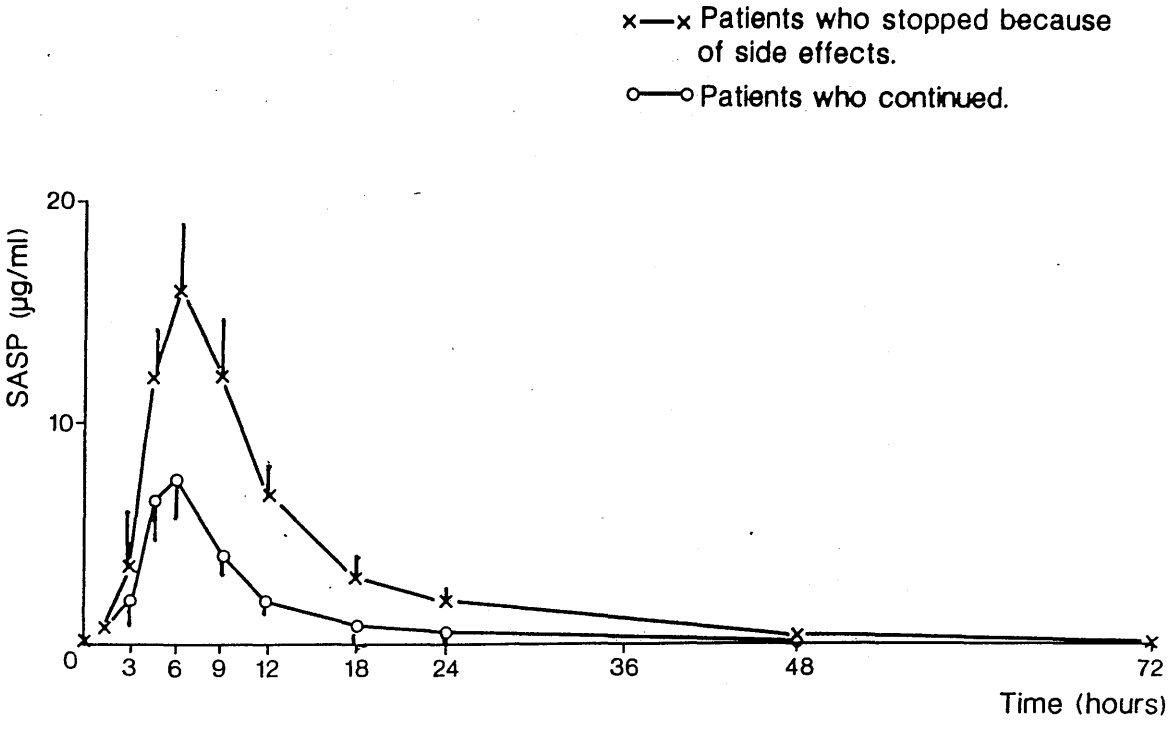


Fig. XXVIII Mean (±S.E.M.) sulphasalazine concentration-time curves for those patients who continued treatment and those who stopped because of side effects.

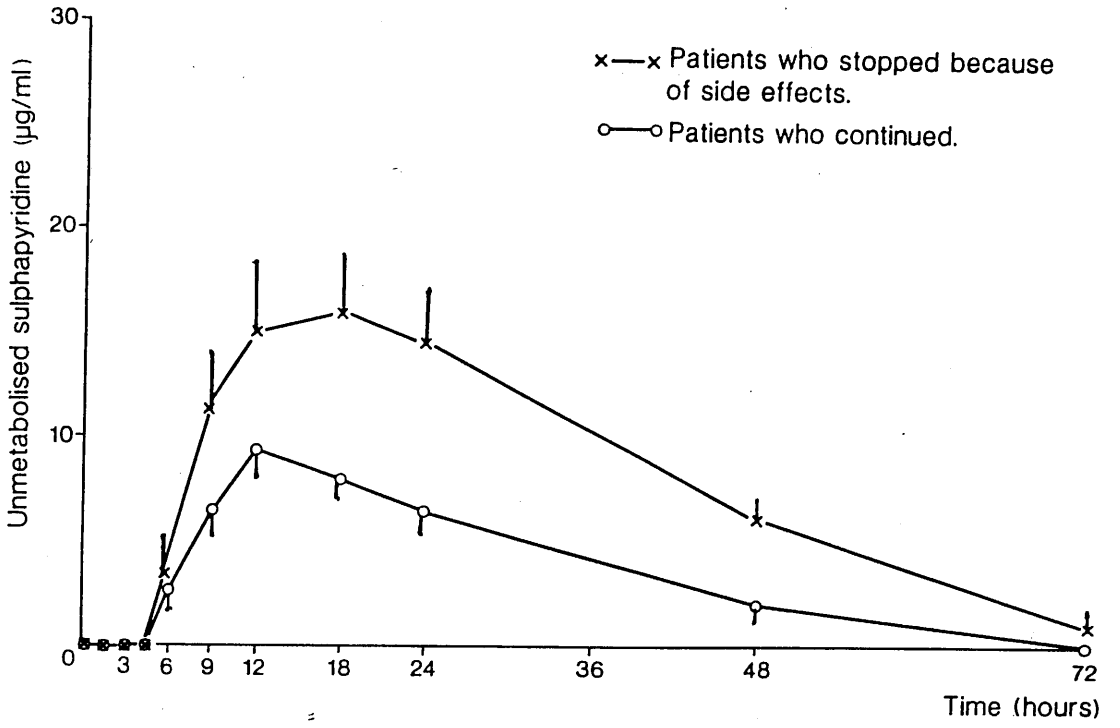


Fig. XXIX Mean (±S.E.M.) unmetabolised sulphapyridine concentration-time curves for those patients who continued treatment and those who stopped because of side effects.

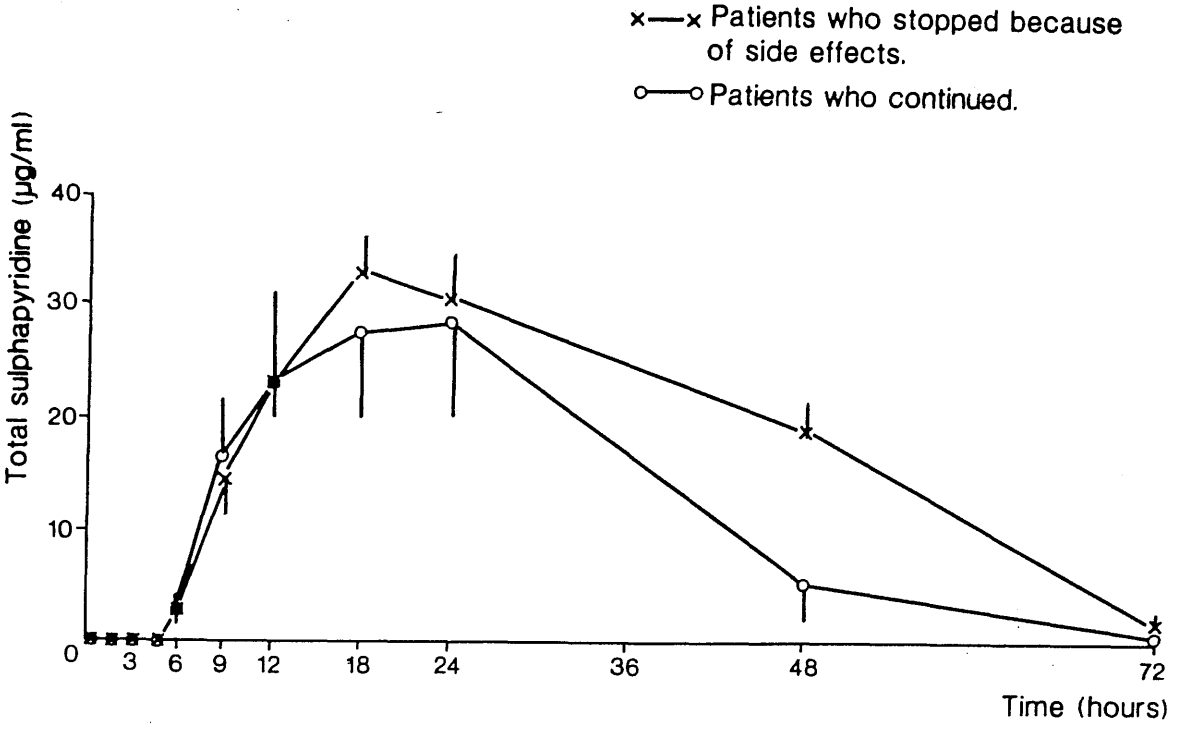


Fig. XXX Mean (±S.E.M.) total sulphapyridine concentration-time curves for those patients who continued treatment and those who stopped because of side effects.

	< 65	≥ 65
n	127	23
Age (yrs)	52.4 (28-64)	69 (65-80)
Disease duration (yrs)	7.5 (1-47)	9.2 (1-57)
ESR (mm/hr)	63.5 (2-150)	72 (17-132)
Hb (g/dl)	11.6 (7.6-16.6)	11.3 (7.4-17.0)
Plats (x 10^9/l)	395 (118-880)	382 (203-780)
RF titre	1/128 (0-1/1024)	1/64 (0-1/512)
AI	19.2 (0-61)	20 (2-32)
LUT (mins)	90 (0-all day)	126 (45-all day)
Pain score	2.65 (1-49)	2.75 (2-4)
Grip strength (mmHg)	89.5 (38-190)	76 (39-177)
DAI	16.7 (12-23)	17.3 (12-22)
Creatinine (umol/l)	69 (40-130)	73 (40-202)

Table XLII Pre-treatment values in the group of 150 patients (Studies 1, 2 and 3) divided by age (median and range).

	Age <65	Age ≥65	Total
n	127 (%)	31 (%)	158 (%)
Fast acetylator/ 3g/day	59 (46)	16 (52)	75 (49)
Slow acetylator/ 3g/day	20 (16)	7 (23)	27 (17)
Fast acetylator/ 1.5g/day	8 (6)	0 (0)	8 (5)
Slow acetylator/ 1.5g/day	35 (28)	4 (13)	39 (25)
? Acetylator status			
3g/day	2 (2)	4 (13)	6 (4)
1.5g/day	3 (2)	0 (0)	3 (2)

Table XLIII Distribution of acetylator phenotype and allocated dose by age.

	< 65 n = 127 (%)	≥ 65 n = 31 (%)
Nausea/vomiting	14 (9)	7 (22.5)
Rash/itch	4 (3.2)	1 (3)
Leucopenia	5 (4)	2 (6)
Mouth ulcers	1 (0.8)	1 (3)
Dizziness	0	1 (3)
Drowsiness	1 (0.8)	0
Hepatitis	1 (0.8)	0
Acute dyspnoea	1 (9.8)	0
Poor compliance	-	1 (3)
Inefficacy	5 (4)	0
Others	4 (3)	1 (3)
Total	36 (28)	14 (45)

Table XLIV Reasons for discontinuing sulphasalazine therapy during first 6 months of treatment (by age).

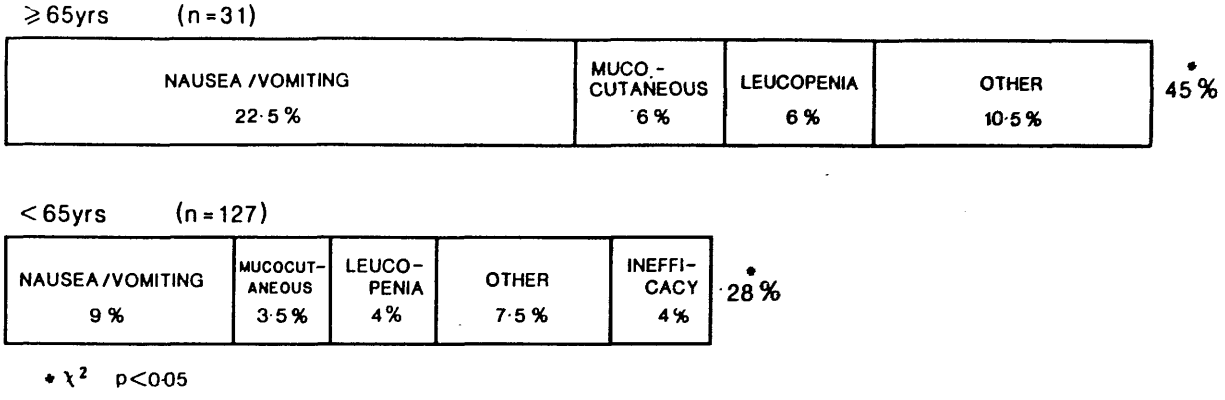


Fig. XXXI Reasons for discontinuing therapy in the elderly and the younger age groups.

	Age < 65		Age ≥ 65		Mann-Whitney % change p values
Week	0	24	0	24	
n	127	91	23	13	
Dose		2.75 (1.0-4.0)		2.8 (1.5-3.0)	
ESR (mm/hr)	63.5 (2-150)	34.5 (2-129)****	72 (17-132)	40 (40-115)	NS
Hb (g/dl)	11.6 (7.6-16.6)	11.8 (8.6-17.1)****	11.3 (7.4-17)	12.5 (7.1-17.7)	NS
Plats ($x10^9$/l)	395 (118-888)	320 (134-809)****	382 (203-780)	295 (211-475)	NS
Rose Waaler titre	1/128 (0-1/1024)	1/128 (0-1/1024)	1.64 (0-1/512)	1/16 (0-1/128)	NS
AI	19.2 (0-61)	7 (0-33)****	20 (2-32)	11.7 (0-21)****	NS
LUT (mins)	90 (0-all day)	30 (0-all day)****	126 (45-all day)	62 (0-all day)*	NS
Pain score	2.65 (1-4)	1.7 (0-4)****	2.75 (2-4)	2.4 (1-4)	NS
Grip strength (mmHg)	89.5 (38-190)	93 (9-245)****	76 (39-177)	83 (36-235)*	NS
DAI	16.7 (12-23)	13.5 (7-21)****	17.3 (12-22)	15.2 (7-21)*	NS

Table XLV Change in inflammatory parameters (median & range) in patients <65 yrs old and in patients ≥65 yrs old and comparison of percent change (Mann-Whitney U test). NS = not significant; $p > 0.05$.
Wilcoxon Wk 0 v Wk 24 - *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; ****$p < 0.001$

change over the study period or to the actual 24 week values (Mann-Whitney U Test $p > 0.05$). Serum creatinine concentration was not significantly greater in the 31 elderly patients when compared to the 127 younger patients, nor was there any difference in the serum creatinine in the 21 of the 158 patients who stopped because of gastrointestinal side effects or in the 51 who stopped treatment for any cause nor between those elderly patients who stopped treatment and those who continued (Mann-Whitney U test, all $p > 0.05$).

Section 4

Discussion

The pharmacokinetic study described here has a number of serious limitations. These include the discrepancy in dosage, the absence of an intravenous dose to determine bioavailability, volume of distribution and clearance and the fact that, of necessity, an elderly rheumatoid group exhibit an astounding degree of polypharmacy. In addition the fact that slow acetylators happened, coincidentally, to have a lower creatinine clearance complicates any attempt to separate these variables. Finally the small numbers make a statistical approach to comparison impossible.

Within the bounds of these limitations the pharmacokinetic study does suggest, in elderly patients, a relationship between upper gastrointestinal side effects and the ability to achieve high values for C max and AUC for sulphasalazine and unmetabolised sulphapyridine and, to a lesser extent, total sulphapyridine. From the evidence available in previous studies in non-rheumatoid patients such a

relationship between sulphapyridine levels and toxicity was not entirely unexpected (25, 161), although a relationship has never previously been demonstrated between sulphasalazine levels and side effects.

Although it is only a crude index of renal function the lack of relationship between serum creatinine concentration and toxicity in the 158 patients suggests that perhaps increased toxicity in the elderly is related to some factor other than renal function (perhaps absorption, metabolism or distribution). In this context it is of interest to note that in a group of 12 young normal volunteers (M. Ryde personal communication) only 61% of the ingested dose was recoverable from the urine whereas in the 8 elderly patients studied here 75% of the ingested dose was recoverable in the urine suggesting, perhaps, increased absorption, reduced biliary excretion or altered distribution. The peak serum levels, serum half lives and areas under the curve for sulphasalazine, sulphapyridine and its metabolites were not significantly different between the elderly patients and the young healthy volunteers.

When one examines the pattern of improvement in inflammatory indices (Table XLV) it is apparent that despite the failure to show any statistically significant difference between the 2 age groups more parameters improve in the <65 age group and in particular none of the haematological indices improve in the $\geq$65 age group despite both groups receiving a similar dose. This may be due to small numbers in this group but this is unlikely to be the entire explanation as with similar numbers in study 1 (Chapter 4) significant improvement was seen in these parameters.

Section 5

Conclusions

In summary, elderly rheumatoid patients show a higher incidence of toxicity necessitating withdrawal of sulphasalazine while they display a tendency towards lesser improvement. Elderly patients who discontinue treatment because of upper gastrointestinal side effects achieve higher C max and AUC for sulphasalazine and its metabolites on single dose studies. Elderly patients, however, also appear to excrete a larger proportion of the ingested dose in the urine which, among other possibilities, may suggest more complete absorption. This, in conjunction with the fact that, overall, serum creatinine concentration does not correlate with toxicity suggests that a mechanism other than poor renal function may be responsible for the increased toxicity rate in the elderly. In practical terms one may expect greater toxicity and less efficacy in elderly rheumatoids. Toxicity, however, tends not be of a serious nature and therefore, if one decides to use this drug in an elderly patient, one should probably still attempt to reach the optimum therapeutic dose of > 40mg/kg.

Summary

Chapter 7

In general drug toxicity tends to be more common in elderly patients. This is frequently due to pharmacokinetic differences resulting in higher drug levels and accumulation of drug in the body. I have investigated the single dose pharmacokinetics of sulphasalazine in 8 elderly patients prior to chronic dosing. The 4 patients who eventually had to discontinue therapy because of nausea and/or vomiting achieved, on single dosing, higher peak levels and greater areas under the curve especially for sulphasalazine but also for unmetabolised sulphapyridine and total sulphapyridine when compared to those who were able to continue treatment. In addition elderly patients tended to excrete in their urine (as sulphasalazine, sulphapyridine and metabolites) a higher percentage of the ingested dose than did a previously documented group of young normal volunteers.

The total group of 158 patients studied contained 31 patients who were 65 years old or over. These patients had a greater incidence of discontinuing therapy for all reasons 45% v 28% (Chi squared, $p < 0.05$) and for adverse effects 39% v 20% (Chi squared, $p < 0.05$) although the incidence of no single adverse event showed a significant difference between the groups. No relationship could be demonstrated between serum creatinine concentration, either in the elderly alone or in all patients, and adverse effects.

In addition, fewer inflammatory parameters improved significantly in the elderly patients. Thus, drop out rates from sulphasalazine are

greater in elderly patients but no single adverse effect can be demonstrated as more common in this age group.

CHAPTER 8

Investigations of the influence of a number of clinical and laboratory variables on the efficacy and toxicity of sulphasalazine in the treatment of rheumatoid arthritis.

Section 1 Introduction

Section 2 Patients and methods

Section 3 The investigation of individual subgroups

3.1 The influence of gender on efficacy and toxicity of sulphasalazine in rheumatoid arthritis

3.2 The relationship of efficacy and toxicity of sulphasalazine to duration of rheumatoid disease

3.3 The influence of previous second line therapy on efficacy and toxicity of sulphasalazine

3.4 The influence of initial ESR on efficacy and toxicity of sulphasalazine

3.5 The influence of initial disease activity index (DAI) on efficacy and toxicity of sulphasalazine

Section 4 The use of prochlorperazine to treat sulphasalazine related upper gastrointestinal problems.

Section 5 Discussion

Section 6 Conclusions

Summary

Section 1

Introduction

The previous two chapters have examined the effect of a number of variables which, either from experience of sulphasalazine in other conditions or from knowledge of the effect of these variables on other drugs, seemed likely to affect the efficacy or toxicity of sulphasalazine in the treatment of rheumatoid arthritis. There are also, however, a number of other variables which might affect one or both of these aspects of sulphasalazine therapy in rheumatoid patients and may help to identify a subgroup in whom this drug displays special properties.

It has been stated that, in general, drug side effects occur more frequently in females (174) and it has also been observed that sulphasalazine is more effective in male rather than in female rheumatoid patients (175). It is theoretically likely that patients with longer disease duration or who have previously used a large number of second line agents may show a poor response to treatment with sulphasalazine and also that those who have had a large number of previous agents may be more susceptible to complications of therapy.

Initial disease activity may also affect the response to second line drugs in that people with milder initial disease may show less overall improvement. Finally, in studies 2, 3 and 4 prochlorperazine was prescribed freely for upper gastrointestinal symptoms and this may have influenced the overall toxicity pattern.

This chapter, therefore, examines the influence of gender, disease duration, previous second line drugs, initial ESR and initial disease activity on the efficacy and toxicity of sulphasalazine and also examines the impact of allowing patients access to the anti-emetic preparation prochlorperazine.

Section 2

Patients and methods

Unless otherwise stated, data on drop-out rates are from all patients in studies 1, 2, 3 and 4 (n = 158), whereas, because of inadequate data in study 4, efficacy was assessed only in patients from studies 1, 2 and 3 (n = 150). All follow-up periods refer to 24 weeks. Methods of assessing disease activity are described in Chapter 4.

Statistical analysis is carried out using the Chi squared test and the relevant non-parametric tests (148), all tests are two tailed.

Section 3

The investigation of individual subgroups

3.1 The influence of gender on efficacy and toxicity of sulphasalazine in rheumatoid arthritis

Thirty-six (23%) of the 158 patients treated were males. At entry to the study males had, as expected, a significantly higher haemoglobin level, a significantly higher hand grip strength (Mann-Whitney U test $p < 0.001$ in both instances), and also a significantly lower platelet

count (Mann-Whitney U test $p < 0.001$).

Rather surprisingly males had a significantly lower articular index (Mann-Whitney U test $p < 0.001$). Other inflammatory indices and demographic parameters showed no significant difference (Mann-Whitney U test $p > 0.05$) and there was no significant difference between the groups as regards distribution of acetylator phenotypes ($\chi^2 = 0.41$; $p > 0.05$) or allocated dose ($\chi^2 = 0.75$; $p > 0.05$). Over the 24 week follow up 9 (25%) males and 41 (36%) females discontinued treatment ($\chi^2 = 0.92$; $p > 0.05$). When only drop outs because of toxicity are considered again there is again no significant difference between groups either for total toxicity or nausea/vomiting alone ($\chi^2 = 0.86$; $p > 0.05$). A similar pattern of improvement in inflammatory parameters was seen in the two groups and no difference could be demonstrated between groups in the percent change in the various values for inflammatory indices (Mann-Whitney U test $p > 0.05$) (Table XLVI) although significant differences were still apparent between groups by week 24 for haemoglobin, platelet count and grip strength (Mann-Whitney U test $p < 0.001$, $p < 0.05$, $p < 0.001$ respectively) but not articular index.

3.2 The relationship of efficacy and toxicity of sulphasalazine to duration of rheumatoid disease

Forty-seven (30%) of the 158 patients had suffered rheumatoid arthritis for less than 5 years. At the commencement of the study patients with a disease duration of < 5 years had a significantly lower articular index and disease activity index (Mann-Whitney U test $p < 0.001$ and $p < 0.05$ respectively) and a significantly higher

	Males		Females		Mann-Whitney % change p value
Wk	0	24	0	24	
n	36	27	114	77	
ESR (mm/hr)	63 (2-115)	24 (2-90)****	67 (7-150)	37 (5-129)****	NS
Hb (g/dl)	12.4 (8.6-17.0)	13.4 (97-17.7)	11.3 (7.4-16.1)	11.7 (7.1-15)**	NS
Plats ($x10^9/l$)	338 (118-740)	288 (189-603)****	421 (126-888)	345 (134-809)****	NS
Rose Waaler Titre	1/64 (0-1/1024)	1/128 (0-1/1024)	1/128 (0-1/1024)	1/64 (0-1/1024)	NS
Articular index	12.5 (0-57)	3.5 (0-20)****	22.5 (0-61)	8 (0-33)****	NS
5 point pain score	2.6 (1-4)	1.7 (0-4)***	2.7 (1-4)	1.8 (0-4)****	NS
Grip strength (mmHg)	117.5 (51-190)	130.5 (77-245)***	75 (38-155)	85 (9-182)****	NS
LUT (mins)	95 (0-all day)	30 (0-all day)*	91 (0-all day)	30 (0-all day)****	NS
DAI	15.7 (12-21)	11.7 (7-21)****	17.5 (12-23)	14 (9-21)****	NS

Table XLVI Pattern of change in inflammatory parameters (median and range) over 24 weeks in males and females and comparison of the percent change between weeks 0 and 24 in the two groups (Mann-Whitney U test). Wilcoxon wk 0 v wk 24 - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001. NS = not significant; $p < 0.05$

rheumatoid factor titre (Mann-Whitney U test $p < 0.05$). There was no significant difference between groups with respect to other markers of disease activity, age, acetylator phenotype or allocated dose (Mann-Whitney U test $p > 0.05$; $\chi^2 = 1.23$ and 0.62; $p > 0.05$). Both groups showed a similar improvement in inflammatory indices and no differences were demonstrated in the percent change in these indices between the groups (Table XLVII).

Neither total drop out rate nor drop out rate because of side effects (either total or nausea/vomiting) differed between groups ($\chi^2 = 0.53$ and 0.71; $p > 0.05$).

3.3 The influence of previous second line therapy on efficacy and toxicity of sulphasalazine

Of the 158 patient studied, 67 had received at least one second line agent or cytotoxic drug previously (30 - 1 previous agent, 11 - 2 previous agents, 17 - 3 previous agents, 5 - 4 previous agents and 4 - 5 previous agents). Table XLVIII shows how many people had received each individual drug.

For the purposes of analysis 3 groups of patients were compared:- those who had received no previous second line drug (91 patients), those who had received 1 previous second line drug (30 patients) and patients who had previously received 2 or more second line drugs (37 patients). No significant difference could be found between groups in the distribution of acetylator phenotype or allocated dose ($\chi^2 = 0.42$ and 0.66; $p > 0.05$) but patients who had received 2 or more previous drugs had a significantly higher initial articular index (Kruskall-

	< 5 years duration		≥ 5 years duration		Mann-Whitney % change p value
Wk	0	24	0	24	
n	46	30	104	74	
ESR (mm/hr)	60 (18-131)	38 (7-97)****	63 (2-150)	32 (2-129)****	NS
Hb (g/dl)	11.6 (9-15.3)	11.9 (9.3-15.6)	11.4 (8.6-17.0)	11.8 (8.6-17.7)*	NS
Plats ($x10^9/l$)	465 (230-798)	359 (89-502)****	377 (118-888)	307 (134-809)****	NS
Rose Waaler Titre	1/256 (0-1/1024)	1/128 (0-1/1024)	1/64 (0-1/1024)	1/128 (0-1/1024)	NS
Articular index	11.5 (0-39)	4.5 (0-29)****	22.6 (2-61)	8.8 (0-33)****	NS
5 point pain score	2.4 (1-4)	1.4 (0-4)***	2.8 (1-4)	1.9 (0-4)****	NS
Grip strength (mmHg)	90 (38-145)	115 (45-200)****	80 (40-190)	90 (9-245)****	NS
LUT (mins)	77 (0-all day)	29 (0-all day)****	118 (0-all day)	59 (0-all day)****	NS
DAI	16.2 (13-22)	12.5 (9-19)****	17.3 (12-23)	14 (7-21)****	NS

Table XLVII Pattern of changes in inflammatory parameters (median and range) over 24 weeks in patients with < 5 yrs disease duration and in patients with ≥ 5 yrs disease duration and comparison of the percent change between week 0 and 24 in the two groups (Mann-Whitney U test). NS = not significant; $p > 0.05$ Wilcoxon wk 0 v wk 24 - *$p<0.05$; **$p<0.01$; ***$p<0.005$; ****$p<0.001$.

Previous 2nd/3rd line agent	No
Sodium aurothiomalate	48
D-penicillamine	38
Levamisole	17
Chloroquine (as hydroxy C or C-phosphate)	17
Azathioprine	10
Auranofin	6
"Clozic"*	3
Ketotifen**	4

Table XLVIII Number of patients (n = 158) receiving individual second line agents prior to the present studies.

* an ICI potential second line drug which because of toxicity was never marketed.

** A lipoxygenase inhibitor which was tested for second line activity but which failed to display any.

	No previous 2nd line drugs		1 previous 2nd line drug		≥ 2 previous 2nd line drug		Kruskal-Wallis % change p value
Wk	0	24	0	24	0	24	
n	84	55	29	20	37	29	
ESR (mm/hr)	63 (7-150)	35 (5-118)****	68 (12-132)	40 (6-129)**	63 (2-140)	33 (2-84)****	NS
Hb (g/dl)	11.4 (7.8-17.0)	12.1 (8.6-17.7)***	11.6 (7.4-14.7)	11.8 (7.1-13.8)	11.4 (8.9-15.6)	11.7 (8.6-15.2)	NS
Plats ($x10^9/l$)	419 (203-888)	312 (134-809)****	349 (226-781)	300 (196-465)*	385 (118-798)	334 (143-605)***	NS
Rose Waaler Titre	1/256 (0-1/1024)	1/128 (0-1/1024)	1/128 (0-1/1024)	1/128 (0-1/1024)	128 (0-1/1024)	1/32 (0-1/1024)	NS
Articular index	16 (0-54)	3.4 (0-33)****	20.5 (3-57)	7.5 (0-30)****	24 (9-61)	9.8 (0-30)****	NS
5 point pain score	2.6 (1-4)	1.7 (0-4)****	2.5 (1-4)	2 (0-4)	2.9 (1-4)	1.9 (0-4)****	NS
Grip strength (mmHg)	87 (38-150)	95 (9-240)****	77.5 (49-160)	91 (55-230)**	70 (43-167)	82 (46-245)****	NS
LUT (mins)	75 (0-all day)	30 (0-all day)****	135 (0-all day)	32 (0-all day)**	116 (0-all day)	59 (0-all day)****	NS
DAI	16 (12-29)	13 (7-21)****	17 (14-22)	14 (8-21)***	18 (12-23)	15.2 (7-21)****	NS

Table XLIX Pattern of change in inflammatory indices (median and range) in patients who had received no previous 2nd line drugs, in patients who had received 1 previous 2nd line drug and in patients who had received 2 or more 2nd line drugs and a comparison between the percent change in the 3 groups (Kruskal-Wallis). NS = not significant; $p > 0.05$
Wilcoxon wk 0 v wk 24 - *$p<0.05$; **$p<0.01$; ***$p<0.005$; ****$p<0.001$.

Wallis $p < 0.005$) and lower hand grip strength (Kruskall-Wallis $p < 0.05$). There was no significant difference between groups with respect to age, disease duration or other inflammatory indices (Kruskall-Wallis $p > 0.05$). Significant improvement in clinical and laboratory parameters were seen in all 3 groups and no significant difference could be demonstrated in the percent change in inflammatory parameters over 24 weeks (Table XLIX). At week 24 patients who had received 2 or more previous second line drugs retained a higher articular index (Kruskall-Wallis $p < 0.05$) but the groups displayed no demonstrable difference in any of the other parameters.

In total 33 (36%) patients who had received no previous second line agent stopped treatment over the first 24 weeks (15 [16%] because of upper gastrointestinal symptoms) compared to 9 (30%) (5 [17%] because of upper gastrointestinal symptoms) in the 1 previous drug group and 8 (22%) (1 [3%] because of upper gastrointestinal symptoms) who had previously received 2 or more second line agents (χ^2 = 2.08 and 4.3; $p > 0.05$).

3.4 The influence of initial ESR on efficacy and toxicity of sulphasalazine

At the commencement of treatment 134 (84%) patients had an ESR > 30mm/hour.

No significant difference could be demonstrated in the distribution of acetylator phenotype or allocated dose between those patients and the 24 patients with an initial ESR $\leq$30mm/hour (χ^2 = 1.32 and 0.91; $p > 0.05$). As one might expect, however, the patients in the low ESR

group also had a significantly higher haemoglobin level (Mann-Whitney U test $p < 0.01$), grip strength (Mann-Whitney U test $p < 0.05$) and functional index (Mann-Whitney U test $p < 0.05$) and a significantly lower platelet count (Mann-Whitney U test $p < 0.005$) and disease activity index (Mann-Whitney U test $p < 0.001$).

Statistically significant improvement was seen in the clinical parameters of inflammation but not the haematological parameters in the low ESR group but in both clinical and haematological parameters in those patients with a high initial ESR. The improvement in haematological parameters expressed as a percentage of the initial value was significantly greater in patients starting with an ESR > 30mm/hour (Table L). At week 24 patients who started with an ESR $\leq$ 30mm/hour still had a significantly lower ESR (Mann-Whitney U test $p < 0.001$) and articular index (Mann-Whitney U test $p < 0.05$) and a significantly higher haemoglobin (Mann-Whitney U test $p < 0.005$) and grip strength (Mann-Whitney U test $p < 0.05$). Seven (29%) of the low ESR patients stopped treatment over the first 24 weeks (5 (21%) because of nausea/vomiting) compared to 43 (32%) of those with an initial ESR of > 30mm/hour (16 (13%) because of nausea/vomiting). No difference could be demonstrated in the pattern of drop out ($\chi^2 = 0.09$ and 1.35; $p > 0.05$).

3.5 The influence of initial disease activity index (DAI) on efficacy and toxicity of sulphasalazine

A calculation of initial disease activity index was available in 142 patients (patients in study 4 did not have enough data available for this calculation and 8 patients from studies 1, 2 and 3 had data

	ESR ≤ 30 mm/hr		ESR > 30 mm/hr		Mann-Whitney % change p value
Wk	0	24	0	24	
n	23	17	127	87	
ESR (mm/hr)	19 (2-30))	13 (2-38)	71 (31-150)	40 (5-129)****	< 0.01
Hb (g/dl)	13.7 (9.6-17)	12.9 (11.1-17.7)	11.3 (7.4-16.6)	11.8 (7.1-17.1)****	< 0.001
Plats ($x10^9/l$)	329 (118-626)	305 (143-448)	419 (203-888)	341 (134-809)****	< 0.01
Rose Waaler Titre	1/256 (0-1/1024)	1/128 (0-1/1024)*	1/128 (0-1/1024)	1/128 (0-1/1024)	NS
Articular index	17.2 (0-57)	2.8 (0-18)****	19.5 (0-61)	8.2 (0-33)****	NS
5 point pain score	2.7 (2-4)	1.5 (0-4)*	2.7 (1-4)	1.8 (0-4)****	NS
Grip strength (mmHg)	90 (55-190)	130 (9-245)**	80 (38-190)	87 (36-240)****	NS
LUT (mins)	124 (0-all day)	32 (0-240)****	90 (0-all day)	30 (0-all day)****	NS
DAI	14.8 (12-19)	11.8 (7-20)****	17.3 (12-23)	14 (8-21)****	NS

Table L Pattern of change in inflammatory parameters (median and range) over 24 weeks in patients with ESR ≤ 30mm/hr and in patients with ESR > 30mm/hr and comparison of the percent change between weeks 0 and 24 in the two groups (Mann-Whitney U test). NS = not significant; $p > 0.05$
Wilcoxon wk 0 v wk 24 - *$p<0.05$; **$p<0.01$; ***$p<0.005$; ****$p<0.001$.

missing).

Two groups of patients were compared, those with a DAI of ≤ 14 and those with a DAI of > 14. The former corresponds to Mallya & Mace's (122) "inactive" and "slightly active" disease groups and the latter to the "moderately" and "very active" groups. Of those patients with DAI $\leq$ 14 all fell into the "slightly active" group and none into the "inactive groups". Twenty four (17%) patients had an initial DAI $\leq$ 14 (range 12-14). At the outset of therapy these patients had a significantly higher haemoglobin and grip strength (Mann-Whitney U test $p < 0.001$ and $p < 0.05$ respectively) and a significantly lower ESR, platelet count, articular index, pain score and duration of morning stiffness (Mann-Whitney U test $p < 0.005$, $p < 0.001$, $p < 0.001$, $p < 0.05$, $p < 0.001$ respectively). Over the 24 week period a significant improvement was seen in most indices in those patients with a DAI > 14 but only clinical parameters improved in those patients with a DAI $\leq$ 14. Little difference was seen between groups, however, in the percent change in inflammatory indices over the follow up period (Table LI). At week 24 disease activity as indicated by haemoglobin, grip strength, articular index, duration of morning stiffness and DAI was less in the patients with initially only slightly active disease (Mann-Whitney U test $p < 0.01$, $p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.001$ respectively). Eight (33%) patients with initial DAI $\leq$ 14 discontinued treatment (4 because of nausea/vomiting) compared to 35 (30%) (11 because of nausea/vomiting) of those with initially more active disease. Again no difference in the pattern of drop outs could be demonstrated ($\chi^2 = 0.12$ and 1.18; $p > 0.05$).

	DAI ≤ 14		DAI > 14		Mann-Whitney % change p value
Wk	0	24	0	24	
n	24	16	118	83	
ESR (mm/hr)	31 (2-130)	16 (7-75)	68 (10-150)	33 (5-129)****	NS
Hb (g/dl)	13.4 (8.7-17)	13.6 (11-17.7)	11.3 (7.4-15.6)	12.0 (9.3-15.6)****	NS
Plats ($x10^9/l$)	315 (118-626)	305 (206-418)	416 (126-888)	343 (134-809)****	< 0.01
Rose Waaler Titre	1/256 (0-1/1024)	1/512 (0-1/1024)	1/128 (0-1/1024)	1/128 (0-1/1024)	NS
Articular index	7.5 (0-34)	3 (0-9)**	21 (3-61)	8.6 (0-33)****	NS
5 point pain score	2.3 (1-4)	1.4 (1-3)*	2.8 (1-4)	1.9 (0-4)****	NS
Grip strength (mmHg)	95 (56-190)	125 (85-235)***	79 (38-178)	85 (9-200)****	NS
LUT (mins)	46 (0-all day)	1.25 (0-60)*	119 (0-all day)	30 (0-all day)****	NS
DAI	13.6 (12-14)	10 (7-13)***	17.4 (15-23)	14.2 (9-21)****	NS

Table LI Pattern of change in inflammatory parameters (median and range) over 24 weeks for patients with DAI ≤ 14 and those with DAI > 14 and comparison of the percent change in the two groups (Mann-Whitney U test). NS = not significant; $p > 0.05$
Wilcoxon wk 0 v wk 24 - *$p<0.05$; **$p<0.01$; ***$p<0.005$; ****$p<0.001$.

Section 4

The use of prochlorperazine to treat sulphasalazine related upper gastrointestinal problems

One hundred and twenty-eight of the 158 patients were allowed access, through their general practitioner, to prochlorperazine (up to 10mg tid) for the symptoms of nausea and/or vomiting.

In the first 24 weeks of therapy 59 (46%) of those with access to prochlorperazine experienced upper gastrointestinal symptoms. Twenty (33%) of these patients availed themselves of the offer of prochlorperazine and of these 3 (15%) eventually stopped sulphasalazine because of their symptoms, 3 (15%) continued sulphasalazine at a reduced dose and 14 (70%) achieved their allocated dose of sulphasalazine. Of the 39 patients who had access to prochlorperazine but failed to receive it, 12 (31%) discontinued sulphasalazine because of nausea/vomiting and a further 12 (31%) continued at a reduced dose. Of the 30 patients not given access to prochlorperazine 17(57%) suffered symptoms of nausea and/or vomiting and of those 6 (35%) stopped therapy for this reason and 2 (12%) failed to achieve the allocated dose. Fig. XXXII represents this information as a flow diagram.

Section 5

Discussion

As might be expected patients who have mild initial disease as identified by either a low ESR or a low DAI tend to have milder

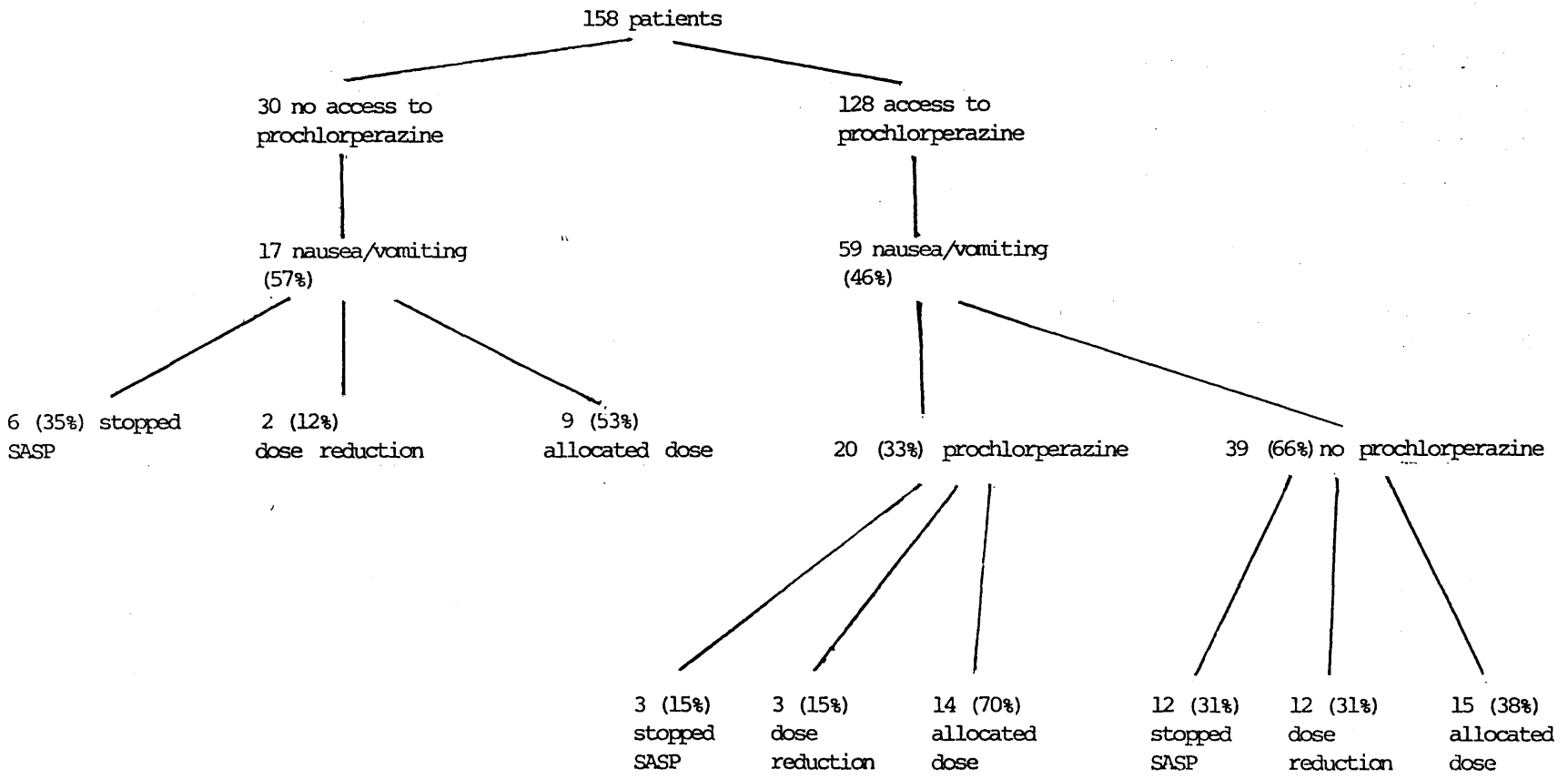


Fig. XXXII Effect of access to prochlorperazine on drop out rate and achievement of allocated dose in sulphasalazine treated patients.

disease as represented by other inflammatory parameters. These patients, however, when given sulphasalazine, are still capable of showing a clinical response of a similar proportion to those with more severe disease. The lack of improvement in haematological measures of inflammation is of interest in that it demonstrates the ability of a second line agent, under certain circumstances, to improve the clinical findings independent of laboratory parameters. In terms of patient treatment it also demonstrates that in a small number of cases a low ESR or DAI is not a contraindication to second line therapy provided it is felt to be clinically indicated. This finding has conflicting implications for clinical trial design in that, on the one hand, if one is interested only in clinical improvement there is no need to restrict entry only to patients with a raised ESR (as is often done), on the other hand, if a trial was carried out predominantly in patients with a low ESR it would be impossible to differentiate first and second line drugs. This second argument is mainly theoretical, however, in that the majority of patients in a random group with disease severe enough to merit second line therapy will have a raised ESR.

As might also be expected patients who have a longer disease duration or who have had a number of previous second line drugs have some evidence of more severe disease although the observation that this evidence consists largely of a higher articular index perhaps indicates that this particular parameter may relate more closely to joint damage rather than to reversible synovitis.

There is some suggestion from data presented here that men are commenced upon second line drugs with a lower articular index value

than are women. This could relate either to some difference in the perception of pain (either men are more stoical during the assessment of articular index or complain more at a set level of pain) or to some unconscious bias of the physician initiating treatment, perhaps in terms of "bread winning capacity". Despite these differences demonstrated in the initial disease severity between various groups this chapter fails to identify any particular group in whom treatment is clinically indicated who would appear to benefit more or less from sulphasalazine therapy.

The pattern of drop out and dose reduction because of upper gastrointestinal problems is of interest. Despite supposed access to prochlorperazine via their family doctor, only one third of patients who had these symptoms actually received it and although the trend was for fewer of these patients to discontinue therapy this did not reach statistical significance. There is therefore no evidence that access to prochlorperazine allows patients to continue sulphasalazine (certainly if one considers "intention to treat" with prochlorperazine). This failure may be explained by reluctance of patients to take more tablets (especially if they are feeling nauseated) or reluctance of GP's to prescribe medications to treat iatrogenic symptoms. Any trend towards a better outcome in patients who received prochlorperazine might be explained by a process of selection of those patients whose gastrointestinal symptoms were mild enough to enable them to obtain a prescription from their GP and to take the prochlorperazine tablets.

Section 6

Conclusions

This chapter fails to identify any subgroup who show an altered clinical response to sulphasalazine therapy although patients who commence therapy with a low ESR show only a clinical but not a haematological response. Because of this lack of influence of any of these factors on drug efficacy when tested individually a multivariate analysis would not contribute any further information.

It also fails to demonstrate any definite advantage to allowing patients access to prochlorperazine via their GP for the treatment of drug induced sickness.

Summary

Chapter 8

In this chapter I have examined the influence of a number of other factors on the efficacy and toxicity of sulphasalazine in rheumatoid arthritis.

Gender, disease duration, number of previous second line agents, seropositivity, initial ESR and initial disease activity index all influenced some aspects of initial disease activity. Only initial ESR, however, was related to the degree of improvement seen. Patients with a low ($\leq$30mm/hour) initial ESR showed no significant improvement in ESR, haemoglobin or platelet count and patients with a higher initial ESR showed a greater degree of improvement in these indices. No difference was seen between these groups in the degree of improvement in clinical indices. This has implications both for day to day practice and clinical trial design as it shows that even patients with an initial low ESR are capable of showing clinical but not haematological improvement with a second line drug.

One hundred and twenty eight patients were given access, via their GP's, to prochlorperazine if they experienced nausea and/or vomiting. Only 33% of those who experienced upper gastrointestinal symptoms availed themselves of the offer. More of these patients, however, were able to continue sulphasalazine treatment and to reach their allocated dose. The self selection of this 33% of eligible patients makes this finding difficult to interpret.

CHAPTER 9

The effect of sulphasalazine on scavengers of oxygen-derived free radicals in rheumatoid arthritis

Section 1

Introduction

Table II shows some of the many and varied actions of second line drugs at the cellular level by which means they may exert their clinical effect. One action of these drugs which has proved constant for all drugs tested is the alteration of intracellular and extracellular thiol or gluthione levels (64, 176, 177, 178, 179). Thiols (-SH groups) are strong reducing agents and are involved in maintaining several aspects of cell function and integrity such as active transport and protein synthesis (180). In addition thiols participate in the protective mechanisms directed against the effects of oxygen-derived free radical damage (181).

Free radicals in biological systems consist primarily of the superoxide (O_2^-) and hydroxyl ($OH^\cdot$) radicals the latter being produced from a reaction of the former with hydrogen peroxide (182). Production of these radicals may occur spontaneously or they can be produced much more rapidly by activated polymorphonuclear leucocytes (183) or as a result of ionising radiation (180). These species are highly reactive and can produce tissue damage in a number of ways. They can cause lipid peroxidation of cell walls resulting in cell damage and perhaps prostaglandin and leukotriene production (184, 185, 186). Oxygen-derived free radicals are also involved in the process of neutrophil chemotaxis (187) and can produce damage to a wide range of biological molecules including DNA (188), hyaluronic acid and collagen (189, 190, 191). They have also been shown to inhibit proteoglycan synthesis (192). In general it is the hydroxyl rather

than the superoxide radical which is thought to be responsible for tissue damage.

Superoxide radicals are removed in a reaction catalysed by the copper containing enzyme superoxide dismutase (SOD):-

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

The hydrogen peroxide thus produced is then removed in a further reaction involving either catalase:

$$2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O$$

or the glutathione (GSH)/glutathione peroxidase system:

$$2RSH + H_2O_2 \xrightarrow[\text{peroxidase}]{\text{glutathione}} RSSR + 2H_2O$$

If these reactions do not remove hydrogen peroxide then in the presence of superoxide radicals and ferrous iron or another transitional metal ion a hydroxyl radical will be produced in the following reactions:

$$Fe^{+++} + O_2^- \longrightarrow O_2 + Fe^{++}$$

$$Fe^{++} + H_2O_2 \longrightarrow Fe^{+++} + OH^- + OH^{\cdot} \qquad (193)$$

It has also been suggested that thiol groups themselves can reduce O_2^- concentrations either by a direct effect or an effect on the O_2^- generating system (181). In rheumatoid arthritis red cell lysate superoxide dismutase activity is low, red cell lysate thiol levels are high and extracellular thiol levels are low (194, 195). Extracellular thiol levels have been shown to bear an inverse relationship with

disease activity (100). Although most of the extracellular thiol is bound to albumin, alterations in plasma thiol levels in rheumatoid arthritis do not merely reflect changes in albumin concentration (195). It is possible, therefore, that an alteration of the levels and ratios of these various scavengers of oxygen-derived free radicals could explain many of the apparently disparate actions of second line drugs. It has even been suggested that to demonstrate "second line" qualities a drug must possess an aliphatic (non-aromatic) thiol group (196, 197).

D-penicillamine has been shown to produce an early rise in erythrocyte glutathione levels which correlates with and precedes clinical improvement in rheumatoid arthritis (78, 198). A similar rise in red cell lysate thiols and concomitant fall in superoxide dismutase activity has been observed in patients responding to sodium aurothiomalate but not in those who failed to respond or who received placebo (176). By 24 weeks these changes are reversed, with responders having a lower lysate thiol level and higher superoxide dismutase activity than at week 0 (176). Furthermore, a close relationship between red cell lysate thiol concentration and superoxide dismutase activity within erythrocytes has been demonstrated (176, 181, 194).

Plasma thiol concentrations show a negative correlation with disease activity (100) and have been shown to increase towards normal with second line drugs such as sodium aurothiomalate (64) and d-penicillamine (178, 195, 199) and with cytotoxic drugs such as cyclophosphamide (179). First line drugs, such as alclofenac, however, fail to produce such a change (178). In addition to the

assessment of individual parameters certain relationships between these parameters may be of value. In particular LSH concentration is raised and plasma thiol concentration lowered in rheumatoid arthritis. After an initial further rise (78, 198) red cell thiol levels fall in patients who respond to second line therapy, whereas extracellular thiols rise (176). Thus the ratio alters during second line drug treatment. Together these compounds are a measure of the reduction capacity or, conversely, the oxidative stress, across cell membranes and the relationship between them is of importance in terms of protection against free radicals.

In order to define further the second line qualities of sulphasalazine and also to test the hypothesis that second line activity is linked to an effect on free radical scavengers an investigation into the action of sulphasalazine on these scavenging systems was carried out. An effect of sulphasalazine on those systems would be of great interest as, unlike many second line drugs, neither it nor its metabolites contain a free thiol group.

Section 2

Patients and methods

2.1 Patients and treatment

Red cell lysate thiol levels, red cell lysate superoxide dismutase activity and plasma thiol levels were measured in 32 consecutive patients who were given sulphasalazine. All patients had definite or classical rheumatoid arthritis and had active disease which required the addition of a second line agent. Patients studied comprised the

last 7 patients randomised to sulphasalazine in Study 1 (Sulphasalazine v sodium aurothiomalate v placebo) - data on patients randomised to placebo or sodium aurothiomalate were insufficient to draw conclusions, and the first 25 patients to be recruited to Study 2 (1.5g/day v 3.0g/day). The studies were recruited consecutively. Twenty-two patients (13 allocated to 3.0g/day and 9 to 1.5g/day) completed the 24 week follow up period and only data from these patients are further described. In view of the relatively small numbers on each dose the patients are analysed as a single group.

2.2 Sampling and analysis

Samples were taken for estimation of red cell lysate superoxide dismutase activity, red cell lysate thiol concentration and plasma thiol concentration. Samples were taken at weeks 0, 6, 12 and 24. Weeks 0, 6 and 24 were chosen as they represent the times at which maximal change was seen (W E Smith personal communication) in previous studies.

Venous blood (10ml) was placed in a lithium heparin container and stored at $4^{o}C$ for a maximum of 4 hrs until analyses was carried out. Blood was centrifuged at 3000 rpm for 10 minutes and the supernatant removed. Red cells were lysed using distilled water. After 2 hours lysis at $4^{o}C$ 2ml haemolysate was removed and haemoglobin precipitated by a 0.8ml 3/5 v/v mixture of chloroform and ethanol followed by 0.3ml distilled water. The mixture was then centrifuged and the resulting clear supernatant used for analysis of thiols and SOD activity. Superoxide dismutase was measured in the red cell lysate by following its effect on the photochemically induced auto-oxidation of O-

dianisidine (200). Red cell lysate and plasma thiol levels were measured using Ellman's reagent (201). Plasma thiols were measured at pH 7.6 and pH 6.5, the former representing "total" plasma thiols and the latter "fast reacting" plasma thiols. The difference between the two is referred to as "slow reacting" plasma thiols. The variation in thiol concentration at different pH's is thought to represent change in the configuration of the albumin molecule (202). Thiol and superoxide dismutase measurements were carried out at the University of Strathclyde by Mr Farid Khan under the supervision of Drs W E Smith and D Brown. Statistical analysis was carried out using the appropriate non-parametric tests.

Section 3

Results

Table LII shows the pattern of change in the various inflammatory indices and Table LIII shows the measured scavengers of oxygen derived free radicals over the 24 weeks of the study. Figs XXXIII, XXXIV and XXXV show the pattern of change in superoxide dismutase activity, lysate thiol and total plasma thiol concentrations repsectively.

By 6 weeks a significant improvement was already seen in ESR, articular index, hand grip strength, albumin, total globulins and DAI and by 24 weeks significant improvement was seen in most inflammatory indices. At 6 weeks a significant fall in lysate superoxide dismutase activity and rise in lysate thiol concentration was apparent (Wilcoxon $p < 0.01$ and $p < 0.05$ respectively). By 24 weeks, however, the direction of these changes had reversed with the result that lysate

	Wk 0	Wk 6	Wk 12	Wk 24
Age	55.5 (43-69)	- -	- -	- -
Duration	7.25 (1-25)	- -	- -	- -
Haemoglobin (g/dl)	11.8 (9.7-15.6)	12.1 (8.6-16.5)	12.3 (8.6-15.6)	13.0* (10.8-15.6)
ESR (mm/hr)	70 (22-131)	54**** (15-137)	40**** (17-85)	27**** (6-118)
Plats (x 10^9/l)	386 (203-888)	397 (211-845)	342** (243-941)	340*** (34-809)
Rose Waaler Titre	1/64 (0-1/1024)	1/256 (0-1/1024)	1/256 (0-1/1024)	1/128 (0-1/1024)
Articular Index	19.5 (8-39)	12.2**** (0-27)	12**** (0-41)	6.5**** (0-33)
Pain Score	2.7 (1-4)	2.6 (1-4)	2.1** (1-4)	1.9*** (0-4)

Table LII Medians (ranges) and P values (Wilcoxon) for inflammatory parameters in sulphasalazine treated patients who had thiols measured at Wks 0, 6, 12, and 24 (n = 22). Wilcoxon v Wk 0 - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001.

	Wk 0	Wk 6	Wk 12	Wk 24
Hand Grip Strength (mmHg)	85 (48-190)	94* (50-254)	95*** (55-290)	95*** (59-215)
Duration of a.m. stiffness (mins)	77.5 (0-all day)	62.5 (0-all day)	45* (0-all day)	30* (0-all day)
Albumin (g/l)	37 (30-44)	38* (33-46)	39.5**** (34-44)	41**** (36-47)
Globulins (g/l)	34 (27-45)	31.5*** (27-42)	30.5**** (25-40)	28**** (21-42)
Bilirubin (umol/l)	6 (4-12)	6 (4-9)	7.1 (3-10)	7 (4-11)
Alk Phos (u/l)	265 (5-610)	262 (215-630)	257 (130-690)	251 (145-850)
AST (u/l)	14 (10-21)	16 (12-25)	20.25**** (12-39)	19*** (7-39)
ALT (u/l)	10 (5-30)	11.5 (5-25)	16**** (5-75)	17** (7-69)

Table LII (Cont) Medians (ranges) and P values (Wilcoxon) for inflammatory parameters in sulphasalazine treated patients who had thiols measured at Wks 0, 6, 12, and 24 (n = 22). Wilcoxon v Wk 0 - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001.

	Wk 0	Wk 6	Wk 12	Wk 24
IgA (g/l)	3.2 (0.7-5.7)	4.0 (1.5-6.0)	3.1 (1.5-6.8)	3.0 (0.3-7.9)
IgG (g/l)	12.75 (8.7-19.2)	13.5 (7.9-19.0)	12.3 (8.3-19.0)	12.4* (6.7-19.1)
IgM (g/l)	1.4 (0.7-2.3)	1.3 (0.7-3.0)	1.1 (0.6-2.6)	1.1 (0.4-2.7)
Creatinine (umol/l)	67 (40-120)	57.5 (45-145)	67.5 (50-120)	67 (50-110)
MCV (fl)	82 (71-98)	- -	84 (77-99)	89**** (74-108)
Disease activity index	17 (12-20)	16.5*** (10-20)	14**** (9-18)	12.5**** (9-18)

Table LII (Cont) Medians (ranges) and P values (Wilcoxon) for inflammatory parameters in sulphasalazine treated patients who had thiols measured at Wks 0, 6, 12, and 24 (n = 22). Wilcoxon v Wk 0 - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001.

	Wk 0	Wk 6	Wk 12	Wk 24
Red cell lysate superoxide dismutase -SOD (ug/ml)	51 (13 - 93)	27** (6 - 98)	59 (7 - 91)	57^{+} (2 - 98)
Red cell lysate thiols (LSH) (umol/l)	181 (81 - 344)	268* (76 - 537)	221 (63 - 511)	145* (20 - 386)
Plasma "fast reacting" thiols (umol/l)	192 (71 - 323)	190 (26 - 316)	209* (68 - 349)	292**** (158 - 413)
Plasma "slow reacting" thiols (umol/l)	131 (25 - 211)	161 (14 - 248)	126 (48 - 219)	167 (54 - 292)
Plasma "total" thiols (PSH) (umol/l)	343 (194 - 473)	345 (199 - 459)	351*** (271 - 444)	411**** (245 - 491)

Table LIII Changes in scavengers of oxygen-derived free radicals in patients completing 24 weeks sulphasalazine therapy (n = 22)

Wilcoxon v Wk 0 - *p<0.05; **p<0.01; ***p<0.005; ****p<0.001. $^{+}$ p<0.05 Wilcoxon v Wk 6.

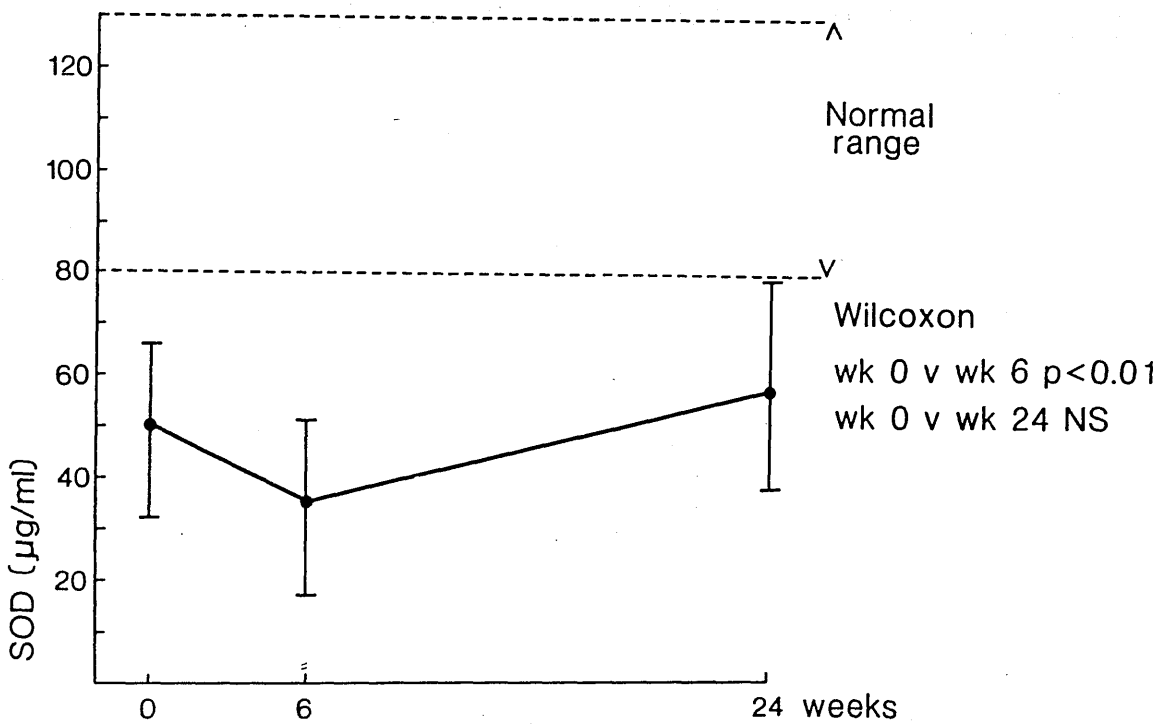


Fig. XXXIII Median (range) red cell lysate superoxide dismutase (SOD) activity in 22 sulphasalazine treated patients over a 24 week treatment period.

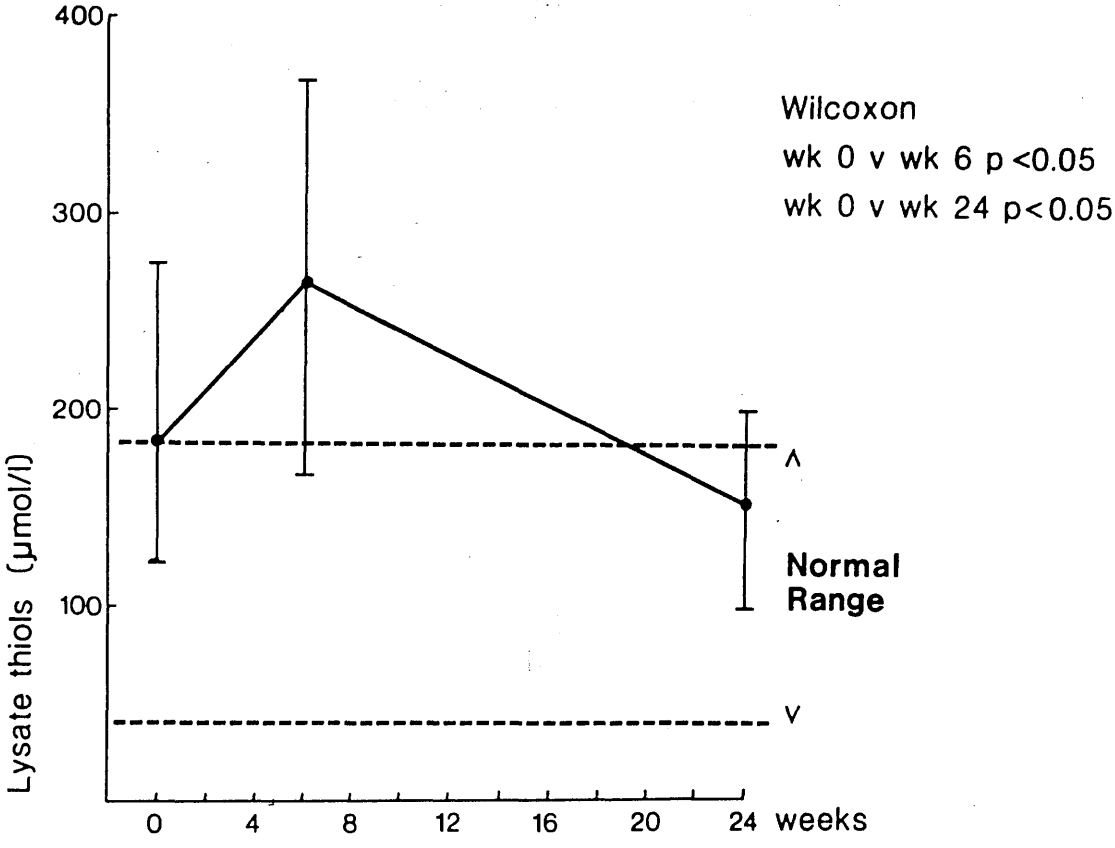


Fig. XXXIV Median (range) red cell lysate thiol (LSH) levels in 22 sulphasalazine treated patients over a 24 week treatment period.

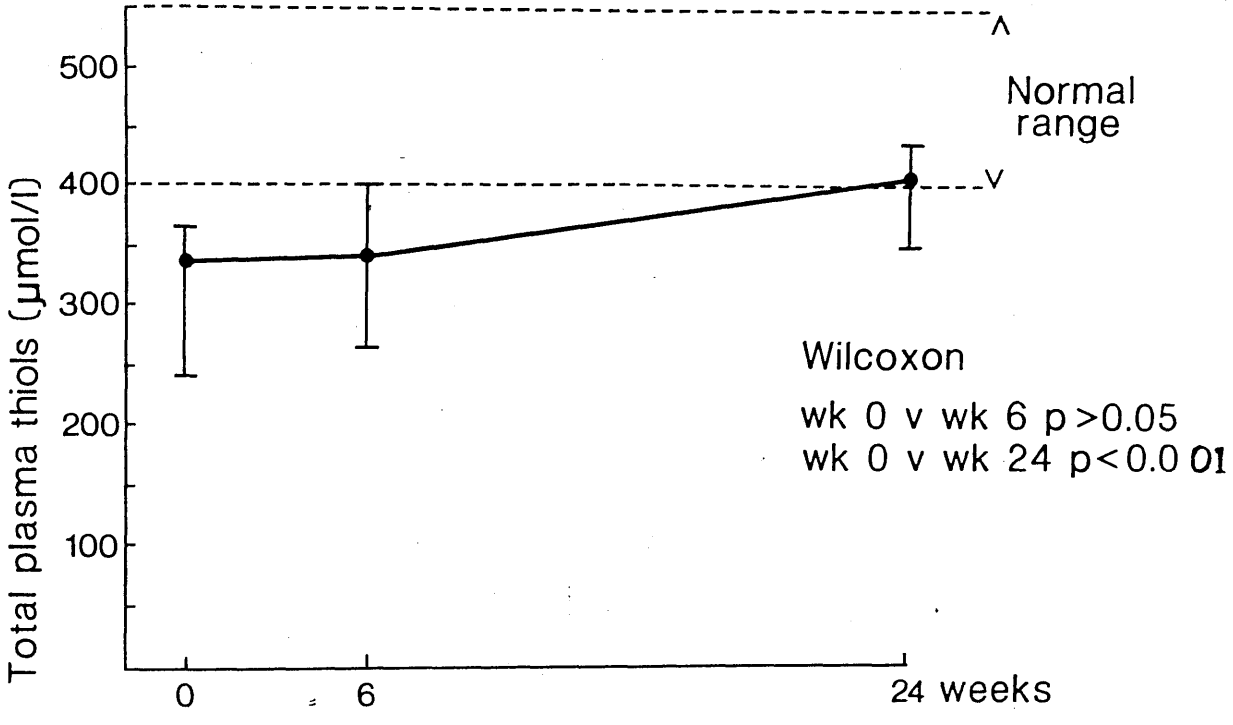


Fig. XXXV Median (range) plasma thiol (PSH) levels in 22 sulphasalazine treated patients over a 24 week treatment period.

thiol was significantly lower than both the pre-treatment and 6 week values (Wilcoxon $p < 0.05$ and $p < 0.01$ respectively) and although superoxide dismutase activity was no different from the pre-treatment value (Wilcoxon $p > 0.05$) it was significantly higher than the 6 week value (Wilcoxon $p < 0.05$). In contrast plasma thiol concentration showed a statistically insignificant rise by week 6 (Wilcoxon $p > 0.05$) but by week 24 was significantly higher than the pre-treatment value (Wilcoxon $p < 0.001$). This rise in plasma thiol concentration consisted almost entirely of a rise in fast reacting thiols (Wilcoxon $p < 0.001$) whereas slow reacting thiols showed no significant change (Wilcoxon $p > 0.05$).

A marked negative correlation was seen between the change in total plasma thiol concentration and the change in ESR over the 24 week follow up period (Spearman-Rank correlation $r_s = -0.61$, $p < 0.02$) (Fig XXXVI) and also between the change in plasma thiol and the change in disease activity index ($r_s = -0.53$, $p < 0.05$). Neither the change in lysate superoxide dismutase activity nor the change in lysate thiol concentration at either 6 or 24 weeks correlated with clinical or laboratory parameters of inflammation .

At week 0 a strong relationship was noted between superoxide dismutase activity and lysate thiol concentration (Spearman-Rank correlation $r_s = -0.75$, $p < 0.01$) but by week 6 this correlation was lost ($r_s = -0.43$, $p > 0.05$) and this loss of correlation persisted through to week 24 ($r_s = 0.32$, $p > 0.05$). At no point during the study could a significant correlation between extracellular thiol levels and intracellular indices be demonstrated. No difference between 1.5g/day and 3.0g/day could be demonstrated at any point in either the absolute

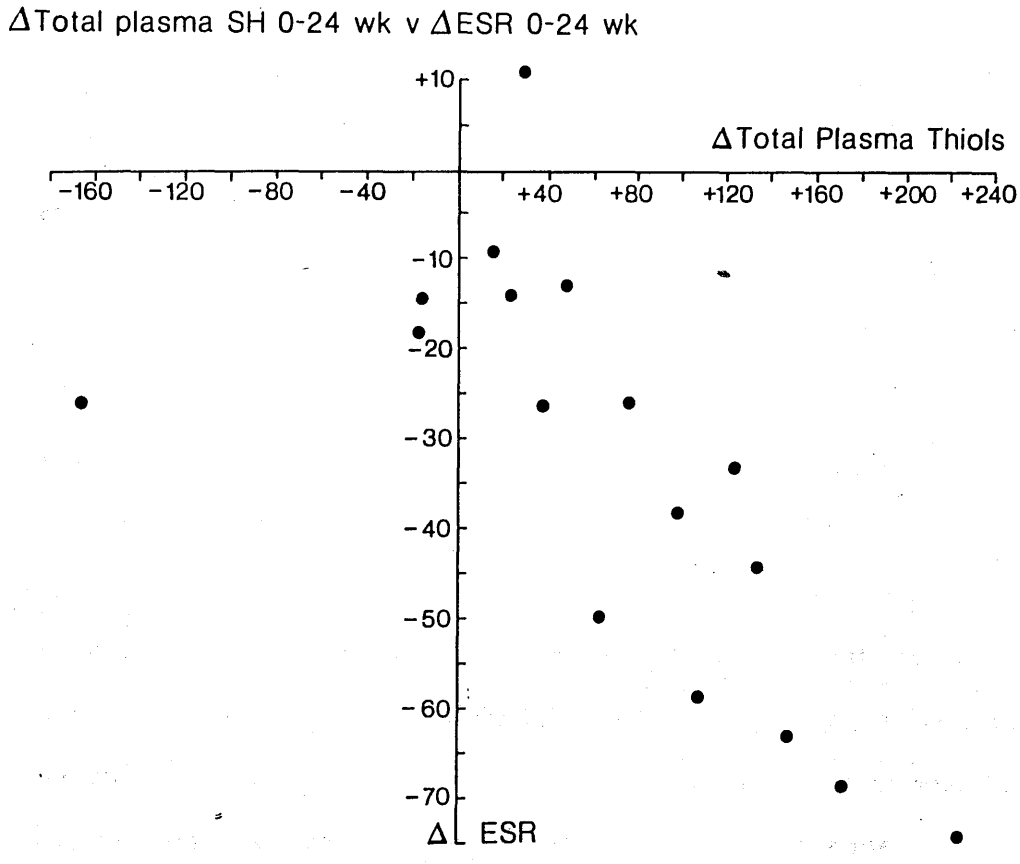


Fig. XXXVI Correlation between the change in total plasma thiol (PSH) and the change in ESR in 22 sulphasalazine treated patients over a 24 week treatment period (Spearman-Rank).

value or the degree of change in any parameter (Mann-Whitney U test $p > 0.05$).

Section 4

Discussion

The results of this study demonstrate that sulphasalazine has a significant effect on plasma thiol levels similar to that found with gold salts and penicillamine (64, 178) and disagrees with previous work which could demonstrate no such effect (140). This finding of a rise in plasma thiol concentrations lends further evidence to the claim that sulphasalazine has "second line" properties. The main effect of sulphasalazine on plasma thiol concentrations is an increase in the fast reacting thiols. This suggests a change in albumin conformation (202) and is similar to the effect of penicillamine which also causes an increase in the concentration of "fast reacting" thiols (195). Using different techniques an increase in the reactivity of serum thiol groups has been shown with various other second line drugs but not with first line agents (177, 119). The change in serum thiol levels over 24 weeks correlates with change in ESR and in disease activity index but follows rather than precedes the improvement in these indices. This implies that alterations in serum thiol levels are more likely to reflect change in disease activity rather than produce it.

The changes in intracellular thiol concentration and superoxide dismutase activity may be more fundamental to the action of sulphasalazine. Most patients remaining on therapy for 24 weeks

showed clinical improvement and thus, overall, the patients studied can be regarded as an "improver" group. In this group, however, the initial changes in the intracellular measurements indicate a "pro-inflammatory" change, namely an increase in thiol levels and reduction in superoxide dismutase activity. Again similar early "pro-inflammatory" changes have been seen previously with sodium aurothiomalate in responders but not in non-responders or placebo treated patients (176) and it may be that cellular defence against free radical attack is enhanced by these changes in the early stages of therapy.

In patients with rheumatoid arthritis undergoing therapy with NSAIDs alone, there is usually a good correlation between red cell lysate superoxide dismutase activity and lysate thiol levels (194) and again this was noted at the beginning of the present study. This correlation, however, breaks down with sulphasalazine therapy indicating, once more, that it interferes in some subtle way with erythrocyte free radical defence mechanisms. Interestingly sodium aurothiomalate alters the nature of this correlation but does not abolish it thus suggesting some difference in the activity of these two drugs (176). None of these intracellular changes (either early or late) show significant correlation with clinical or simple laboratory parameters of inflammation. The early parodoxical changes in intracellular thiol concentration and superoxide dismutase activity taken in conjunction with the change in the relationship between the two measurements and especially the failure to demonstrate a significant correlation between them and the clinical indices suggest, perhaps, that changes in intracellular scavengers of free radicals may be fundamental to the action of sulphasalazine rather than merely a

reflection of disease activity. Such alterations in these potent scavenging compounds could be related to major changes in cellular metabolism and protective mechanisms and, taken in conjunction with the changes in serum thiol concentrations may represent major changes in the redox potential across the cell membrane. Unfortunately because of the volume of blood required it was not possible to carry out similar studies in leucocytes but it would seem probable that similar changes occur in these cells. It is also of interest to note that a drug which is not a thiol compound nor is metabolised to a thiol compound has second line properties and produces profound changes in both extracellular and intracellular thiol concentrations. This disproves previous claims that a thiol group or even an ethane thiol group is necessary for such activity (196, 197). It is unclear which portion of the sulphasalazine molecule is responsible for producing changes in these scavengers of oxygen derived free radicals. Theoretially the most likely candidate would be an aromatic ring but similar rings are found in numerous NSAIDs which do not share the above properties.

Section 5

Conclusions

The major conclusions from this study are that sulphasalazine affects intracellular and extracellular thiol concentrations and intracellular superoxide dismutase activity. This gives further support to the claim that it is a second line drug. The facts that changes in intracellular parameters are initially of a paradoxical nature, precede extracellular changes and do not correlate directly with

disease activity suggest that the intracellular effect may reflect a more basic action of the drug and may represent a mechanism of action. Finally it dispels the myth that a thiol group is necessary before a drug can affect thiol levels.

Summary

Chapter 9

Second line drugs such as penicillamine and sodium aurothiomalate alter the levels of available thiol groups both within and outwith the cells and also affect the intracellular superoxide dismutase activity. This is thought to be related to the possession by these drugs of an aliphatic thiol group. Among other functions of these various intra and extracellular substances are the "scavenging" of oxygen-derived free radicals and the maintenance of cell membrane integrity.

Similar changes to those seen with penicillamine and sodium aurothiomalate were seen here in 22 rheumatoid patients treated with sulphasalazine for 24 weeks. These patients showed an initial fall in red cell lysate superoxide dismutase activity with a rise in red cell lysate thiol levels ("pro-inflammatory" changes). These changes subsequently reversed and by 24 weeks lysate thiol levels were significantly lower than the pre-treatment levels. Over this period a gradual rise was seen in extracellular thiol levels.

These changes may represent the mode of action of sulphasalazine and they certainly show that the possession of an aliphatic thiol group is not a necessary pre-requisite to the production of marked changes in the free radical scavenging system.

Chapter 10

A study to determine the active therapeutic moiety of sulphasalazine in rheumatoid arthritis

Section 1

Introduction

The preceding chapters have demonstrated that sulphasalazine is an effective second second line agent in rheumatoid arthritis and, although a relationship between dose and efficacy has been demonstrated, no relationship could be demonstrated between the measured circulating levels of various metabolites and efficacy of the drug. Hence no light has been shed as to which component (if either alone) of the sulphasalazine molecule is responsible for its second line action.

A small proportion of ingested sulphasalazine is absorbed directly, reaches the systemic circulation and is then excreted unchanged in the urine. Most of the ingested dose, however, reaches the large bowel intact and is split at its azo bond by bacterial action to sulphapyridine and 5-ASA (Fig XXXVII). Most of the sulphapyridine is subsequently absorbed, reaches the systemic circulation and is eventually excreted via the kidneys either in its unchanged form or after hepatic metabolism. The 5-ASA, on the other hand, remains largely within the large bowel lumen and is excreted in the faeces. The little 5-ASA which is absorbed is metabolised by the liver and then rapidly excreted by the kidney. Very low levels of 5-ASA are achieved in venous blood.

The aetiopathogenesis of rheumatoid arthritis is unknown although it is generally thought to be associated with some undefined antigenic stimulus. Many and varied antigens are produced by the bacterial flora of the bowel and it has been proposed that this is a likely

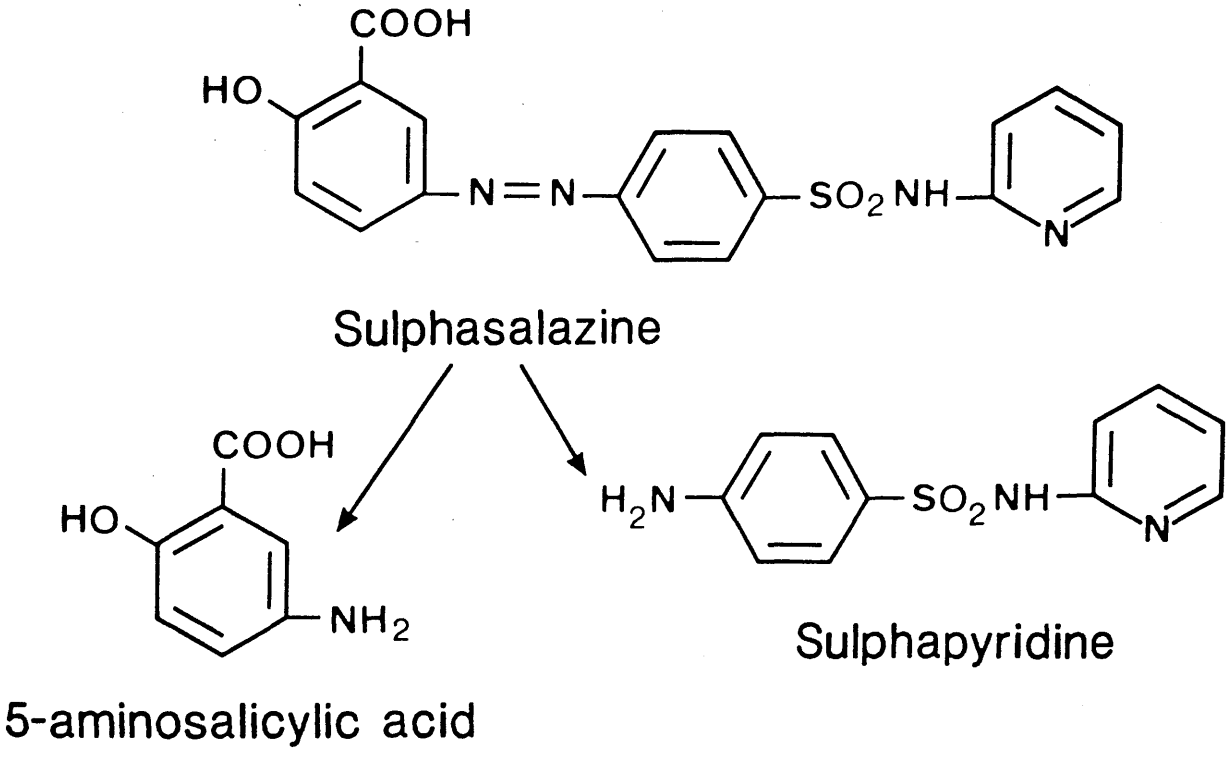


Fig. XXXVII Sulphasalazine and its azo cleavage products.

source of antigen production (154). In addition, gut wall permeability seems to be altered in rheumatoid patients either as a feature of the disease or its treatment (203, 204, 205). It is conceivable, therefore, that sulphasalazine acts by some local action within the bowel either by altering bowel flora by virtue of the sulphapyridine component (38), by an effect on bowel wall prostaglandins and perhaps permeability as a result of the action of 5-ASA (34) or possibly sulphasalazine itself. It should be noted, however, that 5-ASA differs from para aminosalicylic acid (an antituberculous drug) only by the presence of the amino group on C5 rather than C4 and thus it too may have some antibiotic action. Although only a small percentage of ingested sulphasalazine is absorbed unchanged, because of the large doses used, a significant amount is absorbed as is most of the sulphapyridine. It is also possible that one of these compounds or perhaps a metabolite of sulphapyridine exerts its effect after absorption and that the second line effect may be unrelated to any local activity within the gastrointestinal tract. As described in Chapter 1 both sulphasalazine, and to a lesser extent, its metabolites also display a spectrum of immune modulating activity which may represent a mode of action in rheumatoid arthritis.

Both products of azo cleavage of sulphasalazine are available commercially; sulphapyridine as an antibiotic and 5-ASA for the treatment of ulcerative colitis. In this chapter I have used oral preparations of both drugs separately in an attempt to identify the active compound and hopefully separate the active and toxic moieties and also perhaps to comment on and improve our understanding of the aetiological and pathological mechanisms of rheumatoid arthritis.

Section 2

Patients and methods

Sixty patients with active definite or classical rheumatoid arthritis (146) were randomly allocated to sulphapyridine 2g/day or 5-ASA (Asacol; Tillots Laboratories) 1.2g/day (30 patients per group). The sulphapyridine compound used differs from that in sulphasalazine in that it is available for absorption much higher up the gastrointestinal tract, thus most is absorbed before it reaches the terminal ileum and therefore may not be able to exert a local effect in the lower gastrointestinal tract. The 5-ASA preparation used (Asacol) contains 5-ASA bound to an acrylic resin (Eudragit S) which is carried intact to the terminal ileum and colon before the 5-ASA is released and thus closely parallels the kinetics of the 5-ASA portion of sulphasalazine (206, 207). These particular doses were chosen as they represent, to the nearest whole tablet, a dose equimolar to 3g sulphasalazine. During the course of the study no patients received other second line agents or corticosteroids and none had received such drugs in the preceding 3 months. Patients continued to receive their NSAID's throughout the study period.

Patients were initially commenced on 1 tablet per day (500mg sulphapyridine or 400mg 5-ASA) and the dose was increased by weekly increments of 1 tablet per day until the allocated dose was reached. Although the physician looking after the patients was aware of treatment allocation and the patient was able to identify the colour and number of tablets taken (but not the name of the treatment) all assessments were carried out blind by the metrologist (constant throughout the study) and the appropriate laboratory.

Clinical assessment comprised Ritchie articular index, 5 point pain score, mean hand grip strength and duration of morning stiffness while laboratory assessment consisted of Westergren ESR, haemoglobin level, platelet count and IgM rheumatoid factor measured by an ELISA technique. Disease activity index was calculated as described in Chapter 4.

Section 3

Results

At the start of the study the two groups of patients were comparable for age, disease duration and all inflammatory indices (Table LIV) ($p > 0.05$ Mann-Whitney U test). Twenty one patients remained on 5-ASA and 17 on sulphapyridine for the 24 week follow up period and were available for assessment. Reasons for and week of discontinuation of therapy are shown of Table LV. The most frequent reason for discontinuing sulphapyridine was upper gastrointestinal symptomatology while in 5-ASA treated patients the most common reason for discontinuing therapy was inefficacy. One patient in each group stopped therapy at the week 24 visit and was thus available for assessment. No significant differences in inflammatory or demographic parameters between patients who discontinued therapy and those who continued therapy were demonstrable.

Table LIV shows the medians and ranges for various parameters at wk 0 and 24. There was significant improvement by 24 weeks for most indices in the sulphapyridine treated patients but not the 5-ASA treated patients (with the exception of articular index) and, in fact,

	Sulphapyridine		5-ASA	
Week	0	24	0	24
n	30	17	30	21
Age (years)	59 (29-74)		59 (46-74)	
Duration (years)	7 (1-40)		12 (1-29)	
ESR (mm/hour)	55 (20-119)	18 (5-51)	51 (7-114)	58 (5-108)
Haemoglobin (g/dl)	11.8 (9.5-14.7)	12.7 (9.4-15.5)	12.1 (8.9-17.5)	11.3 (6.5-17.0)
Platelets (x 10^9/l)	377 (230-556)	337 (231-448)	416 (196-679)	394 (194-638)
Rheumatoid factor (u/ml)	2,470 (0-10,324)	1,230 (0-4,650)	4,864 (0-31,400)	4,625 (0-14,800)
Ritchie articular index	15 (0-37)	5 (0-23)	18 (4-39)	11 (0-31)

Table LIV Medians (and ranges) for various parameters at week 0 and week 24 for sulphapyridine and 5-ASA treated patients.

	Sulphapyridine		5-ASA	
Week	0	24	0	24
n	30	17	30	21
Pain score	2.5 (1-4)	2 (0-4)	2 (1-4)	2 (1-3)
Duration of a.m. stiffness (mins)	120 (0-all day)	30 (0-all day)	60 (0-all day)	60 (5-all day)
Hand grip strength (mmHg)	78 (43-142)	104 (36-240)	73 (48-111)	71 (45-138)
Disease activity index (DAI)	16.5 (11-21)	12 (6-19)	13.5 (9-20)	16 (12-19)

Table LIV (Cont) Medians (and ranges) for various parameters at week 0 and week 24 for sulphapyridine and 5-ASA treated patients.

	Sulphapyridine		5-amino salicylic acid	
n	30		30	
	No stopped	Week stopped	No stopped	Week stopped
Nausea/vomiting	6	1, 1, 4, 6, 7, 9	0	-
Skin rash	4	2, 4, 6, 6	0	-
Dizziness	0	-	1	4
Anxiety	1	6	0	-
Raised transaminases	1	8	0	-
Abdominal pain	1	16	0	-
Inefficacy	1	24	7	14, 16, 18, 18, 18, 18, 24
Defaulted from clinic	0	-	2	4, 18
Total	14		10	

Table LV Reasons for and time of discontinuation of therapy in sulphapyridine and 5-ASA treated patients.

in the latter group there was significant deterioration in haemoglobin and IgM rheumatoid factor levels (Table LVI). Data for ESR, haemoglobin and hand grip strength are displayed in Figs XXXVIII, XXXIX and XL. Despite the failure to demonstrate any difference between the groups at the outset, at 24 weeks there was a significant difference between the groups in respect to ESR, articular index, mean hand grip strength, IgM rheumatoid factor and disease activity index and in all cases this was in favour of milder disease activity in the sulphapyridine treated patients (Table LVI).

The improvement in the ESR in the sulphapyridine treated patients was not apparent by week 6 but had appeared by week 12 (Table LVII).

Section 4

Discussion

The results described here demonstrate unequivocally that sulphapyridine possesses second line activity in rheumatoid arthritis. The improvement in articular index seen with 5-ASA suggests that this drug may have a mild first line effect. The fall in haemoglobin may possibly represent gastrointestinal blood loss commonly seen with this class of drug or, like the rise in IgM rheumatoid factor, may represent increased disease activity in the 5-ASA treated patients. It is of interest to note that a difference could be demonstrated in the absolute values for inflammatory indices at 24 weeks between the two groups whereas this could not be demonstrated between 2nd line drugs and placebo in previous studies (126). This, paradoxically, could also be related to a mild first line effect of 5-ASA which by

	Sulphapyridine Wk 0 v Wk 24	5-ASA Wk 0 v Wk 24	Sulphapyridine Wk 24 v 5-ASA Wk 24
n	17	21	
ESR	< 0.005	NS	< 0.005
Haemoglobin level	NS	< 0.01 (deterioration)	NS
Platelets	< 0.005	NS	NS
Rheumatoid factor	< 0.1	< 0.05 (deterioration)	< 0.002
Ritchie articular index	< 0.005	< 0.01	< 0.05
Pain score	< 0.05	NS	NS
Duration of am stiffness	< 0.02	NS	NS
Hand grip strength	< 0.005	NS	< 0.002
Disease activity index (DAI)	< 0.005	NS	< 0.005

Table LVI p values for week 0 versus 24 (Wilcoxon matched-pairs signed-rank test) and for sulphapyridine week 24 values versus 5-ASA week 24 values (Mann-Whitney U test). (NS = not significant)

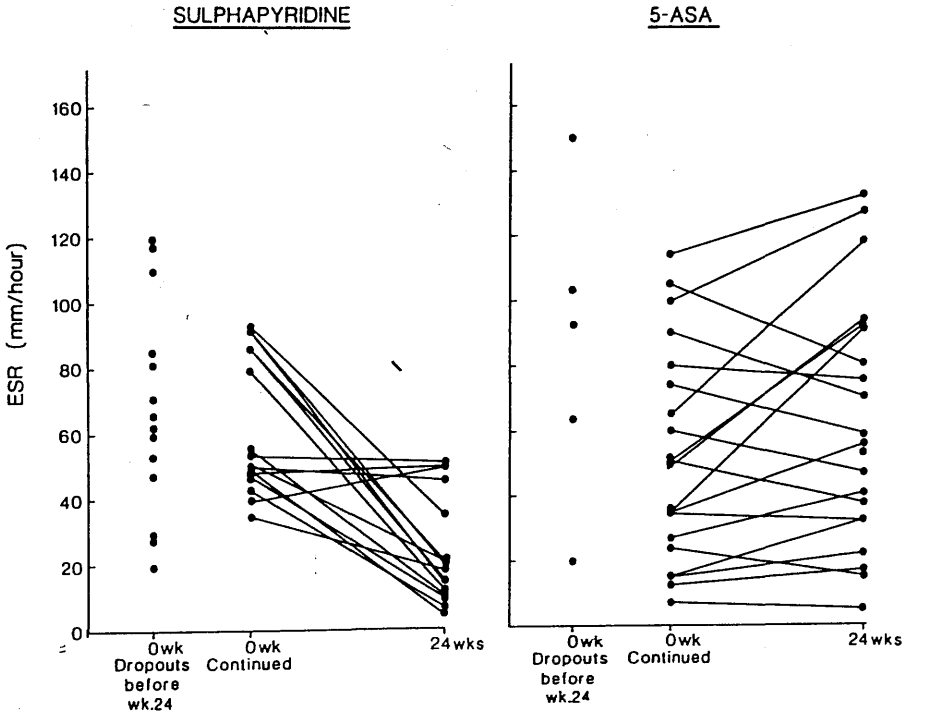


Fig. XXXVIII Change in ESR over 24 week period in sulphapyridine and 5-ASA treated patients.

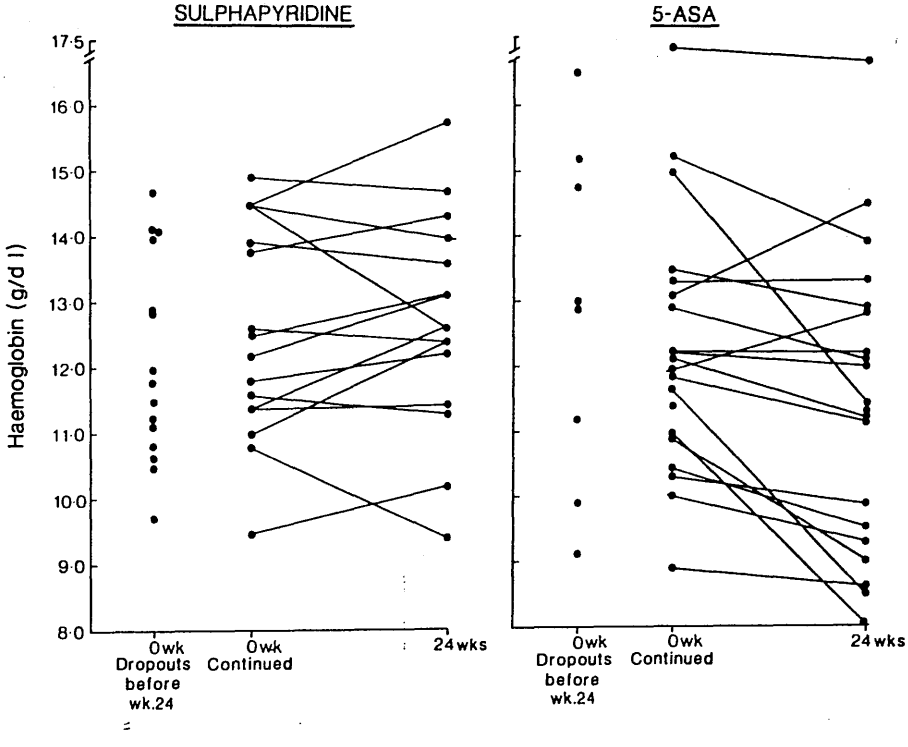


Fig. IXL Change in haemoglobin over 24 week period in sulphasalazine and 5-ASA treated patients.

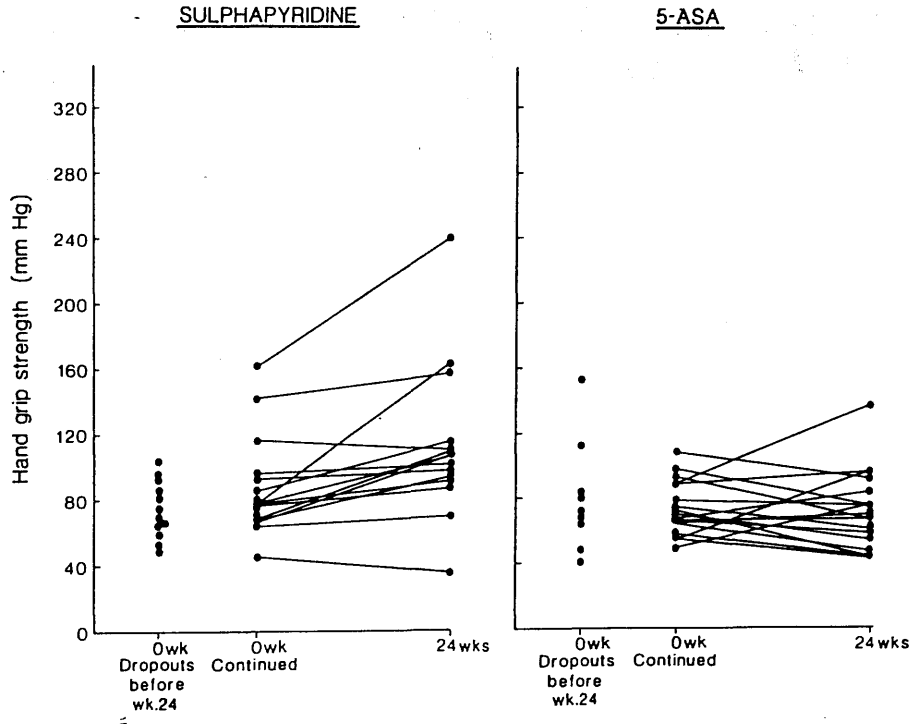


Fig. XL Change in hand grip strength over 24 week period in sulphapyridine and 5-ASA treated patients.

	Sulphapyridine		5-ASA	
	0 v 6	0 v 12	0 v 6	0 v 12
ESR	NS	< 0.01	NS	NS
Haemoglobin	NS	NS	NS	< 0.005*
Platelets	NS	NS	NS	NS
IgM rheumatoid factor	-	-	-	-
Ritchie articular index	-	< 0.001	-	< 0.01
Pain score	-	NS	-	NS
Duration morning stiffness	-	NS	-	NS
Hand grip strength	-	< 0.01	-	NS
Disease activity index (DAI)	-	< 0.01	-	NS

Table LVII p values for Wilcoxon matched-pairs signed-rank test wk 0 v wk 6 and wk 0 v wk 12 for both treatments. NS = not significant; $p > 0.05$
* deterioration

providing a degree of symptomatic benefit may have allowed patients with more severe disease to remain on treatment.

When compared to the 30 sulphasalazine treated patients in the first study (Table X) sulphapyridine shows a similar pattern of toxicity with a high incidence of early drop out because of upper gastrointestinal side effects with both drugs and a similar pattern of efficacy (Table XII). Subsequent studies of sulphasalazine have, however, shown a lower drop out rate. In addition the rate of onset of action appears, if anything, slower with sulphapyridine than with sulphasalazine. No statistically significant improvement could be demonstrated in haematological indices at 6 weeks with sulphapyridine (although the improvement in ESR fell just short of statistical significance) but with sulphasalazine some improvement was seen by this stage. It would thus appear that the direct administration of sulphapyridine rather than sulphasalazine confers no benefit either in terms of reduced toxicity or more rapid rate of onset of action and numbers studied are insufficient to comment on any difference in efficacy. The absence of a more rapid onset of action with sulphapyridine is hardly surprising as, with the administration of sulphasalazine, sulphapyridine is detectable in venous blood after 4-6 hours. The lag phase of several weeks before onset of action must therefore either be associated with equilibration of the serum levels with some other compartment or with the time taken to alter either directly or indirectly some as yet undefined biological process.

This study used "to the nearest whole tablet" an equimolar amount of sulphapyridine to that in 3g sulphasalazine. The exact molar equivalent to 3g sulphasalazine is 1.8g of sulphapyridine whereas in

this study I gave 2g (tablet size = 500mg). In addition, a proportion of ingested sulphasalazine is absorbed as such and excreted unchanged and thus the bioavailable sulphapyridine in sulphasalazine will probably be less than in a sulphapyridine preparation. This discrepancy in bioavailable sulphapyridine in the two studies makes direct comparison difficult although it seems unlikely that there is a marked difference between the compounds.

In practical terms, therefore, there would seem to be little point in changing from sulphasalazine to sulphapyridine in the treatment of rheumatoid arthritis and instead effort should perhaps be expended in finding an equally effective but less toxic sulphonamide. Initial reports on the use of sulphamethoxazole suggest that it too has a second line effect but again toxicity appears to be a major problem (175). During the previous phase of interest in sulphonamides in the treatment of rheumatoid arthritis in the late '30s and early '40s the concensus agreement appeared to be that these drugs were ineffective (26) although one study on an intramuscular preparation, soluseptasine, suggested a beneficial effect (208). It was Nana Svarz's belief in the activity of sulphonamides, despite these discouraging results, however, that eventually led to the production of sulphasalazine.

The findings of this study are in direct contrast to the situation in the treatment of inflammatory bowel disease where it is thought that 5-ASA is the active agent (36, 37, 209). Potentially more important, however, than an attempt to find a less toxic second line agent in rheumatoid arthritis is the fact that sulphapyridine, an established antibiotic, is an effective antirheumatic second line agent and this

in turn raises the question as to the role of infection in the initiation or perpetuation of the rheumatoid disease process. Sulphapyridine was first introducted as an antibacterial agent in 1938 by May & Baker and was known as M & B 693. Sulphonamide drugs produce their antimicrobial effect by acting as competitive antagonists of para amino benzoic acid (PABA) in the bacterial synthesis of folic acid. Replication of certain bacteria which require to synthesise their own folic acid is therefore inhibited and sulphonamides thus have a bacteriostatic effect. In addition to being bacteriostatic against certain gram negative and gram positive organisms sulphonamides are also effective against nocardia, chlamydia and a number of protozoa including plasmodium. It is interesting to note that many other second line drugs (ie gold, chloroquine, levamisole and dapsone) also have antimicrobial activity and were all, in fact, initially introduced for the treatment of infection (210, 211, 212). It has often been suggested that rheumatoid arthritis may have a microbiological origin and although viral infections are most often implicated (213) many non viral organisms have also been implicated in the aetiology and pathogenesis of rheumatoid arthritis. It has been suggested that in rheumatoid disease tissues are affected by direct invasion by fastidious organisms such as corynebacterium (214), mycoplasma (215), bacterial L forms (216) or even free living amoebae (217) and that such organisms may either directly produce an inflammatory reaction or cause some alteration of the immune response. Although evidence of infection by these organisms appears from time to time in the literature their role in the rheumatoid disease process is unproven and their presence may merely reflect increased susceptibility to these infections in rheumatoid arthritis. Another

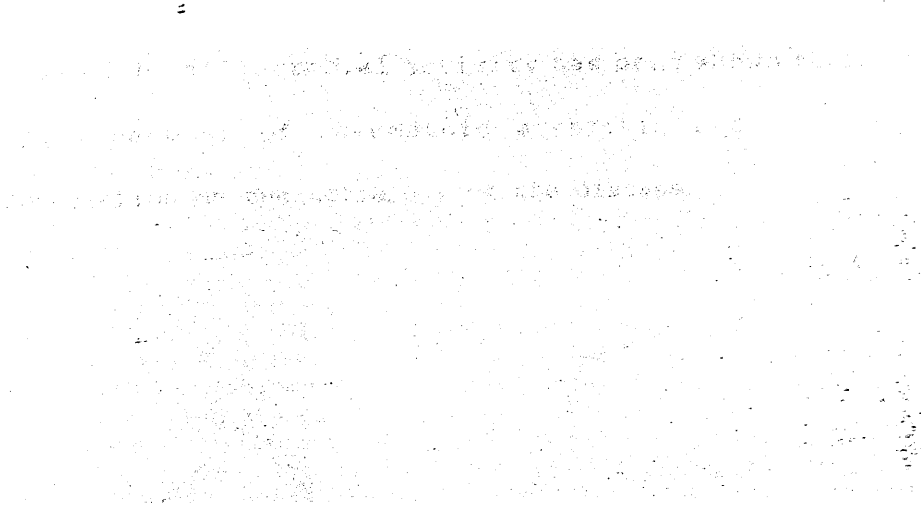
hypothesis for the role of bacteria in the rheumatoid disease process involves the passage of bacterial antigenic material from the gut and it has been suggested that peptidoglycans from bacterial cell walls play a role in the production of immune complexes in rheumatoid arthritis (218). Again only circumstantial evidence is available. It has been proposed that there is increased permeability of the gut wall in rheumatoid arthritis although this may merely be an effect of NSAIDs (203, 204, 205). In addition, overgrowth of colonic clostridium perfringens in swine is associated with a chronic nodular arthropathy resembling rheumatoid arthritis (219). Such an association in human rheumatoid disease is, however, much more controversial (220, 221).

It is possible, however, that sulphapyridine and sulphasalazine are working via some other mechanism such as an effect on the immune response or an alteration of folic acid metabolism. Many of the anti-inflammatory and immune regulating properties of sulphasalazine, however, do not seem to be shared to a great extent with sulphapyridine (31, 34), although the inhibition of killer cell activity is an action of the sulphapyridine rather than the 5-ASA component (30). One of the other actions of sulphapyridine is upon folate metabolism and there is certainly some well documented but ill defined abnormality of folate metabolism in rheumatoid arthritis (222) and it is just conceivable that sulphapyridine exerts its effect via a direct action on folate metabolism.

Section 5

Conclusions

In conclusion the findings from this study have shown sulphapyridine to have a second line effect with a broadly similar pattern of efficacy and toxicity to its parent compound, sulphasalazine. More work is needed to demonstrate what, if any, advantage one drug exerts over the other in clinical use. In contrast 5-ASA shows at best only a mild NSAID type activity but no second line properties. Previous attempts to identify a pathogenic role for micro-organisms in the aetiopathogenesis of rheumatoid arthritis have failed but the finding that yet another antimicrobial drug has second line properties once more raises questions regarding the role of an infective process in the causation or perpetuation of rheumatoid arthritis although alternative mechanisms of action are equally likely.



Summary

Chapter 10

Although it is effective in the treatment of rheumatoid arthritis sulphasalazine is a relatively toxic drug. In an attempt to separate toxicity from efficacy I have investigated separately the two components of sulphasalazine, namely, sulphapyridine and 5-aminosalicylic acid (5-ASA) for antirheumatic activity. In addition such an approach might throw some further light on the aetiopathogenesis of rheumatoid arthritis. Of 30 patients randomly allocated to 2.0g/day sulphapyridine, 17 continued treatment for 24 weeks, whereas 21 of the 30 patients allocated to 1.2g/day 5-ASA continued for 24 weeks. Significant improvement was seen in most inflammatory parameters in the sulphapyridine but not the 5-ASA treated patients and although initially comparable the sulphapyridine treated group had milder disease by 24 weeks. The toxicity profile of sulphapyridine, however, was very similar to that of sulphasalazine and no obvious advantage was observed in the use of sulphapyridine over that of sulphasalazine. It is of interest to note that another drug with antimicrobial activity has been shown to be beneficial in the treatment of rheumatoid arthritis and this promotes some speculation on the aetiology of the disease.

CHAPTER 11

GENERAL DISCUSSION

Section 1 Introduction

Section 2 Discussion of various aspects of the thesis

2.1 The efficacy of sulphasalazine in the treatment of rheumatoid arthritis

2.2 The influence of a number of variables on the efficacy and toxicity of sulphasalazine in rheumatoid arthritis

2.3 The single dose pharmacokinetics of sulphasalazine in elderly rheumatoid patients in relation to toxicity

2.4 The clinical use of sulphasalazine in rheumatoid arthritis

2.5 The effect of sulphasalazine on scavengers of oxygen derived free radicals in rheumatoid arthritis

2.6 The active moiety of sulphasalazine in rheumatoid arthritis

2.7 The place of a placebo group in the study of a new potential second line agent for rheumatoid arthritis

Section 3 The work presented in a broader context

3.1 A summary of the novel aspects of this work, its contributions to the overal body of knowledge and its implications for rheumatology

3.2 Fruitful areas for further reasearch

Section 4 Conclusions

Summary

Section 1

Introduction

In general most of the proposed aims which I outlined in Chapter 3 have been fulfilled. These are discussed below. In addition Study 1 raised some interesting questions on the place of a placebo group in a trial of a new second line agent and this too is discussed below.

Section 2

Discussion of various aspects of the thesis

2.1 The efficacy of sulphasalazine in the treatment of rheumatoid arthritis

Study 1 demonstrated significant improvements in laboratory and clinical indices of inflammation in the sulphasalazine treated patients. A similar improvement was seen in the sodium aurothiomalate treated patients but no such change was seen in those patients allocated to placebo. A difference was also demonstrated between sulphasalazine and placebo groups and between sodium aurothiomalate and placebo groups in the degree of improvement produced in the various inflammatory parameters at both 6 and 12 months. There, therefore, seems to be little doubt that, over the short and medium term, sulphasalazine is an effective second line drug in the treatment of rheumatoid arthritis. Analysis of drug efficacy in Studies 2 and 3 confirm this finding. In addition a number of open studies of sulphasalazine published before (8, 9, 140) and after (143, 144, 145) my own work also show it to be an effective drug. Only two other

controlled studies have been carried out, one against penicillamine (141) and one (published in abstract form only) against placebo (142). Again, both of these studies have shown sulphasalazine to have second line properties. It is perhaps noteworthy, however, that in my series of 90 patients available for 1 year follow up 10 (11%) discontinued therapy because of inefficacy between 6 months and 1 year. Other work has also pin-pointed this high failure rate on prolonged follow up (145) although it was singularly lacking in Farr's study (144). Further long term studies will be necessary to examine this problem.

Although from what is published in the gastroenterology literature sulphasalazine appears to be a relatively safe drug, a number of serious or potentially serious side effects (leucopenia, mucocutaneous toxicity, hepatitis) were found in patients studied in this series. Other workers in the rheumatology field, however, have found either little serious toxicity (141, 144, 145) or serious toxicity of a different nature (macrocytic anaemia) (8, 9). Thus, although serious side effects do occur in rheumatoid patients, the exact incidence is unclear and further (multicentre) studies of large numbers of patients will be required to define the incidence of these problems.

2.2 The influence of a number of variables on the efficacy and toxicity of sulphasalazine in rheumatoid arthritis

Study 2 showed that the efficacy of sulphasalazine in rheumatoid arthritis bears a direct relationship to the dose expressed as mg/kg body weight and the most effective dose would appear to be in excess of 40mg/kg. This dose relationship has not been previously described

with sulphasalazine and, in fact, there is no well documented relationship between dose and efficacy with any second line agent. In order to define the maximum tolerable dose at which a dose response relationship can be demonstrated further studies need to be carried out. In addition, the fact that the dose response relationship of this particular second line drug is not of the "all or none" variety suggests that it may be useful to investigate the dose response relationship of other established second line drugs. Despite this relationship between dose and efficacy no association between serum levels of sulphasalazine, sulphapyridine, acetyl sulphapyridine or total sulphapyridine and efficacy could be demonstrated. A similar situation prevails with other second line drugs (155, 156, 157, 158).

A greater total drop out rate and a greater drop out rate because of adverse effects was observed in elderly patients. A greater drop out rate because of upper gastrointestinal symptoms was also seen in slow acetylators. In addition there was a suggestion that older patients may not show such a good therapeutic response to sulphasalazine and, although the overwhelming evidence is that there is no difference in the degree of improvement seen between slow and fast acetylators, discontinuation of therapy because of inefficacy in the first 6 months was confined to fast acetylators.

No effect of gender, disease duration, number of previous second line drugs or initial disease severity on either toxicity or clinical response could be demonstrated and, interestingly, even patients with an initial low ESR or "slightly active" disease showed a clinical improvement. This suggests that the only indication for this form of therapy should be the overall assessment, by an experienced physician,

of a potential for response to the drug. In the context of second line studies this finding has conflicting implications. Although response is seen in patients with an initial low ESR and thus these patients can benefit from therapy, the absence of a haematological response in these patients would make it difficult to differentiate first and second line drugs. In practical terms, however, most patients eligible for second line therapy will have a raised ESR and thus this phenomenon would probably not influence the final result of a randomised trial.

No relationship was discovered between toxicity and serum creatinine concentrations and no predictors of serious toxicity were found.

Patients who actually received prochlorperazine from their GP for their upper gastrointestinal symptoms were more successful at continuing therapy and reaching their allocated dose, but most patients eligible for prochlorperazine did not take it and it may be that those who did take it were a self selected group with less severe gastrointestinal symptoms who were well enough to await a prescription.

2.3 Single dose pharmacokinetics in elderly rheumatoid patients with special reference to toxicity

In single dose studies elderly patients who, with eventual chronic dosing, experienced upper gastrointestinal symptoms achieved greater peak serum levels and areas under the serum concentration-time curve than those who suffered no upper gastrointestinal symptoms. This suggests that these troublesome side effects are related to serum

levels of sulphasalazine or its metabolites. Elderly patients excrete a greater proportion of the ingested dose in their urine than did a previously documented group of young normal volunteers and this implies increased absorption, reduced biliary excretion or altered distribution. Further studies with intravenous dosing and also steady state pharmacokinetics would be useful in further elucidating this problem.

2.4 The clinical use of sulphasalazine in rheumatoid arthritis

The most important recommendations for the clinical use of sulphasalazine in rheumatoid arthritis concern dose and monitoring. One should aim to achieve a dose in excess of 40mg/kg body weight and, if dose related upper gastrointestinal symptoms are a problem, it is probably of use to allow access to prochlorperazine to help achieve this dose. Monitoring of white cell count (and platelet count) should be carried out regularly (fortnightly) for the first 12 weeks (all cases of leucopenia occurred within this period). Thereafter until more data are available it should probably be monitored six weekly. In addition, in view of the risk of drug induced hepatocellular damage, liver function tests should be monitored at weeks 0, 6 and 12 and 12 weekly thereafter. Despite the finding of an increased incidence of problems necessitating drug withdrawal in slow acetylators and in the elderly, these groups did not appear to have any higher risk of serious, potentially life threatening, side effects. Thus, in the present state of knowledge, there is no justification for regarding old age or slow acetylator phenotype as a contraindication to treatment.

Other important practical considerations are that prolonged disease duration, low ESR or the number of previous second line treatments have no demonstrable effect on the response to sulphasalazine in patients in whom second line therapy is clinically indicated.

2.5 The effect of sulphasalazine on scavengers of oxygen-derived free radicals in rheumatoid arthritis

Patients described in Chapter 9 showed a rise in extracellular thiol levels. Although previous work failed to demonstrate such a change (140) it is not surprising to find a rise in extracellular thiol levels as these are thought merely to reflect disease activity (100). What, perhaps, is more surprising is that sulphasalazine produces alterations in the intracellular thiol levels and superoxide dismutase activity similar to those seen with sodium aurothiomalate and penicillamine (78, 176, 198) although neither sulphasalazine nor its metabolites contain an aliphatic (non-aromatic) thiol group which, it has previously been suggested (196, 197), is an important determinant of second line function, especially for drugs which are thought to act on the free radical scavenging system of cells.

Although this finding is of interest in proving that an aliphatic thiol group is not a pre-requisite of second line drugs which affect the free radical scavenging system, the present study has shed no light upon the necessity of these cellular changes for a second line effect although the lack of correlation of intracellular changes with various inflammatory parameters suggests that these changes represent something other than merely a reflection of disease activity. To answer this question fully it would be necessary to dissociate the

biochemical and clinical effects or alternatively to study the effect of a drug whose sole action is to alter the free radical scavenging system.

2.6 The active moiety of sulphasalazine in rheumatoid arthritis

The final study described in Chapter 10 shows, beyond doubt, that sulphapyridine is the active component of sulphasalazine in the treatment of rheumatoid arthritis. Unfortunately this is also the component of sulphasalazine responsible for most of its toxic effects and thus it displays no obvious practical benefit over sulphasalazine in the treatment of rheumatoid arthritis. This contrasts directly with the finding in inflammatory bowel disease that 5-ASA is the active component (36, 37, 209).

In terms of the mode of action of sulphasalazine or the aetiopathogenesis of rheumatoid arthritis the finding that sulphapyridine and not 5-ASA is active adds little although it does raise the possibility that a microbial process is involved. Further studies of antimicrobial drugs in the treatment of rheumatoid arthritis are indicated and these studies should probably first of all concentrate on the comparison of absorbable and non-absorbable agents. Other possible mechanisms of action of sulphapyridine also exist.

2.7 The place of a placebo group in the study of new second line agents in rheumatoid arthritis

In addition to the above comments pertaining to the stated aims of the

thesis the results obtained in Study 1 also raise questions about the usefulness of a placebo group in studies of new second line drugs. In this study only 6 (20%) patients remained on placebo at 1 year and, despite all groups being comparable at the start of the study, those placebo patients who achieved 1 year treatment had a significantly lower initial ESR than both the sulphasalazine group and those placebo patients who discontinued therapy. This implies that, largely because of the symptomatic benefit of second line drugs, even in a blind situation, there is selection within the placebo group throughout the course of the study so that those placebo patients available for comparison at the final analysis had mild initial disease thus invalidating any direct comparison with groups treated with active drugs. In addition, within group comparisons make it obvious when a potential second line drug is effective whereas, even in the self-selected group who continue treatment, no improvement is seen in the placebo group. These two facts question the usefulness or, indeed, the need for a placebo group in this type of study.

Section 3

The work presented in a broader context

3.1 A Summary of the novel aspects of this work, its contributions to the overall body of knowledge and its implications for rheumatology

This thesis contains several novel features both in the approaches used and in the findings. Approaches which were new to the field of second line drug research include attempts to relate the degree of

efficacy of a second line drug to dose (previous studies of other agents have concentrated upon the demonstration of efficacy with low dose therapy on the assumption that response is "all or none") and attempts to relate variations in drug handling to efficacy and toxicity, although subsequently interest has been displayed in the relationship between sulphoxidation status and the efficacy of d-penicillamine.

In this thesis I also explore the effect of sulphasalazine upon the group of substances (thiols and superoxide dismutase) which are largely responsible both for maintaining the redox potential across cell membranes and for scavenging oxygen-derived free radicals.

In addition to those methods mentioned above several standard approaches which have not previously been used in the investigation of sulphasalazine in the treatment of rheumatoid arthritis were employed, eg. a double blind comparison of sulphasalazine with placebo, the clinical use of individual drug metabolites in an attempt to identify the active moiety and a systematic investigation of variables which may affect the efficacy or toxicity of the drug.

As a result of these and other approaches several new findings have emerged:-

1) Sulphasalazine compares favourably with placebo in producing an improvement in both clinical and laboratory indices of inflammation in rheumatoid arthritis.

2) The efficacy of sulphasalazine is related to dose but is not related to serum levels of sulphasalazine or its metabolites and is unrelated to acetylator phenotype.

3) Nausea and vomiting, the most common adverse effect, bears a relationship to the serum levels of sulphasalazine and its metabolites and is commoner in slow acetylators.

4) The possession of a low ESR or previous multiple second line therapy does not preclude response in clinically suitable individuals.

5) Sulphasalazine affects the concentrations of various scavengers of oxygen derived free radicals. Previously it has been suggested that for a second line drug to exert such an effect either the drug itself or a metabolite must contain an aliphatic thiol group.

6) Sulphapyridine is the active moiety of sulphasalazine in rheumatoid arthritis. 5-ASA has no demonstrable second line effect.

7) Despite adequate matching at the beginning of the study, because of a differential in the rate of drop out due to inefficacy, patients who could continue placebo to the 24 week assessment were a self selected group with milder initial disease. This questions the applicability of a placebo group to this sort of trial.

These findings have significantly added to our knowledge and understanding of sulphasalazine in rheumatoid arthritis and have several important implications in a number of fields. In the day to day practice of rheumatology, sulphasalazine will be a useful addition to the available group of second line drugs and information from this thesis regarding dosage and monitoring schedules will be of value in

allowing the most efficient use of this drug. In our understanding of the rheumatoid disease process and how this may be altered, sulphasalazine has been shown to be capable of altering the oxygen-derived free radical scavenging system in a manner other than merely reflecting disease activity. This gives some further weight to the hypothesis that such an effect may be a necessary part of the second line effect and disproves the theory that an aliphatic thiol group is a pre-requisite for alteration of this system. In addition, the finding that sulphapyridine alone is a second line drug is of interest and may be of potential importance in understanding the rheumatoid process. Finally, in the area of recognition of new second line agents doubt has been cast upon the place of a placebo control group.

3.2 Fruitful areas for further research

All research asks more questions than it answers and the work described above is no exception. I have listed below several areas in which further research may be of use in answering some of these questions.

1) A large (almost certainly multicentre) prospective study is required to define the exact incidence of some of the less common side effects of sulphasalazine and long term follow up is required to further define the problem of late relapse.

2) Further investigation of the dose/effect relationship is required to identify the maximum tolerated dose at which such a relationship exists.

3) Studies of other second line drugs are required to investigate possible dose/effect relationship.

4) Studies of the effect of other potential second line drugs on the free radical scavenging system are required to investigate further the relationship between this system and second line activity. In addition, attempts should be made to measure those scavenging systems within the leucocyte where their relevance to inflammation may be more apparent.

5) Studies of other antimicrobial agents in the treatment of rheumatoid arthritis and a further search for a causative micro-organism may yield useful information. Alternatively investigation of folate metabolism in rheumatoid arthritis or of the immunological effects of sulphasalazine and sulphapyridine in rheumatoid arthritis may be of use.

Section 4

Conclusions

From work carried out in this thesis I can state the following conclusions:-

1) Sulphasalazine is an effective second line drug in the treatment of rheumatoid arthritis.

2) Potentially serious toxicity may occur with the use of sulphasalazine in rheumatoid patients. These problems tend to occur in the first few weeks of treatment.

3) Sulphasalazine exhibits a dose/response relationship in the treatment of rheumatoid arthritis but no relationship can be demonstrated between serum levels of sulphasalazine, sulphapyridine, acetylsulphapyridine or total sulphapyridine and efficacy of the drug. The optimum dose is in excess of 40mg/kg body weight/day.

4) Other than dose there are no good clinical predictors of efficacy.

5) No clinical predictors of potentially serious toxicity can be demonstrated. Upper gastrointestinal symptoms, however, are more common in slow acetylators. The overall drop out rate due to adverse effects is higher in elderly patients and those elderly patients who stop therapy because of nausea and/or vomiting achieve higher peak levels and areas under the curve for sulphasalazine, sulphapyridine, and total sulphapyridine on single dosing.

6) Sulphasalazine alters the oxygen-derived free radical scavenging and redox status of red blood cells. Such changes, therefore, are not solely dependent upon the thiol content of a drug. Intracellular parameters appear to change independently of disease activity thus suggesting such changes are more fundamental to the action of sulphasalazine.

7) Sulphapyridine is the active (and toxic) component of sulphasalazine in the treatment of rheumatoid arthritis.

Summary

Chapter 11

In this chapter I discuss the relevance of the results of the foregoing studies and suggest further possible areas of research.

Study 1 showed sulphasalazine to be an effective second line drug in the treatment of rheumatoid arthritis and it is thus a useful addition to the small supply of such agents. This finding is confirmed in subsequent studies. Unfortunately it has more potentially serious side effects than anticipated (especially leucopenia, mucocutaneous toxicity and hepatitis). The haematological and liver problems occur in the first 12 weeks of treatment and thus the most intensive monitoring should be concentrated in this period. There is also some suggestion of a high late failure rate and further large long term studies are required to help answer this question.

In Study 2 a direct relationship is shown between dose of sulphasalazine and the degree of improvement although no relationship is seen between serum levels of the various metabolites and efficacy. The optimum dose would appear to be in excess of 40mg/kg body weight/day. A relationship is apparent between old age and both total drop out rate and drop out rate because of toxicity and between slow acetylator phenotype and drop out rate because of upper gastrointestinal symptoms. However, neither of these variables are useful predictors of serious toxicity. Elderly patients who experience upper gastrointestinal problems attain higher peak levels and areas under the curve for sulphasalazine, sulphapyridine and total sulphapyridine. The rate of drop out in elderly patients or in the

whole population is not, however, related to renal function as measured (crudely) by serum creatinine concentration. There is, in fact, a suggestion that older rheumatoid patients excrete a greater proportion of the ingested dose in their urine. No other factors were found to be related to efficacy or toxicity.

Sulphasalazine, although it does not contain a thiol group, has been shown in these studies to alter both the intra- and extracellular thiol concentrations and also intracellular superoxide dismutase activity in a similar pattern to that found with sodium aurothiomalate and penicillamine. These substances are involved in free radical scavenging and in maintaining cell membrane integrity and these changes may represent the basic mode of action of second line drugs. In order to investigate further this point similar measurements need to be made with other second line drugs.

The final study shows sulphapyridine and not 5-ASA to be the active component of sulphasalazine. Unfortunately it is also the toxic component and offers little, if any, advantage over sulphasalazine. The fact that an antimicrobial agent produces benefit in rheumatoid arthritis allows us, once more, to consider an infectious aetiology of the disease. Further studies of antibiotics, both absorbable and non-absorbable, may be of use in further investigating this problem.

Appendix I

Functional Index

In Studies 1 and 2 functional index was assessed using the following administered questionnaire. Three points are scored for a "yes", one for a "no" and 2 for a "sometimes". The maximum possible score is 90 (147).

NAME: HOSPITAL NUMBER:

ADDRESS:

AGE:

We are interested to know how you are managing at home with normal daily activities without the help of aids or appliances:

Please put a tick in answer to each of these questions "yes", "no" or "sometimes".

MOBILITY	YES	NO	SOMETIMES
(1) I am able to walk about out of doors.			
(2) I can manage any steps and stairs I have at home.			
(3) I can use public transport.			
TRANSFER			
(4) I can get in and out of bed.			
(5) I can take a bath.			
(6) I can get on and off the toilet.			
PERSONAL CARE			
(7) I am able to put on my own make up.			
(8) I can manage to wipe myself after using the toilet.			

PERSONAL CARE (CONT) YES NO SOMETIMES

(9) I can manage to dress and undress my top half.

(10) I can manage to dress and undress my lower half.

(11) I can manage to brush and comb my hair.

(12) I can manage to do up and undo fastenings on my clothing.

EATING

(13) I can manage to cut my food up.

HOUSEHOLD AND KITCHEN

(14) I can turn my taps at home.

(15) I can manage to prepare vegetables.

(16) I can manage to unscrew jars and bottles.

(17) I can manage to lift saucepans.

(18) I can manage to use the top of the cooker.

(19) I can manage to use the oven.

(20) I can manage to open tins.

(21) I can manage to open packets (eg bacon or cheese).

(22) I can manage washing clothes.

(23) I can manage ironing clothes.

HOUSEHOLD AND KITCHEN (CONT) YES NO SOMETIMES

(24) I can pick up objects from the floor.

(25) I can grip electric plugs.

(26) I can manage my front door key.

OTHER ACTIVITIES

(27) I can write or type a letter.

(28) I can use scissors.

(29) I can open my purse and handle change.

(30) In spite of my arthritis I can visit my friends.

ANYTHING YOU WOULD LIKE TO ADD?

Appendix 2

Statistical Tests

Statistics used are mainly non-parametric and all tests are two-tailed. A significance level of $p < 0.05$ is regarded as significant. Statistical analysis was carried out either manually using standard methods (148) or by computer using the Statistical Package for the Social Sciences programme (SPSS) (223) on the Glasgow University Main Frame Computer. Formulae used in statistical analysis are as follows:-

(1) Wilcoxon matched-pairs signed-rank test:-

This test is used when comparing paired data at different time points, eg, ESR at week 0 and week 24.

It is expressed as:-

$$Z = \frac{T - \frac{(n + 1)}{4}}{\sqrt{\frac{n\ (n + 1)\ (2n + 1)}{24}}}$$

where n = number of pairs.

T = sum of the ranks with the less frequent sign.

Z can be converted to a p value using standard statistical tables.

(2) Mann-Whitney U test.

This is used to compare two sets of unrelated data, e.g. initial ESR in patients randomly allocated to 1.5g SASP with initial ESR in patients randomly allocated to 3g SASP. It is expressed as:-

$$U = n_1 n_2 + \frac{n_1 (n_1+1)}{2} - R_1$$

where n_1 = number in the smaller group.

n_2 = number in the larger group.

R_1 = sum of the ranks in the smaller group.

When $n_2 \leq 20$ the p value can be calculated directly from a statistical table. When $n_2 > 20$ then Z is calculated as follows:-

$$Z = \frac{U - \frac{n_1 n_2}{2}}{\sqrt{\frac{(n_1)\ (n_2)\ (n_1 + n_2 + 1)}{12}}}$$

The Z value can then be converted to a p value using statistical tables.

(3) Kruskal-Wallis one-way analysis of variance. This test is used in a similar situation as the Mann-Whitney U test when there are > 2 groups, eg, when comparing the initial ESR's in the patients randomly allocated to sulphasalazine, sodium

aurothiomalate or placebo. It is expressed as:-

$$H = \frac{12}{n\ (n+1)} \sum_{j=1}^{k} \frac{Rj^2}{nj} - 3\ (n+1)$$

where k = the number of groups.

nj = the number of cases in the j^{th} sample.

n = total number in all groups ($=\sum nj$).

Rj = the score of ranks in the j^{th} sample.

the p value can then be read from standard statistical tables.

(4) Spearman-Rank Correlation Coefficient.

All correlations were carried out using this test, eg. the correlation of the change in ESR with the change in plasma thiol concentration. It is expressed as:

$$r_s = 1 - \frac{6 \sum_{i=1}^{n} di^2}{n^3 - n}$$

where di = the difference between the ranks of two variables in an individual.

n = the number of individuals.

The r_s (the Spearman-Rank Correlation Coefficient), can then be converted to a p value using statistical tables.

(5) Chi Squared.

This test is used to compare two or more groups with reference to the proportions of individuals falling into a particular category or categories. eg, the number of patients $\geq$ 65 years old who discontinued therapy with the number of patients < 65 who discontinued therapy. It is expressed as:-

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

where O = the observed number of individual cases to fall within a particular category.

E = the number of individual cases expected to fall within a particular category.

The χ^2 value can then be converted to a p value using statistical tables.

(6) Life table analysis:- Log rank test (224).

This test is used to compare the life table curves of two treatment groups e.g. the number of patients discontinuing sulphasalazine because of inefficacy with the number discontinuing placebo for this reason. The log rank test involves counting the number of drop outs observed in each group (O) and comparing it with the extent of exposure to the risk of drop out in that group (E). The extent of exposure for each treatment group can be calculated at the time each drop out occurs using the formula:-

Extent of exposure = $\frac{e\ a}{r}$

Where e = the number of drop outs occurring in all groups at the particular time point.

a = the number at risk in the individual treatment group at that particular time.

r = the total number at risk in all treatment groups at that particular time.

The overall extent or risk of exposure to drop out (E) over the entire study period can then be calculated for each treatment group by summating the individual extents of exposure at each time point, ie, $E = \sum \frac{e\ a}{r}$

This value of E can then be inserted into the equation:-

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

The χ^2 value can then be converted to a p value using standard statistical tables.

(7) Power calculations.

The power of a clinical trial is the probability that if the experimental treatment produces a real difference that this difference will be demonstrated and will reach a predetermined level of statistical significance. The arithmetic complement of the power is the β error which represents the probability of a "false negative" result, ie our failure to reject a null hypothesis which is in fact

false. The calculation of power can only be carried out on parametric data and for this reason when I made a power calculation comparing the sulphasalazine treated group with the placebo group and the sodium aurothiomalate group, I assumed normal distribution and thus the power of the test calculated is merely on approximation although any differences will be slight.

As an example the calculation of the power of the test to show a significant difference between the change in ESR between sulphasalazine and placebo groups is shown below.

Placebo	-	mean change in ESR	= - 0.765
n = 17		standard deviation	= 19.515
Sulphasalazine	-	mean change in ESR	= - 29.667
n = 18		standard deviation	= 27.808
Sodium aurothiomalate	-	mean change in ESR	= - 37.267
n = 15		standard deviation	= 32.010

The combined estimate of the standard deviation (θ) is therefore calculated as:-

$$\theta = \sqrt{\frac{(17-1)\ 19.515^2 + (18-1)\ 27.808^2 + (15-1)\ 32.010^2}{17 + 18 + 15 - 3}}$$

$$= 26.7$$

$$\theta^2 = 713$$

We know that the distribution of the ESR difference in the placebo group can be expressed as

$\bar{x}p \sim N\ (-\ 0.8,\ 713/17)$

The null hypothesis is:-

$Ho : \mu p - \mu s = 0$

where:- μs represents the mean ESR difference in the sulphasalazine group and

μp the mean ESR difference in the placebo group.

If Ho is true then:-

$\bar{x}s \sim N\ (-\ 0.8,\ 713/18)$

$\Rightarrow \bar{x}p - \bar{x}s \sim N\ (0,\ 713/17 + 713/18)$

$= N\ (0,\ 81.6)$

Hence we must calculate the critical value (C) which we would have to obtain before we could reject Ho under these circumstances at the 5% level.

Let $y = \bar{x}p - \bar{x}s$

$\Rightarrow y \quad N\ (0,\ 81.6)$

We want to find C such that:-

$P\ (Y < C) = 0.975$

$\Rightarrow p \left[z < \frac{c - 0}{\sqrt{81.6}} \right] = 0.975$ where Z = normal deviate

$\Rightarrow \frac{C}{\sqrt{81.6}} = 1.96$ (from area under curve standard tables)

$\Rightarrow \quad C = 17.71$

The alternative hypothesis is:

$Hi : \mu p - \mu s = -\ 0.8 - (-\ 29.7) = 28.9$

Let $y \sim n\ (28.9,\ 81.6)$

The power of the test is

Pr (reject Ho when Ho is false)

$= P\ (y < -17.71) + P\ (y > 17.71)$

$= P\ (y < -17.71) + 1 - P\ (y < 17.71)$

$$= 1 + P\left[z < -\frac{17.71-28.9}{\sqrt{81.6}}\right] - P\left[z < \frac{17.71-28.9}{\sqrt{81.6}}\right]$$

$= 1 + \Phi\ (-5.160) - \Phi\ (-1.239)$

$1 + 0 - 0.107$ (from area under curve standard tables)

$= 0.893$

ie, when comparing the change in ESR in the placebo group with the change in ESR in the sulphasalazine group using parametric statistics, the likelihood we will demonstrate a difference at the 5% level if a true difference exists is 89%.

Appendix 3

Case reports of serious toxic events.

Section 1	Leucopenia	(1)
		(2)
		(3)
		(4)
		(5)
		(6)
		(7)
Section 2	Hepatitis	(1)
		(2)
Section 3	Thrombocytopenia	
Section 4	Acute dyspnoea	

Section 1

Leucopenia

Case 1

JD, a 64 year old housewife with a 1 year history of erosive seropositive rheumatoid arthritis was commenced on sulphasalazine 0.5g increasing by 0.5g/day increments each week to 1.5g/day. She had received no previous 2nd line drugs.

Concurrent medications were atenolol and bendrofluazide for her hypertension plus indomethacin for her rheumatoid. She was noted to be a slow acetylator. At the commencement of therapy WBC = 8.1 x 10^9/l; Hb = 10.0g/dl; plats = 664 x 10^9/l; ANA was negative. Five weeks after commencement of sulphasalazine (dose 1.5g/day) WBC had fallen to 3.8 x 10^9/l. Sulphasalazine was continued and 2 weeks later WBC had fallen to 1.1 x 10^9/l (10% polymorphs). At this time the patient had mouth ulceration. She was admitted to hospital, all medications were stopped and she was treated by reverse barrier nursing and intravenous gentamicin. She subsequently developed a pyrexia of 38.4^{o}C. Repeated cultures of urine, faeces, blood, mouth swabs and vaginal swabs failed to grow any pathogens. Bone marrow examination showed depressed granulocytopoeisis with only occasional myelocytes, no segmented forms were seen. ANA was negative. White blood count gradually rose to 3.5 x 10^9/l over the next 3 weeks. She then developed leucocytosis of 20.4 x 10^9/l (neutrophilia) but again no organisms were grown. Clinically she improved and was discharged from hospital 6 weeks after admission. HLA haplotype showed her to be A1, 3, B7, 8, Dr3, 4. Eight months later she was clinically well on

fenclofenac and sodium aurothiomalate.

Case 2

AC, a 70 year old female with a 2 year history of seropositive erosive rheumatoid arthritis was commenced on sulphasalazine 0.5g/day gradually increasing to 3g/day. She had received no previous 2nd line therapy. Concurrent therapy consisted of indomethacin and cimetidine. At the commencement of therapy total WBC was 9.8 x 10^9/l and she was noted to be a fast acetylator. Nine weeks later (on 3g/day) she presented with a WBC = 0.5 x 10^9/l with a profound neutropenia, platelet count and haemoglobin were within normal limits. Three weeks before the acute presentation WBC was 7.3 x 10^9/l. She was treated conservatively with reverse barrier nursing and antibiotics and within 5 days of stopping sulphasalazine WBC rose to 10.9 x 10^9/l. As her WBC rose she developed a purulent tonsillar and peritonsillar discharge. Bone marrow film on the day following admission showed arrest at the myelocyte stage with a few metamyelocytes present. Blood cultures were consistently negative and sputum culture revealed a mixed growth of staphylococcus, pneumococcus and streptococcus. No viral aetiology of the neutropenia was demonstrated and ANA was consistently negative. Eighteen months later she remained well on d-penicillamine.

Case 3

JF, a 54 year old female with a 20 year history of active seropositive erosive rheumatoid arthritis, previously well controlled on first line drugs, was commenced on sulphasalazine 0.5g/day increasing by weekly

increments of 0.5g/day to reach 3g/day at 6 weeks. At initiation of treatment haematological parameters were as follows: Hb = 10.3g/dl, plats = 385 x 10^9/l, WBC = 6.6 x 10^9/l, ESR = 50mm/hr and ANA was negative. At this time serum B12 was noted to be low at 82pg/ml, serum folate 1.6ng/ml and red cell folate 80ng/ml (lower limit of normal = 150pg/ml, 2.2ng/ml and 106ng/ml respectively). She was noted to be a fast acetylator.

Other medications consisted of fenclofenac 600mg b.d., dihydrocodeine 60mg b.d., ferrous sulphate 200mg t.i.d. After 8 weeks of treatment WBC fell to 3.8 x 10^9/l (54% polymorphs, 40% lymphocytes, 6% monocytes). Sulphasalazine was continued and 2 weeks later WBC had fallen further to 3.3 x 10^9/l (61% polymorphs, 30% lymphocytes, 7% monocytes, 2% eosinophils). Sulphasalazine was stopped 1 week later when the patient was reviewed although by this time the WBC had risen to 4.0 x 10^9/l. Three weeks after stopping sulphasalazine WBC was 5.2 x 10^9/l. Schilling test showed normal absorption of B12 without the addition of intrinsic factor. Unfortunately ANA titres and reticulocyte counts were not carried out during the period of leucopenia. Two months following the cessation of sulphasalazine the patient's disease activity necessitated another 2nd line agent and she was commenced on hydroxychloroquine. There has been no recurrence of her leucopenia.

Case 4

AF, a 41 year old female with a 2 year history of seropositive erosive rheumatoid arthritis was commenced on sulphasalazine in a dose of 0.5g/day to be increased in weekly increments of 0.5g/day to 3g/day.

Her only other medication was fenclofenac in a dose of up to 1200mg/day. At commencement of therapy WBC = 4.8 x 10^9/l, Hb = 10.7g/dl, plats = 451 x 10^9/l, ESR = 80mm/hr, ANA = 1/256 with normal DNA binding, serum B12 and folate levels were within normal limits. Ten weeks after commencing treatment (dose = 3g/day) she complained of perioral paraesthesia and at the same visit WBC was noted to be 2.5 x 10^9/l (74% polymorphs, 20% lymphocytes, 6% monocytes) with a Hb of 11.2g/dl and plats of 295 x 10^9/l, ESR 60mm/hr. ANA titre at this time was 1/1000 with normal DNA binding. Apart from a rather "bizarre personality" she had never exhibited any clinical features of SLE and she had no evidence of Sjogren's syndrome. Within 1 week of stopping treatment WBC rose to 4.6 x 10^9/l. Three months later she was receiving only intermittent fenclofenac therapy and had shown no recurrence of her leucopenia.

Case 5

RH, a 67 year old female with a long history of seropositive erosive rheumatoid arthritis was commenced on sulphasalazine 0.5g/day increasing gradually to 3g/day. Previous second line treatment consisted of sodium aurothiomalate injections which were stopped because of lack of efficacy. Concurrent medication consisted of ketoprofen. At commencement of therapy WBC was 6.5 x 10^9/l and she was noted to be a fast acetylator. Within 2 weeks of starting treatment WBC had fallen to 2.2 x 10^9/l (26% neutrophils). Sulphasalazine was discontinued and WBC rose within 3 weeks to 4.9 x 10^9/l. Bone marrow examination in the recovery stage was normal and ANA was negative. The patient remained clinically well throughout.

Case 6

EC, a 43 year old female with a 3 year history of seropositive rheumatoid arthritis who was receiving indomethacin 150mg/day plus naproxen 1000mg/day in addition to diazepam 4mg nocte was commenced on sulphasalazine 0.5g/day eventually aiming at 1.5g/day. At the time of commencing sulphasalazine WBC was 3.4 x 10^9/l (polymorphs 79%, lymphocytes 13%, monocytes 8%) with a relative lymphopenia. Haemoglobin was 12.3g/dl and platelets 319 x 10^9/l. Rheumatoid factor titre was strongly positive with a Rose Waaler titre of 1/1024 and ANA was positive in a titre of 1/64 with a membranous pattern. She was a slow acetylator. White count was monitored fortnightly and 4 weeks after commencing sulphasalazine WBC had fallen to 2.6 x 10^9/l (38% polymorphs, 46% lymphocytes, 10% monocytes, 6% basophils). Sulphasalazine was stopped on this occasion but recommenced in a dose of 1.5g/day 2 weeks later when WBC had risen to 4.0 x 10^9/l. Within 2 weeks, however, WBC had fallen again to 2.5 x 10^9/l (44% polymorphs, 41% lymphocytes, 12% monocytes, 2% eosinophils, 1% basophils) and sulphasalazine was stopped. There was no change in haemoglobin or platelet count over this period. Six months later while on hydroxychloroquine WBC was 4.8 x 10^9/l.

Case 7

AN, a 41 year old lady with a 31 year history of seropositive rheumatoid arthritis who was receiving indomethacin 200mg/day was commenced on sulphasalazine 0.5g/day aiming at an eventual dose of 1.5g/day. At the time of commencing treatment WBC was 5.0 x 10^9/l, haemoglobin of 10.9g/dl and platelet count 441 x 10^9/l. She had

previously received penicillamine, auranofin and chlorambucil but had stopped the latter 3 months previously because of leucopenia of 3.2 x 10^9/l. Auranofin had been stopped because of proteinuria and penicillamine because of inefficacy. On starting sulphasalazine ANA was 1/256 with normal DNA binding and serum B12 was low at 115pg/ml. She was a slow acetylator. WBC was monitored weekly and after 8 weeks had fallen to 3.8 x 10^9/l. Sulphasalazine was discontinued and following this WBC rose to 4.7 x 10^9/l and 6 months later she was doing well on hydroxychloroquine with WBC 5.9 x 10^9/l. Schilling test showed normal absorption of Vitamin B12.

Information on the 7 leucopenia patients is summarised on Table LVIII.

Section 2

Hepatitis

Case 1

AK, a 69 year old female with a 2 year history of seropositive erosive rheumatoid arthritis and known Paget's disease of bone was commenced on sulphasalazine 0.5g/day increasing by weekly increments of 0.5g/day to 3g/day. Other medications consisted of indomethacin 50mg t.i.d. At commencement of therapy serum aspartate transaminase (AST) and alanine transaminase (ALT) were within normal range at 21u/l and 13u/l respectively. Serum bilirubin level was normal at 7umol/l and alkaline phosphatase was raised at 880u/l (this had remained constant since presentation 18 months previously and had been shown by heat inactivation studies to be of bone origin). After 5 weeks treatment she developed nausea (dose 2.5g/day) and discontinued treatment.

Patient	Age (yrs)	Disease Duration (yrs)	Acetylator Phenotype	Pre-treatment ANA (DNA binding)	Allocated dose (g/day)	Pre-treatment WBC (% polys)	Week of discovery of leucopenia	Dose at time of leucopenia (g/day)	
JD	64	1	S	-ve	1.5	8.1 x 10^9/l	5	1.5	Cont/.
AC	70	2	F	-ve	3.0	9.8 x 10^9/l	9	3.0	Cont/.
JF	54	20	F	-ve	3.0	6.6 x 10^9/l	8	3.0	Cont/.
AF	41	2	F	1/256 (normal)	3.0	4.8 x 10^9/l	10	3.0	Cont/.
RH	67	30+	F	-	3.0	6.5 x 10^9/l	2	0.5	Cont/.
EC	43	3	S	1/64	1.5	3.4 x 10^9/l (79%) (13% lymphos)	4	1.5	Cont/.
AN	41	31	S	1/256 (normal)	1.5	5.0 x 10^9/l	8	1.5	Cont/.

Table LVIII Pattern of leucopenia in sulphasalazine treated patients. Cont/.

Patient	Trough WBC (% polymorphs)	Marrow	ANA (DNA binding)	Concurrent medications	Clinical course	Outcome
JD	1.1 x 10^9/l (< 10%)	Depressed granulocyto-poiesis Occasional myelocytes	-ve	Atenolol Bendrofluazide Indomethacin	Pyrexia Mouth ulcers Negative cultures. Antibiotic.	WBC recovery after 3 weeks
AC	0.5 x 10^9/l	Arrest at myelocyte stage Few metamyelocytes	-ve	Indomethacin Cimetidine	Staph.Aureus Pneumonoccus Streptococcus from sputum. Antibiotics.	Recovery of WBC within 5 days
JF	3.3 x 10^9/l (61%)	-	-	Fenclofenac Dihydrocodeine Fe SO_4	Asymptomatic	WBC recovered after 3 weeks
AF	2.5 x 10^9/l (74%) (20% lympho-cytes)	-	1/1000 (normal)	Fenclofenac	Asymptomatic	WBC recovered after 1 week

Table LVIII (Cont) Pattern of leucopenia in sulphasalazine treated patients.

Patient	Trough WBC (% polymorphs)	Marrow	ANA (DNA binding)	Concurrent medications	Clinical course	Outcome
RH	2.2 x 10^9/l (26%)	Normal (during recovery stage)	-ve	Ketoprofen	Asymptomatic	WBC recovered after 3 weeks
EC	2.6 x 10^9/l (38%)	-	-	Indomethacin Naproxen Diazepam	Asymptomatic Recurred on on re-challenge	Leucopenia recovered after 2 weeks
AN	3.8 x 10^9/l	-	-	Indomethacin	Asymptomatic	Rapid recovery

Table LVIII (Cont) Pattern of leucopenia in sulphasalazine treated patients.

Routine liver function tests checked 3 days later revealed a slight increase in AST and ALT to 49u/l and 39u/l respectively with a marked rise in alkaline phosphatase to 1210u/l. Bilirubin remained normal at 6u/l. Two weeks later a further rise is AST, ALT and alkaline phosphatase to levels of 79u/l, 99u/l and 2000u/l respectively had occurred but bilirubin remained normal. Within 2 months levels had all returned to pre-treatment values. At no time was she clinically jaundiced and apart from nausea had no symptoms attributable to liver disease. Other investigations showed negative ANA, negative hepatitis B surface antigen, negative antimitochondrial and anti-smooth muscle antibody titres and titres against both Epstein-Barr and cytomegalovirus were less than 1/16.

One year later LFTs remained normal on indomethacin and fenclofenac.

Case 2

A 50 year old female with a 22 year history of erosive seropositive rheumatoid arthritis was commenced on sulphasalazine 0.5g/day increasing to 3g/day (fast acetylator). Previous 2nd line drugs had consisted of chloroquine and sodium aurothiomalate. Other drugs at the time of commencing sulphasalazine were ketoprofen and indomethacin. At the beginning of sulphasalazine treatment liver function tests were normal (AST 26u/l, ALT 29u/l, alkaline phosphatase 107u/l, bilirubin 4u/l, γGT 17u/l, albumin 40g/l), ANA was positive in a titre of 1/64 with normal DNA binding, serum IgA, IgG and IgM levels were within normal limits. Eight weeks later repeat liver function tests remained normal. After 12 weeks treatment, however, transaminases had risen dramatically (AST 609u/l, ALT 1449u/l) with a raised γGT

(211u/l). Alkaline phosphatase and bilirubin remained within normal limits. Sulphasalazine was stopped. Shortly after this she developed severe upper abdominal discomfort with nausea. Within 3 weeks of stopping sulphasalazine, liver biochemistry had largely returned to normal except for a γGT of 68u/l. Other investigations at the time of the maximum rise in liver enzymes revealed negative titres for Epstein-Barr and cytomegalovirus, hepatitis B surface antigen was negative, ANA was positive at 1/64, anti-mitochondrial and anti-smooth muscle antibodies were negative. Liver biopsy was reported as showing acute hepatitis with perivenular confluent necrosis and foci of liver cell necrosis within the parenchyma suggestive of possible drug toxicity.

Section 3

Thrombocytopenia

A 73 year old female with a 30 year history of seronegative erosive rheumatoid arthritis was commenced on sulphasalazine 0.5g/day with the aim of gradually increasing the dose to 3g/day (Study 4). In addition she was receiving naproxen, paracetamol and ferrous sulphate. Sodium aurothiomalate therapy had been discontinued 1 month previously because of leucopenia (3.6 x 10^9/l; sternal marrow aspirate 3 weeks later was normal). On commencement of sulphasalazine haematology showed WBC = 4.6 x 10^9/l, Hb = 10.4g/dl, platelets = 291 x 10^9/l. ANA had been positive on 2 previous occasions in a titre of 1/16. She was started on sulphasalazine but stopped after 4 weeks because of upper gastrointestinal symptoms. Three weeks later she presented with a history of easy bruising and 1 episode of epistaxis. On examination

she had a petechial rash on her lower legs with echymoses on her upper and lower limbs. Two small fundal haemorrhages were seen. Spleen was not palpable. Platelet count was 30 x 10^9/l and the following day was < 5 x 10^9/l (10 days previously platelets = 163 x 10^9/l). WBC = 3.5 x 10^9/l. Clotting factors were normal, no circulating platelet specific antibodies were demonstrable, sternal marrow aspiration suggested peripheral platelet destruction. ANA was raised at 1/1000 (homogeneous) and DNA binding 6.5% (normal). Radioisotope spleen scan showed spleen size to be upper limit of normal. She was commenced on prednisolone 40mg/day and by 6 days her platelet count had risen to 120 x 10^9/l. Steroids were slowly reduced and when seen 6 months later was receiving 8.5mg prednisolone/day and was well with a platelet count of 375 x 10^9/l.

Section 4

Acute Dyspnoea

A 37 year old female (AM) with an 18 year history of seropositive erosive rheumatoid arthritis was commenced on sulphasalazine in a dose of 0.5g/day to be increased weekly by 0.5g/day increments to 3g/day. She was also receiving indomethacin 250mg/day. After 3 weeks (dose 1.5g/day) she developed dyspnoea accompanied by a dry cough and 'flu like symptoms (fever, myalgia and nausea). This settled on stopping sulphasalazine but when it was reintroduced 1 week later symptoms recurred and once again disappeared on stopping sulphasalazine. Unfortunately these episodes were managed by her general practitioner and no chest radiograph or eosinophil count was available from this time. She has since been commenced on d-penicillamine and remains well.

REFERENCES

1. Pullar T, Hunter JA, Capell HA. Second line therapy in rheumatoid arthritis - a four year prospective study. Clinical Rheumatology 1985; 4: 133-142.

2. Svartz N. Salazopyrin, a new sulfanilamide preparation. Acta Medica Scandanavica 1942; 110: 577-598.

3. Svartz N. The treatment of rheumatic polyarthritis with acid azo compounds. Rheumatism 1948; 4: 56-60.

4. Sinclair RJG, Duthie JJR. Salazopyrin in the treatment of rheumatoid arthritis. Ann Rheum Dis 1949; 8: 226-231.

5. Kuzell WK, Gardner GM. Salicylazosulfapyridine (salazopyrin or azopyrin) in rheumatoid arthritis and experimental polyarthritis. California Medicine 1950; 73: 476-480.

6. The National Co-operative Crohn's Disease Study. Gastroenterology 1979; 77: 825-944.

7. Dissanayake A S, Truelove SC. A controlled therapeutic trial of long-term maintenance treatment of ulcerative colitis with sulphasalazine. Gut 1973; 14: 923-926.

8. McConkey B, Amos RS, Butler RA et al. Salazopyrin in rheumatoid arthritis. Agents and Actions 1978; 8: 438-441.

9. McConkey B, Amos RS, Durham et al. Sulphasalazine in rheumatoid arthritis. Br Med J 1980; i: 442-444.

10. Schröder H, Campbell DES. Absorption, metabolism and excretion of salicylazosulfapyridine in man. Clin Pharmac Therap 1972; 13: 539-555.

11. Das KH, Eastwood MA. Effect of iron and calcium on salicylazosulphapyridine metabolism. Scott Med J 1973; 18: 45-50.

12. Azad Khan AK, Truelove SC, Aronson JK. The disposition and metabolism of sulphasalazine in man. Br J Clin Pharmac 1982; 13: 523-528.

13. Schröder H, Lewkonia RH, Evans DAP. Metabolism of salicylazosulfapyridine in healthy subjects and in patients with ulcerative colitis. Clin Pharmac Ther 1973; 14: 802-809.

14. Das KM, Eastwood MA, McManus JPA, Sircus W. The role of the colon in the metabolism of salicylazosulphapyridine. Scand J Gastroenterol 1974; 9: 137-141.

15. Peppercorn MA, Goldman P. The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. J Pharmacol Exper Ther 1972; 181: 555-562.

16. Van Hees PAM. Clinical and pharmacological aspects of sulphasalazine. MD Thesis 1979. Kathalieke Universiteit te Nijmegen.

17. Das KM, Eastwood MA, McManus JPA, Sircus W. The metabolism of salicylazosulfapyridine in ulcerative colitis. Gut 1973; 14: 631-641.

18. Das KM, Dubin R. Clinical pharmacokinetics of sulphasalazine. Clinical Pharmacokinetics 1976; 1: 406-425.

19. Das KM, Eastwood MA. Acetylation polymorphism of sulfapyridine in patients with ulcerative colitis and Crohn's disease. Clin Pharmac and Therap 1975; 18: 514-520.

20. Evans DAP, White TA. Human acetylation polymorphism. J Lab Clin Med 1964; 63: 394-403.

21. Fischer C, Klotz U. Is plasma level monitoring of sulfasalazine indicated in the treatment of Crohn's disease or ulcerative colitis? Therapeutic Drug Monitoring 1980; 2: 153-158.

22. Schröder H, Evans DAP. Acetylator phenotypes and adverse effects of sulphasalazine in healthy subjects. Gut 1972; 13: 278-284.

23. Azad Khan AK, Howes DT, Piris J, Truelove SC. Optimum dose of sulphasalazine for maintenance treatment in ulcerative colitis. Gut 1980; 21: 232-240.

24. Azad Khan AK, Truelove SC. Circulating levels of sulphasalazine and its metabolites and their relation to the clinical efficacy of the drug in ulcerative colitis. Gut 1980; 21: 706-710.

25. Das KM, Eastwood MA, McManus JPA, Sircus W. Adverse reactions during salicylazosulfapyridine therapy and the relation with drug metabolism and acetylator phenotype. N Engl J Med 1973; 289: 491-495.

26. Anon. Salazopyrin and some side issues. Lancet 1943; 1: 181-182.

27. Svartz N. Treatment of ulcerative clitis with salazopyrin. International Surgery 1968; 50: 421-427.

28. Rubinstein A, Das KM, Mohamed J, Murphy RA. Comparative analysis of systemic immunological parameters in ulcerative colitis and ideopathic proctitis: effects of sulfasalazine in vivo and in vitro. Clin Immunol 1978; 33: 217-224.

29. Thayer WR, Charland C, Field C. Salazopyrin and its effect on lymphocyte subpopulation. Gastroenterology 1976; 70: 942.

30. Gibson PR, Jewell DP. Sulphasalazine and derivatives, natural killer activity and ulcerative colitis. Clin Sci 1985; 69: 177-184.

31. Stenson WF, Labos E. Sulphasalazine inhibits the synthesis of chemotactic lipids by neutrophils. J Clin Invest 1982; 69: 494-497.

32. Stenson WF, Mehta J, Spilberg I. Sulphasalazine inhibition of binding of N-formyl-methionyl-leucyl-phenylalanine (FMLP) to its receptor on human neutrophils. Biochem Pharmac 1984; 35: 407-412.

33. Laursen ML. The influence of salicyl-azo-sulfapyridine on the immune response to antigenic tumour cells inoculated into the coecal lumen of C3H mice. Scand J Gastroenterology 1978; 13: 991-997.

34. Gould SR, Brash AR, Connolly ML, Leonard Jones JE. Studies of prostaglandins and sulphasalazine in ulcerative colitis. Prostaglandins and Medicine 1981; 6: 165-182.

35. Van Hees PAM, Bakker JH, Van Tongersen JHM. Effect of sulphapyridine, 5-aminosalicylic acid and placebo in patients with ideopathic proctitis: a study to determine the active therapeutic moiety of sulphasalazine. Gut 1980; 21: 632-635.

36. Azad Khan AK, Piris J, Truelove SC. An experiment to determine the active therapeutic moiety of sulphasalazine. Lancet 1977; ii: 892-895.

37. Dew MJ, Hughes P, Harries AD et al. Maintenance of remission in ulcerative colitis with oral preparation of 5-aminosalicylic acid. Br Med J 1982; 285: 1012.

38. West B, Lendrum R, Hill MJ, Walker G. Effects of sulphasalazine (Salazopyrin) on faecal flora in patients with inflammatory bowel disease. Gut 1974; 15: 960-965.

39. Birnie GG, McLeod R, Watkinson G. Incidence of sulphasalazine induced male infertility. Gut 1981; 22: 542-455.

40. Toovey S, Hudson E, Hendry MF, Levi AJ. Sulphasalazine and male infertility: reversibility and possible mechanism. Gut 1981; 22: 445-451.

41. Kanner RS, Tedesco FJ, Kalser MH. Azulfidine (sulfasalazine) induced hepatic injury. Am J Dig Dis 1978; 23: 956-958.

42. Chester AC, Diamond LH, Schreiner GE. Hypersensitivity to salicylazosulfapyridine. Renal and hepatic toxic reactions. Arch Intern Med 1978; 138: 1138-1139.

43. Mihas AA, Goldenberg DJ, Slaughter RL. Sulfasalazine toxic reactions. J Am Med Assoc 1978; 239: 2590-2591.

44. Raforth RJ. Systemic granulomatous reaction to salicylsulfapyridine (azulfidine) in a patient with Crohn's disease. Am J Dig Dis 1974; 19: 465-469.

45. Lennard TWJ, Farndon JR. Sulphasalazine hepatotoxicity after 15 years successful treatment for ulcerative colitis. Br Med J 1983; 187: 96.

46. Block MB, Genant HK, Kirsner JB. Pancreatitis as an adverse reaction to salicylazosulfapyridine. N Engl J Med 1980; 282: 580-581.

47. Wallace IW. Neurotoxicity associated with a reaction to sulphasalazine. The Practitioner 1970; 204: 850-851.

48. Jones GR, Malone DNA. Sulphasalazine induced lung disease. Thorax 1972; 27: 713-717.

49. Davies D, McFarlane A. Fibrosing alveolitis and treatment with salicylazosulfapyridine. Gut 1974; 15: 195-198.

50. Thomas P, Seaton A, Edwards J. Respiratory disease due to sulphasalazine. Clinical Allergy 1974; 4: 41-47.

51. Tydd TF, Dyer NH. Sulphasalazine lung. Med J Aust 1976; 1: 570-573.

52. Alarcon-Segovia D, Herskovic T, Dearing WH et al. Lupus erythematosus cell phenomena in patients with chronic ulcerative colitis. Gut 1965; 6: 39-47.

53. Griffiths ID, Kane SP. Sulphasalazine induced lupus in ulcerative colitis. Br Med J 1977; ii: 1188-1189.

54. Crisp AJ, Hoffbrand BI. Sulphasalazine induced systemic lupus erythematosus in a patient with Sjogren's syndrome. J Royal Soc Med 1980; 73: 60-61.

55. Pounder RE, Craven ER, Henthorn JS, Bannatyne JM. Red cell abnormalities associated with sulphasalazine maintenance therapy for ulcerative colitis. Gut 1975; 16: 181-185.

56. Franklin JL, Rosenberg IH. Impaired folic acid absorption in inflammatory bowel disease. Effects of salicylazosulfapyridine (azulfidine). Gastroenterology 1973; 64: 517-525.

57. Selhub J, Dhar GJ, Rosenberg IH. Inhibition of folate enzymes by sulfasalazine. J Clin Investigation 1978; 61: 221-224.

58. Strom J. Toxic epidermal necrolysis (Lyell's Syndrome). Scand J Infect Dis 1969; 1: 209-216.

59. Cameron AJ, Baron JH, Priestly BL. Erythema multiforme, drugs and ulcerative colitis. Br Med J 1966; 2: 1174-1178.

60. Holdsworth CD. Sulphasalazine desensitisation. Br Med J 1981; 282: 110-111.

61. Farr M, Scott DL, Bacon PA. Sulphasalazine desensitisation in rheumatoid arthritis. Br Med J 1982; 284: 118.

62. Willoughby CP, Truelove SC. Ulcerative colitis and pregnancy. Gut 1980; 21: 469-174.

63. Mogadam M, Dobbins WO, Korelitz BI, Ahmed SW. Pregnancy in inflammatory bowel disease: effect of sulphasalazine and corticosteroids on fetal outcome. Gastroenterology 1981; 80: 72-76.

64. Dixon JS, Bird HA, Pickup ME, Wright V. A human model screening system for the detection of specific antirheumatic activity. Seminars in Arthritis and Rheumatism 1982; 12: 185-190.

65. Martin MFR, Dieppe PA, Jones HE. Serum conconavalin A binding in rheumatoid arthritis. Ann Rheum Dis 1982; 41: 133-136.

66. Farr M, Scott DL, Constable TJ et al. Thrombocytosis of active rheumatoid disease. Ann Rheum Dis 1983; 42: 545-549.

67. Cooperating Clinics Committee of the American Rheumatism Association. A controlled trial of cyclophosphamide in rheumatoid arthritis. Engl J Med 1970; 283: 883-889.

68. Sigler JW, Bluhm GB, Duncan H et al. Gold salts in the treatment of rheumatoid arthritis. Ann Int Med 1974; 80: 21-26.

69. Gibson T, Huskisson EC, Wojtulewski JA et al. Evidence that d-penicillamine alters the course of rheumatoid arthritis. Rheumatol Rehabil 1976; 15: 211-215.

70. Luukkainen R, Isovaki H, Kajander A. Effect of gold on the progression of erosions in RA patients. Scand J Rheumatol 1977; 6: 123-127.

71. Lipsky PE, Ziff M. Inhibition of antigen and mitogen induced human lymphocyte proliferation by gold compounds. J Clin Invest 1977; 59: 455-466.

72. Hopkins SJ, Jayson MIV, Van der Zeil P. Inhibition of lymphocyte activation by gold sodium thiomalate. Br J Pharmac 1983; 79: 617-622.

73. Griffin MMC, Steven FS. Inhibition of trypsin and papain by sodium aurothiomalate mediated by exchange reactions. Br J Pharmac 1982; 75: 333-339.

74. Mowat AG. The effect of specific drug therapy on neutrophil chemotaxis in rheumatoid arthritis. In: Dumonde DC and Jasani MK, eds. Recognition of anti-rheumatic drugs. Lancaster: MTP Press Ltd, 1977; 293-299.

75. Jessop JD, Vernon-Roberts B, Morris J. Effects of gold salts and prednisolone on inflammatory cells. I: phagocytic activity of macrophages and polymorphs in inflammatory exudate studies by a skin window technique in rheumatoid and control patients. Ann Rheum Dis 1973; 32: 294-300.

76. Jessop JD, Wilkins M, Young MH. The effect of anti-rheumatic drugs on the phagocytic activity of synovial macrophages in organ culture. Ann Rheum Dis 1982; 41: 632-634.

77. Scheinberg MA, Santos LMB, Finkelstein AE. The effect of auranofin and sodium aurothiomalate on peripheral blood monocytes. J Rheumatol 1982; 9: 366-369.

78. Munthe E, Kass E, Jellum E. Evidence for enhanced radical scavenging prior to drug response in rheumatoid arthritis. In Goroni S, ed. Advances in Inflammation Research Vol. 3, Rheumatoid Arthritis. New York: Raven Press, 1982; 211-218.

79. Highton J, Panayi GS, Shepherd P et al. Fall in immune complex levels during gold treatment of rheumatoid arthritis. Ann Rheum Dis 1981; 40: 575-579.

80. Kirwan JR, Chaput de Saintonge DM, Joyce CRB, Currey HLF. Clinical judgement in rheumatoid arthritis I. Rheumatologists' opinions and the development of "paper patients". Ann Rheum Dis 1983; 42: 644-647.

81. Kirwan JR, Chaput de Saintonge DM, Joyce CRB, Currey HLF. Clinical judgement in rheumatoid arthritis II. Judging "current disease activity" in clinical practice. Ann Rheum Dis 1983; 42: 648-651.

82. Cooperating Clinics of the American Rheumatism Asociation. A three month trial of indomethacin in rheumatoid arthritis with special reference to analysis and interference. Clin Pharmac Ther 1967; 8: 11-18.

83. Ritchie DM, Boyle JA, McInnes J et al. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Q J Med 1968; 37: 393-406.

84. Balint GP, Mavrikakis KE, Lee P, Rooney PJ, Buchanan WW. Standardisation in clinical measurement. In: Dumonde DC and Jasani MK, eds. Recognition of Anti-Rheumatic Drugs. Lancaster: MTP Press Ltd., 1977; 251-265.

85. McCarthy K. Pain. Update Publications Ltd., 1983.

86. Huskisson EC. Assessment for clinical trials. Clinics in Rheumatic Diseases 1976; Vol 2, No 1: 37-49.

87. Scott J, Huskisson EC. Accuracy of subjective measurement made with or without previous scores:- an important source of error in the measurement of subjective states. Ann Rheum Dis 1979; 38: 558-559.

88. Dixon JS, Bird HA. Reproducibility along a 10cm vertical visual analogue scale. Ann Rheum Dis 1981; 40: 87-89.

89. Lee P, Webb J, Anderson JA, Buchanan WW. A method of assessing the therapeutic potential of anti-inflammatory anti-rheumatic drugs in rheumatoid arthritis. Br Med J 1973; 2: 685-686.

90. De Ceulaer K, Dick WC. The clinical evaluation of anti-rheumatic drugs. In: Kelley WN, Harris ED, Ruddy S, Sledge CB, eds. Textbook of Rheumatology. Philadelphia, London, Toronto: Saunders Co., 1981; 729-739.

91. Lansbury J. Quantitation of the activity of rheumatoid arthritis. Am J Med Sci 1956; 232: 300-310.

92. Wright V, Johns RJ. Physical factors concerned in the stiffness of normal and diseased joints. Bull John Hopkins Hosp 1960; 106: 215-231.

93. Scott JT. Morning stiffness in rheumatoid arthritis. Ann Rheum Dis 1960; 19: 361-368.

94. Wright V. Some observations in diurnal variation of grip. Clin Sci 1959; 18: 17-21.

95. Webb J, Downie WW, Dick WC, Lee P. Evaluation of digital joint circumference measurements in rheumatoid arthritis. Scand J Rheumatol 1983; 2: 127-131.

96. Collins AJ, Ring EFJ, Cash JA, Bacon PA. Quantitation of thermography in arthritis using multiisothermal analysis. Ann Rheum Dis 1974; 33: 113-116.

97. Bacon PA, Collins AJ, Ring FJ, Cash JA. Thermography in the assessment of inflammatory arthritis. Clinics in Rheumatic Dis 1986; Vol 2, No 3: 51-65.

98. Fraser SM, Land DV, Sturrock RD. Microwave thermography - a new technique for the objective assessment of inflammation. Br J Rheumatol 1984; 23: 131.

99. Dick WC, Grennan DM. Radioisotopes in the study of normal and inflamed joints. Clinics in Rheumatic Dis 1976; Vol 2, No 1: 67-76.

100. Haajata M. Evaluation of activity of rheumatoid arthritis. Scand J Rheumatol 1975; Suppl 7; 4: 5-54.

101. Dick WC, Whaley K, St Onge RA et al. Clinical studies on inflammation in human knee joints: Xenon (^{133}Xe) clearances correlated with clinical assessment in various arthritides and studies on the effect of intra-articularly administered hydrocortisone in rheumatoid arthritis. Clin Sci 1970; 38: 123-133.

102. Crockson RA, Crockson AP. Relationship of the erythrocyte sedimentation rate to viscosity and plasma proteins in rheumatoid arthritis. Ann Rheum Dis 1974; 33: 53-56.

103. McConkey B, Crockson RA, Crockson AP, Wilkinson AR. The effects of some anti-inflammatory drugs on the acute-phase proteins in rheumatoid arthritis. Q J Med 1973; 168: 785-791.

104. McConkey B, Davies P, Crockson RA et al. Effects of gold, dapsone and prednisone on serum C-reactive protein and haptoglobin and the erythrocyte sedimentation rate in rheumatoid arthritis. Ann Rheum Dis 1979; 38: 141-144.

105. Multicentre Trial Group. Controlled trial of d-penicillamine in severe rheumatoid arthritis. Lancet 1973; i: 275-280.

106. Amos RS, Constable TJ, Crockson RA, Crockson AP, McConkey B. Rheumatoid arthritis: relation of serum C-reactive protein and erythrocyte sedimentation rates to radiographic changes. Br Med J 1977; 1: 195-197.

107. Arthritis and Rheumatism Council Symposium/Workshop. Advances in assessing rheumatoid arthritis. The London Hospital, 1983.

108. Kinsella TD, Fritzler MJ. CRP: an immunoregulatory protein (editorial). J Rheumatol 1980; 7: 272-274.

109. Walsh L, Davies P, McConkey B. Relationship between erythrocyte sedimentation rate and serum C-reactive protein in rheumatoid arthritis. Ann Rheum Dis 1979; 38: 362-363.

110. Mallya RK, de Beer FC, Berry H et al. Correlation of clinical parameters of disease activity in rheumatoid arthritis with serum concentrations of C-reactive protein and erythrocyte sedimentation rate. J Rheumatol 1982; 9: 224-228.

111. Anon. C-reactive protein or ESR? Lancet 1977; ii: 1166.

112. Decker B, McGuckin WF, McKenzie B, Slocumb CH. A study of some acute phase reactants in rheumatic diseases. Arthritis & Rheumatism 1960; 3: 49-53.

113. McCarty DJ. Clinical assessment of arthritis. In: McCarty DJ, ed. Arthritis and allied conditions (Hollander), 9th edition. New York: Lea and Febiger 1979; 131-147.

114. Crockson RA, Crockson AP. Relationship of the erythrocyte sedimentation rate to viscosity and plasma proteins in rheumatoid arthritis. Ann Rheum Dis 1973; 33: 53-56.

115. Pickup ME, Dixon JS, Hallett C, Bird HA, Wright V. Plasma viscosity - a new appraisal of its use as an index of disease activity in rheumatoid arthritis. Ann Rheum Dis 1981; 40: 272-275.

116. Larkin JG, Lowe GDO, Sturrock RD, Forbes CD. The relationship of plasma and serum viscosity to disease activity and smoking habit in rheumatoid arthritis. Brit J Rheumatol 1984; 23: 15-19.

117. Research Subcommittee of the Empire Rheumatism Council. Gold therapy in rheumatoid arthritis. Ann Rheum Dis 1961; 20: 315-334.

118. Williams HJ, Ward JR, Reading JC. Low dose penicillamine therapy in rheumatoid arthritis. Arthritis & Rheumatism 1983; 26: 581-592.

119. Hall ND, Gillan AH. Effects of antirheumatic drugs on protein sulphydryl reactivity of human serum. J Pharm Pharmac 1979; 31: 676-680.

120. Boyles T, Hall M. A yardstick for rheumatoid arthritis. N Eng J Med 1943; 228: 418-421.

121. Duthie J, Thomson M, Weir M, Fletcher W. Medical and social aspects of treatment of rheumatoid arthritis with special reference to factors effecting prognosis. Ann Rheum Dis 1955; 14: 133-148.

122. Mallya RK, Mace BEW. The assessment of disease activity in rheumatoid arthritis using a multivariate analysis. Rheumatol Rehabil 1981; 20: 14-17.

123. Larsen A, Dale K, Eek M. Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiologica (Diagn) 1977; 4: 481-491.

124. Sharp JT, Lidsky MD, Collins LC, Moreland J. Methods of scoring the progression of radiological changes in rheumatoid arthritis. Arthritis & Rheumatism 1971; 14: 706-720.

125. Pullar T, Hunter JA, Capell HA. Does second line therapy affect the radiological progression in rheumatoid arthritis? Ann Rheum Dis 1984; 43: 18-23.

126. Pullar T Capell HA. Is it posible to modify the course of rheumatoid arthritis? Can we answer the question? Ann Rheum Dis 1985; 44: 134-140.

127. Sharp JT. Radiographic evaluation of the course of articular disease. Clinics in Rheumatic Diseases 1983; 9: 541-557.

128. Sharp JT, Lidsky MD, Duffy J. Clinical responses during gold therapy for rheumatoid arthritis. Arthritis & Rheumatism 1982; 25: 540-549.

129. Buckland-Wright JC. X-ray assessment of activity in rheumatoid disease. Br J Rheumatol 1983; 22: 3-10.

130. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. J Am Med Soc 1949; 140: 659-662.

131. Lee P, Jasani MK, Dick WC, Buchanan WW. Evaluation of a functional index in rheumatoid arthritis. Scand J Rheumatol 1973; 2: 71-77.

132. Fries JF, Spitz P, Kraines RG, Halmon HR. Measurement of patient outcome in arthritis. Arthritis & Rheumatism 1980; 23: 137-145.

133. Meenan RF, Gertman PM, Mason JH. Measuring health status in arthritis. Arthritis & Rheumatism 1980; 23: 146-152.

134. Liang MH, Larson MG, Cullen KE, Schwartz JA. Comparative measurement efficiency and sensitivity of five health status instruments for arthritis research. Arthritis & Rheumatism 1985; 28: 542-547.

135. McKenzie AH, Schirbel AL. Chloroquine and hydroxychloroquine in rheumatological therapy. Clinics in Rheumatic Diseases 1980; Vol. 6, No. 3: 545-564.

136. Whitehouse JMA. Cytotoxic drugs for non-neoplastic disease. Brit Med J 1983; 287: 79-80.

137. Wooley PH, Griffin BJ, Panayi GS et al. HLA Dr antigens and toxic reaction to sodium aurothiomalate and d-penicillamine in patients with rheumatoid arthritis. N Engl J Med 1980; 303: 300-302.

138. Emery P, Panayi GS, Huston G et al. D-penicillamine induced toxicity in rheumatoid arthritis: the role of sulphoxidation status and HLA Dr3. J Rheumatol 1984; 11: 626-632.

139. Pullar T, Hunter JA, Capell HA. Gold and penicillamine therapy: is shared care with general practitioners effective and safe? Rheum Rehabil 1982; 21: 139-144.

140. Bird HA, Dixon JS, Pickup ME et al. A biochemical assessment of sulphasalazine in rheumatoid arthritis. J Rheumatol 1982; 9: 36-45.

141. Neumann VC, Grindulis KA, Hubball S, McConkey B, Wright V. Comparison between sulphasalazine and penicillamine in rheumatoid arthritis. Br Med J 1983; 287: 1099-1102.

142. Chattopadhay C, Swinson DR, Stewart I. A double blind trial of sulphasalazine against placebo in rheumatoid arthritis. Combined Heberden, BARR and RSM meeting, Leeds 1983; Abstract No. 23.

143. Grindulis K, McConkey B. Outcome of attempts to treat rheumatoid arthritis with gold, penicillamine, sulphasalazine or dapsone. Ann Rheum Dis 1984; 43: 398-401.

144. Farr M, Tunn E, Crockson AP, Bacon PA. The long term effects of sulphasalazine in the treatment of rheumatoid arthritis and a comparative study with penicillamine. Clinical Rheumatology 1984; 3: 473-482.

145. Bax DE, Amos RS. Sulphasalazine: a safer, effective agent for prolonged control of rheumatoid arthritis. A comparison with sodium aurothiomalate. Ann Rheum Dis 1985; 44: 194-198.

146. Ropes MW, Bennet GA, Cobb S, Jacob R, Jesser RA. 1958 revision of diagnostic criteria for rheumatoid arthritis. Arthritis & Rheum 1959; 2: 16-20.

147. Mitchell WS, Ledingham A, Bradshaw E, Sturrock RD. Assessment of functional status in a rheumatic disease clinic - a pilot study. Annual General Meeting, British Association for Rheumatology and Rehabilitation 1981; abstract book p 15.

148. Siegal S. Non parametric statistics for the behavioural sciences. McGraw-Hill, New York 1956.

149. Lewis D, Capell HA. Is auranofin preferable to sodium aurothiomalate in the management of rheumatoid arthritis? In "Auranofin", Netherlands: Excerpta Medica 1983; 147-154.

150. Teh LG, Madhok R, Capell HA. Does the addition of ketotifen to non-steroidal anti-inflammatory drugs confer any additional benefit in rheumatoid arthritis? Br J Clin Pharm 1984; 17: 157-159.

151. Capell HA, Hunter JA, Rennie JAN, Murdoch RM. Levamisole - a possible alternative to gold and penicillamine in the long term treatment of rheumatoid arthritis? J Rheumatol 1981; 8: 730-740.

152. Fischer C, Klotz U. High performance liquid chromatographic determination of aminosalicylate, sulfapyridine and their metabolites. J Chromatography 1979; 162: 237-243.

153. Shaw PN, Sivner AL, Aarous L, Houston JB. A rapid method for the simultaneous determination of the major metabolites of sulphasalazine in plasma. J Chromatography 1983; 274: 393-397.

154. Anon. Rheumatoid arthritis and the gut. Br Med J 1979; i: 104.

155. Mascarenhas BR, Granda JL, Freyberg RH. Gold metabolism in patients with rheumatoid arthritis treated with gold compounds re-investigated. Arthritis & Rheumatism 1972; 15: 391-402.

156. Gottlieb NL, Smith PM, Smith EM. Pharmacodynamics of ^{197}Au and ^{195}Au labelled aurothiomalate in blood. Arthritis & Rheumatism 1974; 17: 171-183.

157. Rudge SR, Parratt D, Swannell AJ. Free thiomalate levels in patients with rheumatoid arthritis treated with disodium aurothiomalate: relationship to clinical outcome of therapy. Ann Rheum Dis 1984; 43: 698-702.

158. Brooks PM, Miners JD, Smith K, Smith MD, Fearnley I, Birkett DJ. Dose, plasma concentration and response relationship of d-penicillamine in patients with rheumatoid arthritis. J Rheumatol 1984; 11: 772-775.

159. Evans DAP. An improved and simplified method of detecting the acetylator phenotype. J Med Genet 1969; 6: 405-407.

160. Lunde PKM, Frislid K, Hansteen V. Disease and acetylation polymorphism. In: Gibaldi M, Prescott L, eds. Handbook of Clinical Pharmacokinetics. Sydney: Adis Health Science Press, 1983; Section 3: 150-168.

161. Azad Khan AK, Nurazzaman M, Truelove SC. The effect of acetylator phenotype on the metabolism of sulphasalazine in man. J Med Genet 1983; 20: 30-36.

162. Schroder H. Simplified method for determining acetylator phenotype. Br Med J 1972; ii: 506-507.

163. Eidus L, Hodgkin MM. Simplified screening test for phenotyping of isoniazid inactivators. Int J Clin Pharmac 1973; 7: 82-86.

164. Lawson DH, Henry DA, Lowe J, Reavey P, Rennie JAN, Solomon A. Acetylator phenotype in spontaneous SLE and rheumatoid arthritis. Ann Rheum Dis 1979; 38: 171-173.

165. Ellard GA, Gammon PT, Savin JA, Tan RSH. Dapsone acetylation in dermatitis herpetiformis. Br J Dermatology 1974; 90: 441-444.

166. Mucklow JC, Shadforth MF. Acetylator phenotype and the effect of dapsone in rheumatoid arthritis. J Rheumatol 1983; 10: 805-808.

167. Crooks J, O'Malley K, Stevenson IH. Pharmacokinetics in the elderly. In: Gibaldi M, Prescott L, eds. Handbook of Clinical Pharmacokinetics, Sydney: Adis Health Science Press, 1983; Section II: 168-187.

168. Huskisson EC. Benoxaprofen. Eur J Rheumatol Inflamm 1982; 5: 49-50.

169. Hamdy RC, Murnane B, Perera N, Woodcock K, Koch IM. The pharmacokinetics of benoxaprofen in elderly subjects. Eur J Rheumatol Inflamm 1982; 5: 69-75.

170. Taggart HM, Alderdice JM. Fatal cholestatic jaundice in elderly patients taking benoxaprofen. Br Med J 1982; 284: 1372.

171. Goudie BH, Birnie GG, Watkinson G et al. Jaundice associated with the use of benoxaprofen. Lancet 1982; i: 959.

172. Anon. Benoxaprofen. Br Med J 1982; 285: 459-460.

173. Ahnfelt N-O, Modin R, Odell ML, Ohlson U. Determination of salicylazosulphapyridine, sulphapyridine and its metabolites in serum and urine. J Pharm Biomed Anal (In press).

174. Rawlins MD, Davies DM. Adverse drug reactions. Medicine International 1984; 7: 290-294.

175. Workshop on sulphasalazine in rheumatoid arthritis. Br J Rheumatol 1985; 24: 223-224.

176. Rae KJ, Mackay CNN, McNeil CJ et al. Early and late changes in sulphydryl group and copper protein concentrations and activities during drug treatment with aurothiomalate and auranofin. J Rheumatol (In press).

177. Hall ND, Blake DR, Bacon PA. The significance of serum sulphydryl measurements in patients with rheumatoid arthritis treated with d-penicillamine. In: Maini RN,and Berry H, eds. Modulation of Autoimmunity and Disease. New York: Praeger Publishers, 1981; 143-149.

178. Bird HA, Dixon JS, Pickup ME, Lee MR, Wright V. A biochemical comparison of alclofenac and d-penicillamine in rheumatoid arthritis. Ann Rheum Dis 1980; 39: 281-284.

179. Grimaldi MG. Long term cyclophosphamide treatment in rheumatoid patients: effects on serum sulphydryl levels, technetium index, ESR and clinical response. Br J Clin Pharmac 1981; 12: 505-506.

180. Kosower NS, Kosower EM. The glutathione status of cells. International Review of Cytology 1978; 54: 109-160.

181. McNeil CJ, Banford JC, Brown DH, Smith WE. A relationship between thiols and the superoxide ion. Federation of European Biochemical Socs 1981; 133: 175-177.

182. Rowley DA, Halliwell B. Formation of hydroxyl radicals from hydrogen peroxide and iron salts by superoxide and ascorbate dependent mechanisms. Relevance to the pathology of rheumatoid disease. Clin Sci 1983; 64: 649-653.

183. Babior BM, Kipnos RS, Curnutte JT. Biological defence mechanisms. The production by leucocytes of superoxide, a potential bactericidal agent. J Clin Invest 1973; 52: 741-744.

184. Kellogg EW, Fridovich I. Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J Biol Chem 1975; 250: 8812-8817.

185. Kellogg EW, Fridovich I. Liposome oxidation and erythrocyte lysis by enzymatically generated superoxide and hydrogen peroxide. J Biol Chem 1977; 252: 6721-6728.

186. Niwa Y, Myake S, Sakane T, Shingu M, Yokoyama M. Auto-oxidative damage in Behcet's disease - endothelial cell damage following the elevated oxygen radicals generated by stimulated neutrophils. Clin Exp Immunol 1982; 49: 247-255.

187. McCord JM, Wong K, Stokes SH, Petrone WF, English O. Superoxide and inflammation: a mechanism for the anti-inflammatory activity of superoxide dismutase. Acta Physiol Scand 1980; Suppl. 492: 25-30.

188. Brown K, Fridovich I. Superoxide radical and superoxide dismutases: threat and defence. Acta Physiol Scand 1980; Suppl. 492: 9-18.

189. Del Maestro RF, Thaw HH, Bjork J, Planker M, Arfors K-E. Free radicals as mediators of tissue injury. Acta Physiol Scand 1980; Suppl. 492: 43-57.

190. Greenwald RA, Moy WW. Efect of oxygen-derived free radicals on hyaluronic acid. Arthritis & Rheumatism 1980; 23: 455-463.

191. Greenwald RA. Effects of oxygen-derived free radicals on connective tisue macromolecules. Inhibition by copper-penicillamine complex. J Rheumatol 1981; Suppl. 7: 9-13.

192. Bates EJ, Lowther DA, Handley CJ. Oxygen free radicals mediate an inhibition of proteoglycan synthesis in cultured articular cartilage. Ann Rheum Dis 1984; 43: 462-469.

193. Del Maestro RF. An approach to free radicals in medicine and biology. Acta Physiol Scand 1980; Suppl. 492: 153-168.

194. Banford JC, Brown DH, Hazelton RA et al. Serum copper and superoxide dismutase in rheumatoid arthritis. Ann Rheum Dis 1982; 41: 458-462.

195. Banford JC, Brown DH, Hazelton RA et al. Altered thiol status in patients with rheumatoid arthritis. Rheumatol Int 1982; 2: 107-111.

196. Drury PL, Rudge SR, Perret D. Structural requirements for activity of certain "specific" anti-rheumatic drugs: more than a single thiol group? Br J Rheumatol 1984; 23: 100-106.

197. Huck F, de Medicis R, Lussier A, Dupuis G, Federlin P. Reducing property of some slow acting antirheumatic drugs. J Rheumatol 1984; 11: 605-609.

198. Munthe E, Kass E, Jellum E. D-penicillamine induced increase in intracellular gluthathione correlating to clinical response in rheumatoid arthritis. J Rheumatol 1981; Suppl. 7: 14-19.

199. Dixon JS, Pickup ME, Lowe JR, Hallet C, Lee MR, Wright V. Discriminatory indices of response of patients with rheumatoid arthritis treated with d-penicillamine. Ann Rheum Dis 1980; 39: 301-311.

200. Misra HP, Fridovich I. Superoxide dismutase: a photochemical augmentation assay. Arch Biochem Biophys 1977; 181: 308-312.

201. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959; 82: 308-312.

202. Evans PH. Serum sulphydryl changes in rheumatoid coalworker's pneumoconiosis patients treated with d-penicillamine. Proc Royal Soc Med 1977; 70 (Suppl. 3): 95-97.

203. Bjarnason I, Williams P, So A et al. Intestinal permeability and inflammation in rheumatoid arthritis. Lancet 1984; ii: 1171-1173.

204. Jenkins RT, Goodacre RL, Rooney PJ, Hunt RH, Bienstock J. Intestinal permeability in inflammatory disease. Gut 1984; 25: A1166-1167.

205. Smith MD, Gibson RA, Brooks PM. Abnormal bowel permeability in arthritis. Relationship to disease activity and spondylitis. Br J Rheum 1985; 24: 77.

206. Dew MJ, Hughes PJ, Lee MG, Evans BK, Rhodes J. An oral preparation to release drugs in the human colon. Br J Clin Pharmac 1982; 14: 405-408.

207. Dew MJ, Harries AD, Evans BK, Rhodes J. Treatment of ulcerative colitis with oral 5-aminosalicylic acid in patients unable to take sulphasalazine. Lancet 1983; ii: 801.

208. Shea BF. Soluseptasine in rheumatoid arthritis. Br Med J 1943; i: 30.

209. Dew MJ, Harries AD, Evans N, Evans BK, Rhodes J. Maintenance of remission in ulcerative colitis with 5-aminosalicylic acid in high doses by mouth. Br Med J 1983; 287: 23-44.

210. Fraser TN. Gold treatment in rheumatoid arthritis. Ann Rheum Dis 1945; 4: 71-75.

211. Rollo IM. Chemotherapy of parasitic diseases. In: Goodman and Gillman, eds. The pharmacological basis of therapeutics. New York: Macmillan, 1975; 1018-1044.

212. Weinstein C. Antimicrobial agents. In: Goodman and Gillman, eds. The pharmacological basis of therapeutics. New York: Macmillan, 1975; 1201-1223.

213. Anon. The viral aetiology of rheumatoid arthritis. Lancet 1984; i: 772-774.

214. Duthie JJR, Stewart SM, McBride WH. Do diphtheroids cause rheumatoid arthritis? In: Dumonde DC, ed. Infection and immunology in the rheumatic diseases. Oxford: Blackwell Scientific Publications, 1976; 171-173.

215. Taylor-Robinson D, Taylor G. Do mycoplasmas cause rheumatic disease? In: Dumonde DC, ed. Infection and immunology in the rheumatic diseases. Oxford: Blackwell Scientific Publications, 1976; 177-186.

216. Bartlett R, Bisset KA. Isolation of Bacillus Licheniformis Var endoparasiticus from the blood of rheumatoid arthritis patients and normal subjects. J Med Microbiol 1981; 14: 97-105.

217. Wyburn-Mason R. The free-living amoebic causation of rheumatoid and autoimmune diseases. International Medicine 1979; 1: 20-25.

218. Bennett JC. An infectious aetiology of rheumatoid arthritis. Arthritis & Rheumatism 1978; 21: 531-538.

219. Mansson J, Norberg R, Olhagen B, Bjorklund NE. Arthritis in pigs induced by dietary factors. Clin Exp Immunol 1971; 9: 677-693.

220. Olhagen B, Mansson I. Intestinal clostridium perfringens in rheumatoid arthritis and allied conditions. Acta Med Scand 1968; 184: 395-402.

221. Sapico FL, Emori H, Smith LDS, Bluestone R, Finegold SM. Absence of relationship of fecal clostridium perfringens to rheumatoid arthritis. J Infect Dis 1973; 128: 559-562.

222. Mowat AG. Anaemia in rheumatoid arthritis. In: Hill AGS, ed. Modern trends in rheumatology 2. London: Butterworths, 1971; 106-116.

223. Bent DH. Statistical Package for the Social Sciences. Second Edition, McGraw-Hill, New York, 1975.

224. Peto R, Pike MC, Armitage P et al. Design and analysis of randomised clinical trials requiring prolonged observation of each patient. II Analysis and examples. Br J Cancer 1977; 35: 1-39.