DRUG-INDUCED HEPATIC INJURY

ANALYSIS OF CLINICOPATHOLOGICAL PATTERNS WITH THE HELP OF VOLUNTARY REPORTING

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE ERASMUS UNIVERSITEIT TE ROTTERDAM, OP GEZAG VAN DE RECTOR MAGNIFICUS, PROF. DR. A.H.G. RINNOOY KAN, EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN, IN HET OPENBAAR TE VERDEDIGEN OP WOENSDAG 21 OKTOBER 1987, OM 15.45 UUR

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PROMOTOR: Prof. J.H.P. Wilson

OVERIGE LEDEN: Prof. Dr. V.J. Desmet
Prof. Dr. M.N.G. Dukes
Prof. Dr. H.A. Valkenburg

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In memory of Dr. Kees van Dijke

To Monique, Sanne and Katinka
To my parents

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PART 1

Chapter 1

INTRODUCTION

This thesis consists of two parts. In the first part (Chapters 1-4) adverse reactions to drugs are discussed: the history of the problem (Chapter 1), types of adverse reactions and detection of adverse reactions prior to the marketing of drugs (Chapter 2), and the different types of postmarketing studies (Chapter 3). In Chapter 4 a review is made of the methods and results of the voluntary reporting scheme in The Netherlands.

In the second part (Chapters 5-12) the aims, methods and results are outlined of the studies which form the basis of this thesis. In Chapter 5 the objectives are formulated. The first objective was to demonstrate that suspected adverse reactions to drugs - as reported to a national monitoring centre employing voluntary reporting - can not only be used for the detection of unknown adverse reactions but can also be used for in-depth studies of series of cases. The latter would improve the knowledge of the clinicopathological pattern of a particular adverse reaction. The second objective was to demonstrate that the studies are not necessarily restricted to cases reported to a national centre but that cases from different countries may be used. In this thesis the adverse reaction which was studied was hepatic injury. Glafenine, ketoconazole, nitrofuran derivatives and pirprofen are examples of drugs which may cause this adverse effect. The third objective of this thesis was to study the clinicopathological patterns of hepatic injury attributed to these drugs. The variety of patterns of drug-induced hepatic injury and methods for diagnosis are discussed in Chapters 6 and 7. The studies on the clinicopathological pattern of hepatic injury by three different drugs are given in Chapter 8 (glafenine), Chapter 9 (ketoconazole) and Chapter 10 (nitrofuran derivatives). These studies are the result of the first and third objective. The study of pirprofen-associated hepatic injury (Chapter 11) is the result of the second objective. The results are discussed in Chapter 12.

HISTORY

Phocomelia means "seal extremities", a congenital malformation which consists of the absence of arms and legs. Normal or rudimentary hands and feet are directly attached to the trunk like the flippers of a seal. Phocomelia was - and again is - an extremely rare condition. Only a few cases of phocomelia had been noted between 1949 and 1959 in the various university pediatric clinics in the Federal Republic of Germany. Then there was an explosive growth with 17 cases in 1959, 124 in 1960 and 477 in 1961. Viral causes, radioactive fall-out, X-ray exposure of the mother, food and its preservatives and contraceptives were considered but since the outbreak seemed largely confined to

the Federal Republic of Germany, none of these possible causes seemed to be likely. A German gynaecologist, Lenz, found retrospectively that 20 percent of his patients, who had recently been delivered of a baby with phocomelia, reported to have taken the drug Contergan in early pregnancy. When he requestioned the mothers of these children approximately 50 percent of the mothers admitted taking Contergan. They had not mentioned using the drug at first because they considered the drug to be innocuous. At a pediatric meeting in Düsseldorf on 20 November 1961 Lenz brought his suspicion of a drug-induced cause to public attention without mentioning the name of the product.

> .....That night a physician came up to him and said: "Will you tell me confidentially, is the drug Contergan ? I ask because we have such a child and my wife took Contergan". In the next few days he received a half dozen letters asking the same question and saying, "My wife took Contergan and we have such a child". A couple of days later it was generally known among the doctors that Contergan was the drug under suspicion.....(1).

Thalidomide (Contergan, Distaval, Kevadon, Softenon, Talimol) is a sedative and hypnotic. It was marketed in 1956 in the Federal Republic of Germany and in 1958 in Great Britain and soon gained popularity since - unlike barbiturates - an overdose was not fatal. It was even recommended in children ("Western Germany's baby-sitter") and mixed with several analgesic, antitussive and antipyretic preparations. After its withdrawal from the market in 1961, the Ministry of Health in the Federal Republic of Germany estimated the number of babies with malformations attributed to maternal use of thalidomide during pregnancy at 10000 of whom 5000 survived. In Great Britain these figures were estimated at 500 and 275 respectively (9).

This was not the first iatrogenic epidemic and it will probably not be the last. In the 19th century the toxicity of chloroform had led to its withdrawal from clinical use (2) and in the period 1920-1940 hepatic injury by cinchophen (3), and agranulocytosis by amidopyrine and related agents (4), were recognized. Following the thalidomide episode several drugs have been recognized as the cause of serious disease, e.g.chronic active hepatitis by oxyphenisatin (5), acute hepatocellular necrosis by halothane (6) and sclerosing peritonitis (7) and oculocutaneous reactions (8) by practolol.

The thalidomide disaster, however, was a breakthrough. It used to be easy to market almost any drug. As a result of this epidemic it was made mandatory to perform extensive preclinical and toxicological studies before a drug was marketed. Moreover it was recognized that the marketing of a drug is the decisive toxicological experiment which only stops when the drug is withdrawn from clinical use. In the developed countries national monitoring centres were star-

ted, as the central post for feedback of unwanted experiences with drugs, either as an initiative of medical associations or health authorities.

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Chapter 2

ADVERSE REACTIONS TO DRUGS

The use of drugs is a double-edged sword. Every drug with a pharmacological action may cause adverse reactions. The World Health Organisation defined an adverse effect as "one which is noxious and unintended, and which occurs at doses used in man for prophylaxis, diagnosis or therapy" (1). This definition has two aspects. On the one hand it differentiates the wanted (therapeutic) effect from the unwanted one whereas on the other hand the latter is differentiated from toxic effects by overdosage.

There are several types of adverse effects. As a rule, one may say that every 'natural' disease may be mimicked by an adverse reaction. Several drugs, for instance sulphonamides which may cause a large variety of skin disorders (2), may produce a reaction indistinguishable from the morbilliform rash of measles. Other drugs, e.g. labetalol, may cause fever (3), which is usually attributed to infection before the reaction is recognized as drug-induced. Some clinical manifestations, however, are fairly specific for a drug-related cause. Anaphylactic shock, e.g. to ketoconazole (4) or glafenine (5), and agranulocytosis, e.g. to spironolactone (6) or dipyrone (7), are examples of reactions which are often drug-induced. Adverse reactions have several aspects which may lead to different subdivisions (table 1). A short review will be made of the first 2 aspects, on mechanisms and latent period, whereas for more information the interested reader is referred to the textbooks "Iatrogenic Diseases" (8) and "Textbook of Adverse Drug Reactions" (9) and to the comprehensive reference-book 'Meyler's Side Effects of Drugs'(10), of which there is a Dutch version (11).

1.a Adverse effects which are dose-related

The large majority of drugs do not have a completely specific pharmacological action. Therefore their effects are not only beneficial. Drugs produce a variety of dose-related effects of which the therapeutic action is usually the most important one. With increasing dose, wanted - but also dose-dependent unwanted effects - become more prominent. In principle these effects may occur in every patient. Therefore the reaction is called 'predictable'. Dose-related adverse effects are frequent but usually not very serious. Examples are an excessive therapeutic effect (e.g. hypoglycemia by insulin, hypotension by vasodilators), pharmacological adverse effects (e.g. dry mouth by tricyclic antidepressants), organ-specific toxicity (e.g. vestibular damage and deafness by aminoglycosides) and secondary effects (e.g.interference with bacterial flora by broad-spectrum antibiotics; infections during treatment with immunosuppressants). Sometimes an adverse effect in one situation is a wanted effect in another situation. An example is morphine which causes constipation as an adverse effect when

TABLE 1

SOME EXAMPLES OF THE DIFFERENT ASPECTS OF ADVERSE REACTIONS TO DRUGS

Mechanism	dose-related not dose-related < metabolic / immunoallergic
Latent period	short-term effects (e.g. rash) long-term effects (e.g. cancer)
Localization of injury	organ-specific (e.g. eye, kidney) generalized (multiple organs)
Reversibility	irreversible damage (e.g. cirrhosis) recovery after discontinuation of drug (e.g. headache)
Severity	fatal reaction serious but non-fatal trivial
Objectivity	objective effects (e.g. elevation of serum creatinine) subjective effects (e.g. drowsiness)

employed as an analgesic. In the treatment of severe diarrhoea, however, this adverse effect may find therapeutic use.

1.b Adverse effects which are not dose-related

These reactions are rare and unpredictable. They are not related to the dose but rather to a vulnerability of the individual who develops the reaction. These reactions may be divided into idiosyncratic reactions and immunoallergic reactions. Idiosyncratic reactions are often due to a genetically based abnormality in drug metabolism, e.g. by a decreased quantity of certain enzymes. Typical examples are hemolytic anemia in patients with a deficiency of erythrocytic glucose-6-phosphate dehydrogenase treated with nitrofurantoin and prolonged paralysis in patients with plasma cholinesterase deficiency receiving suxamethonium.

Immunoallergic ("hypersensitivity") reactions are always secondary to antibody formation by the immune-system. Especially large molecules, e.g.dextran, give rise to immunoallergic reactions but also small molecules may do so secondary to the formation of complexes consisting of proteins and drug metabolites. There are at least 4 types of immunological reactions (anaphylactic, cytotoxic, immune-complex and delayed type), which can usually be distinguished by their clinical manifestations (12).

2. Latent period

If adverse effects appear they usually do so shortly (<2 months) after starting treatment. In these cases the temporal relationship will make it relatively easy to ascertain a causal relationship. Urticaria and collapse, for instance, appearing within 20 minutes after ingestion of glafenine or nalidixic acid leave little doubt as regards a causal relationship. In case of unwanted effects after long-term treatment (e.g.cancer, slow-onset intellectual impairment) a causal relationship with use of the suspected drug is usually very difficult to assess. The assessment of a causal relationship between a particular disease and the intake of a drug is based on three points: on the specificity of the clinicopathological pattern, on the temporal relationship and on the exclusion of other potential causes (see Chapter 7). Very few patterns are specific for a drug-induced cause and the temporal relationship is uninformative when assessing long-term effects. There are many unknown genetical-, environmental- and age-related factors which may be held responsible for a particular disease. A suspected drug is often only one of many potential causes. A number of these causes may be unknown and therefore impossible to exclude. When there is a long delay between use of a drug and the appearance of drug-induced injury none of the three aforementioned points are conclusive. In these cases epidemiologic studies should give the answer with prospective and controlled trials, cohort studies or case-control studies. Examples are the association between long-term use of drugs and certain forms of cancer, e.g.between

the use of oral contraceptives and hepatocellular carcinoma (13-17) and breast carcinoma (18-23). Disagreement on the result of these studies may occur and - even when properly performed - this may give rise to heated debates.

Drugs may cause congenital malformations when used during pregnancy. The problems in assessing a causal relationship resemble those of long-term effects but are somewhat different. Although the teratogenic effect is usually not recognized before birth there may be a clear-cut temporal relationship between maternal use of the suspected drug and the congenital defect. Phocomelia by thalidomide, for instance, is secondary to the use of this drug by the mother between 37-54 days after the first day of the last menstruation. The latter is a clearly visible congenital defect. The evidence is much more difficult to deliver when the defect is very subtle or appears after a long delay. These effects may manifest themselves later in life, e.g. vaginal carcinoma in daughters of mothers treated with diethylstilboestrol (23). The cause of most birth defects is unknown which makes it difficult to exclude other potential causes. For obvious reasons it is unethical to test drugs during pregnancy and despite much experimental data in animals little is known about the teratogenic risk of drugs in humans.

DETECTION OF ADVERSE REACTIONS TO DRUGS

The value of a drug depends not only on its therapeutic effect. The potential adverse effects should not outweigh the wanted effects of a drug. In addition to this benefit/risk ratio the nature of the underlying illness and the availability of alternative treatment are important. Bone marrow depression during treatment of cancer with antineoplastic agents is often unavoidable and taken for granted but it is unacceptable when this adverse effect is caused by a simple analgesic. Because some years ago there was no alternative drug for salazosulfapyridine in the treatment of ulcerative colitis (with the exception of corticosteroids), desensitization was often attempted in patients with a hypersensitivity reaction to this drug. When a patient with an infectious disease develops a rash to penicillin, however, it is almost invariably possible to treat him with an alternative antibiotic.

It will be clear that for an adequate treatment both therapeutic and adverse effects of a drug should be known. What should we like to know about the adverse effects of a particular drug? In an ideal situation we know everything, i.e.:

1. Every potential adverse effect of the drug, its potential severity, its reversibility and its risk-groups (qualitative aspect).

2. The incidence of each adverse effect (quantitative aspect).

3. Its treatment (besides discontinuation of the drug).

Unfortunately the actual situation is far from ideal. What then do we know ? And what studies are done to obtain knowledge about adverse effects ?

The development of a drug has several phases. Compounds which have been selected by pharmaceutical firms as potentially successful are submitted to a rigorous test program of several years duration before the drug is marketed. There is a phase of animal testing and there are three clinical phases, which have to be passed successfully before widespread marketing of a drug is started. Promising compounds and large series of related chemicals are screened in animals for their biologic effects. Usually only a few potentially successful drugs remain after testing several thousands of investigational chemicals. Drugs are mostly tested in rats, mice, hamsters, rabbits and guinea pigs (24). For several, often commercial, reasons the use of dogs and apes is limited to second-line toxicity testing or to specific problems such as those in which biliary excretion plays a role in toxicity (25). In animal toxicity testing data on the pharmacokinetic and pharmacodynamic effects of a drug are obtained. Toxic effects of the drug are assessed both after acute overdose (LD50) and after chronic administration. Special emphasis is given to development of tumors and congenital defects. During or following this phase mutagenicity tests in cell cultures or in vivo are performed. Subsequently these drugs are tested in humans. Four phases are distinguished, of which the first 3 phases are usually successfully passed before marketing. The fourth phase starts when the drug is marketed.

- Phase I -

In phase I the drug is tested on a small group of human volunteers in order to obtain data about the pharmacokinetics of the drug and its pharmacodynamic action. Usually it concerns a single administration. The subjects are under close clinical supervision.

- Phase II -

As soon as toxicological studies in animals and phase I studies in volunteers have been brought to a successful end, small groups of selected patients are studied after administration of the investigational drug. The therapeutic action is scrutinized and the optimal dosage assessed. Adverse effects are carefully evaluated in every phase since negligence is not only dangerous for the patient but may lead to financial losses for the company when development has to be abandoned at a later stage. An example of a drug which successfully passed preclinical testing but proved hepatotoxic in humans is FPL 52757. This antiallergic drug which structurally resembles cromoglicic acid, passed animal toxicity testing and human volunteer studies without

TABLE 2

TYPES OF ADVERSE REACTIONS WHICH ARE USUALLY KNOWN FROM PRE-MARKETING STUDIES*

	Known
Dose-dependent	
– excessive therapeutic effect (e.g. insulin-hypoglycemia)	++
– pharmacological side effect (e.g. dry mouth-tricyclic antidepressants)	+
– secondary effect (e.g. infection-immunosuppressants)	–/+
– organ damage (e.g. ototoxicity-aminoglycosides)	–/+
Dose-independent	
– immunoallergy (e.g. rash-penicillin)	–/+
– idiosyncrasy (hepatitis-isoniazide)	–/+
Other types	
– long-term effect (e.g. liver adenoma-oral contraceptives)	–
– teratogenic effect (e.g. phocomelia-thalidomide)	–
– interactions (e.g. rifampicin-coumarines)	–/+
– effect on risk-groups (e.g. benoxaprofen-hepatic injury)	–/+

++ = very well-documented
\+ = well-documented but not all effects known
–/+ = insufficiently documented
– = unknown

* Adapted from van Dijke CPH. 'Postmarketing surveillance': een instrument voor veiligheid? Pharm. Weekbld. 1987; 122:139.

problems but caused an incidence of liver enzyme elevations in patients of 20 percent (25). Another drug discontinued because phase II and III studies showed hepatotoxicity is oxmetidine, an H2-antihistamine (26). This was, however, also demonstrable in rat hepatocyte cultures (27) and in isolated rat hepatocytes (28, 29).

- Phase III -

Subsequently large studies are performed in which many patients are enrolled in prospective controlled clinical trials with the aim to test the therapeutic effects of the drug. In this phase the most frequently encountered adverse effects are assessed. Because of the enormous cost of the development of a new drug only one to several thousands (usually up to approximately 4000) of patients are tested during a limited period. Therefore data are only obtained about adverse effects with a relatively high incidence and appearing after relatively short periods of administration. Moreover drugs are tested in restricted groups of patients. Some patient-groups, e.g.pregnant or elderly patients or children, are not the subject of these tests but may nevertheless be exposed to the drug after marketing.

- Phase IV -

After acceptance by the regulatory authorities of a country the drug is marketed. Even after years of use new therapeutic effects of a drug may be discovered. Postmarketing studies, however, are especially important for the early detection of unknown adverse effects. Although premarketing studies (phases I-III) give some insight into the pharmacological and toxicological, dose-dependent effects of a drug many important adverse effects are unknown at the moment of marketing. Table 2 shows which type of adverse effects are generally known at the moment of marketing. It is clear that adequate data fail on 5 important topics, i.e. the dose-independent (immunoallergic and idiosyncratic) reactions, effect in risk groups (elderly, pregnant women, children etc.), interactions, teratogenic and long-term effects. It is clear that these adverse effects may change the benefit/risk ratio of a drug and may even lead to withdrawal from the market. This has been the case with several drugs in the recent past, e.g.benoxaprofen (hepatic injury), zimelidine (Guillain-Barrē syndrome), nomifensine (hemolytic anemia) and tienilic acid (hepatic injury). In the next chapter types of postmarketing studies are reviewed.

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Chapter 3

MONITORING OF ADVERSE REACTIONS TO DRUGS

It is clear that at the moment of marketing (Phase IV) knowledge about the adverse effects of a particular drug is scanty. This means that the final experiment with a drug is the marketing itself, an experiment which only stops when a drug is no longer in use. There are many examples of drugs which were proven as a cause of a particular adverse effect only after widespread marketing. The discovery that salicylates could induce hepatic injury (1) took almost 60 years of clinical use whereas on the other hand bronchospasm and bleeding tendency were already mentioned in a pharmacological handbook in 1901 (2). Liver damage by cinchophen was reported for the first time in 1923 (3) whereas the drug had been introduced in 1908. Another striking example of a long delay is the association between use of phenacetin and nephropathy. This drug was introduced in general medicine in 1887 but its toxic effect on the kidneys was not reported until 1953 (4). A short delay between marketing and discovery of an adverse effect is mostly possible when the observed clinical effect is very rare. This makes it easy to recognize a sudden increase in the incidence, as was the case with phocomelia by thalidomide and sclerosing peritonitis by practolol. Also when the increase is restricted to a particular geographical area the cause may be recognized relatively easily. An example of this was the sudden increase of cases of obstructive pulmonary hypertension in Switzerland, Germany and Austria, which proved to be caused by the anorectic drug aminorex (5). Also important are the extent of use of the drug and the presence of a 'system' to pool and discuss suspected adverse reactions to drugs (e.g. congresses, medical journals, monitoring centres). Unfortunately many adverse events have a high spontaneous occurrence (e.g.headache) and many other (often unknown) causes, so that only a positive reaction to rechallenge can prove a causal relationship. Although there are exceptions to this rule a rechallenge is in most cases unethical, especially when it concerns severe adverse events. Therefore this type of conclusive proof is rarely obtained, and then often accidentally. Some reactions are so infrequently caused by a particular drug that they are discovered only after years of general use. Pancreatitis by methyldopa (6.7), for instance, and agranulocytosis by spironolactone (8) were demonstrated for the first time more than 20 years after marketing.

By definition, 'postmarketing surveillance' includes studying of wanted as well as unwanted effects (9). This chapter, however, will focus on the latter. There are several methods for studying adverse reactions to drugs after marketing of a drug. Four sources may be distinguished, which are important for the detection of unknown adverse effects, the analysis of associations between

diseases and suspected drugs, and/or the description of adverse reactions to drugs (Table 3). Generally speaking, most adverse reactions are detected with the help of reporting in the medical literature, of voluntary reporting and of cohort studies. The first two of these are useful for descriptive studies of the clinical and histological pattern of a particular adverse effect based on single cases or series. The analysis of associations between diseases and suspected drugs is most effectively performed with cohort and case-control studies. When a monitoring system is staffed by capable scientists and when several complementary methods are employed it should be possible to discover and prevent drug-induced epidemics at an early stage. Even then, however, an unknown adverse effect may be missed and it should be realized that none of these systems is an absolute guarantee against unrecognized drug-induced disease.

A short review of these methods follows (table 4). The interested reader is referred to the books of Inman (10) and Stephens (9). Invariably the aim is to detect unknown adverse effects as early as possible, and/or to estimate their incidence, and/or to determine predisposing or precipitating factors.

LITERATURE REPORTING

An adverse effect remains unknown unless it is recognized as such by someone. This will mostly be the patient, the medical practitioner or pharmacist. The recognized adverse effect may be reported in the literature and/or to the national monitoring centre. Although exact figures are not available most postmarketing data on unknown adverse effects are probably published by the medical practitioner, either as 'letter to the editor' or 'short report' or as a small series of cases. Van Dijke found that almost 80% of reports, published between 1981 and 1986 as "Bijwerkingen van geneesmiddelen" in the Dutch medical journal "Nederlands Tijdschrift voor Geneeskunde", consisted of reports by medical practitioners or pharmacists (11). This makes medical journals an important source of information, especially since computer retrieval facilitates easy access to these data. Reports are usually limited to one or two case-histories since the relatively low incidence of a particular adverse effect complicates generation of large series of cases. Sometimes, however, larger series are generated by co-operation between university medical centres, or by studying manufacturers data (12,13).

VOLUNTARY REPORTING

Most developed countries have an agency which evaluates adverse effects as reported by medical practitioners or pharmacists. Usually these monitoring centres are affiliated with the regulatory authority or inspectorate of drugs but in some countries (e.g.in the Federal Republic of Germany) these are run by the medical association. In a minority of countries (e.g. Sweden) it is mandatory to report

TABLE 3

RELATIVE EFFICACY OF METHODS FOR DETECTION, ANALYSIS AND DESCRIPTION OF ADVERSE REACTIONS TO DRUGS

	Detection	Analysis	Description	
			Cases	Series
Literature reporting	++	−	++	+
Voluntary reporting	++	+	++	++
Cohort studies	+	++	−	−
Case-control studies	−	++	−	−

++ = effective
\+ = may be effective on some occasions
− = usually not effective

TABLE 4

CURRENTLY EMPLOYED AND SUGGESTED METHODS FOR STUDYING ADVERSE REACTIONS TO DRUGS

Literature reporting

Voluntary reporting

Cohort studies

Patient-oriented:
- Intensive hospital monitoring
- Out-patient monitoring
- Medical record linkage

Drug-oriented:
- Prescription event monitoring
- Postmarketing study by manufacturer
- Registered/Monitored/Restricted release

Case-control studies

serious adverse reactions. Most national monitoring centres also have other activities but these are not discussed here. The disadvantage of voluntary reporting is the fact that only a minor part of adverse effects is reported. This means that voluntary reporting gives no insight into the incidence of adverse effects. Not every clinical event is recognized, not every identified clinical event is recognized as an adverse reaction to a drug, not every recognized adverse reaction is reported and sales figures are kept confidential by the manufacturer. This means that both numerator and denominator of the incidence assessment are unknown. The advantage of voluntary reporting schemes is that they are simple to operate, may rapidly react to an alert, are relatively cheap and include the complete population of a country, which means that also rare idiosyncratic and immunoallergic reactions can be discovered. Moreover they can be used for the 'pooling' of patients with a particular adverse effect, groups which can be used for further studying. An other important advantage of voluntary reporting schemes is that they function as "feedback receiver". As already mentioned marketing of a drug is the final experiment and it is very important that the medical practitioner has an address where he can report his suspicions of a drug-induced effect. Especially this function makes a voluntary reporting system indispensable.

Voluntary reporting schemes usually act on a nationwide basis. There are, however, also a few systems acting on a regional basis. A successful example of the latter is the West-Midlands Centre for Adverse Drug Reaction Reporting (Dr.L.Beeley).

Variants of the system of voluntary reporting are the monitoring systems specialized on one organ, e.g. the National Registry of drug-induced ocular side effects (Prof.Dr.F.T.Fraunfelder) (14) and the File on Drug Reaction to the Skin (Dr.W.Bruinsma) (15). Sometimes voluntary reporting schemes are specialized on a particular group of drugs, e.g. radiological contrast media (16) and radiopharmaceuticals (17). Another example is the Federation of Dutch Thrombosis Service Centres, which records interactions with coumarine anticoagulants.

The World Health Organisation has a system, the WHO Collaborating Centre for International Drug Monitoring, which accumulates reports from the national monitoring centres in an abstracted form. Every adverse effect reported to a participating national centre receives a 'preferred term' and is put into a computer data base, which is located in Uppsala, Sweden. The most important aim is to generate early signals as regards adverse effects (18).

Some medical practitioners and pharmacists report their suspicions concerning adverse reactions both to the national monitoring centre and to the manufacturer. Most manufacturers have their own adverse reaction monitoring department. Their data are often kept confidential and are rarely published after the drug has been marketed, unless as part of the revision of a data sheet.

COHORT STUDIES

As may be seen in table 2, there are several types of cohort studies. Cohort studies may be performed prospectively as well as retrospectively. These are disease-oriented when the cohort is based on a group of patients with a particular illness (hereafter called 'patient-oriented') and drug-oriented when all patients on a particular drug form the cohort. In the latter case it means that all patients using a particular drug are enrolled in a study in which all adverse events are recorded and compared with a control group. A usual advantage of cohort studies is the fact that the absolute and relative risk can be estimated fairly accurately and that several adverse effects can be studied concomitantly. Moreover unsuspected risks and benefits may be discovered. Disadvantages are the costs, the limited population and the limited period over which such studies can be performed prospectively. When performed retrospectively data may be incomplete and unreliable and data collection difficult. When performed prospectively bias may be introduced by patient and control selection.

PATIENT-ORIENTED

- Intensive hospital monitoring (19-21)

This system consists of the registration of all ingested drugs and all observed suspected adverse effects in a particular (hospital-based) population. The advantage of this system is that it is relatively easy to estimate the incidence of a particular adverse effect. Moreover the adverse effect can readily be studied by the medical assessor and it is easy to record all relevant data. Disadvantages are the limited period of observation, the fact that the population is usually too small for the detection of unknown - mostly rare - adverse effects and the fact that the costs are relatively high. Another disadvantage is that patients in a hospital often receive many drugs concomitantly and are commonly the subject of several procedures (e.g. operations) which may complicate the assessment of a causal relationship. Intensive hospital monitoring may be a local initiative or be a part of a multicentre study (e.g. the Boston Collaborative Drug Surveillance Program).

Although most intensive hospital monitoring schemes are patient-oriented these may be drug-oriented as well.

- Out-patient monitoring (22,23)

Some Health Maintenance Organizations in the U.S.A. - the Kaiser-Permanente system (22) and the Medicaid Management Information System (23) - register all prescriptions and adverse reactions. By linking these data (also discussed below: medical record linkage) it is attempted to prove a causal relationship between the use of drugs and an adverse event, not only by statistics but also by subsequent evaluation of the original medical records. The first system is

much smaller than the second, which made it unsuitable for relatively rare reactions, e.g. the incidence-comparison of erythromycin estolate with other erythromycines. With the larger systems, however, it may be more cumbersome to return to the original medical records.

- Medical record linkage (24,25)

Medical record linkage refers to the bringing together of different records of individuals, mostly by linkage of two data registers. The register of patients on drug X, for instance, may be linked with the register of malignancies. Then the frequency of a particular diagnosis can be compared between users and non-users. There are several advantages. By systematically searching for associations between drugs and events, unknown adverse effects (but also beneficial effects) are detected and hypotheses arising from other sources may be tested. The relative risk of developing an adverse effect may be estimated. Unlike other systems record linkage may demonstrate the safety of a drug as soon as other sources cause public alarm. With this system it is possible to detect long-term effects of a drug. An important drawback is the price but this may be kept low when the medical record linkage system is part of a large health security system. Medical record linkage systems operate successfully in the United Kingdom (19) and in Finland (20) and gave important results on practolol and rash/conjunctivitis, and clozapine and agranulocytosis respectively.

DRUG-ORIENTED

- Prescription event monitoring (26)

In the United Kingdom, Inman started a new type of post-marketing surveillance. When a new drug is prescribed copies of the forms are obtained from the Prescription Pricing Authority which, as part of the system for reimbursing pharmacists, collects all prescriptions on behalf of the Department of Health and Social Security. Via these forms the general practitioners who have prescribed a particular drug are approached with the request to give data about all events (including not only adverse events and their consequences but also unexpected therapeutic effects) which have happened to the patient during the period of intake and after discontinuation of the drug. The events after discontinuation are compared to those during treatment. As a control the same procedure is done with one or two older drugs from the same therapeutic class. The nature and frequency of adverse events between the two or three groups are compared. The advantage of this system is that it may discover at relatively low costs both new adverse reactions and the incidence but also previously unknown therapeutic effects. Other advantages are the introduction of a sort of control-group (albeit not randomized) by the comparison with a similar drug and the avoidance of discussions about causality since all events are included. The

system is most suitable for the detection of adverse reactions to drugs which are ingested for a longer period. Disadvantages of Prescription Event Monitoring are the fact that only a limited number of drugs can be investigated at a particular moment and that only prescriptions of general practitioners are included. Rare reactions (e.g.with an incidence of 1:10.000) may be missed and even if present these may get lost in the enormous number of events. An other disadvantage is that adequate estimation of the incidence may be jeopardized by a low response rate in the group of doctors, who consider themselves as responsible for an adverse effect. Others may be unwilling to report complicated patient histories because of a lack of time. All this may result in a difference between the incidence of adverse events in patients of general practitioners who respond and those who do not respond to the enquiry. Since a study can only start after a sufficient number of prescription data has been generated, prescription event monitoring may be slower in the detection of adverse effects than voluntary reporting schemes. Nevertheless prescription event monitoring is a sophisticated system. It is, beyond doubt, a promising type of postmarketing study.

- Postmarketing studies by the manufacturer

Ideally, of all patients who receive the drug all adverse events (not necessarily drug-induced) are recorded and the possible causal relationship with the drug is assessed. This may be an expensive method since it usually requires a large medical, pharmaceutical and clerical staff to do a follow-up of several thousands of patients. The population studied should be much larger than the population of the clinical trials in order to discover the more rarely encountered adverse effects. The follow-up should be prolonged to detect any long-term effects. Postmarketing studies are not mandatory. Nevertheless some pharmaceutical industries have attempted to perform such studies.

- Registered (27)/Monitored (28)/Recorded (9) release

These systems were proposed in order to facilitate close supervision on the marketing of new drugs. In registered release free sales are permitted as soon as a registered quota has been completed and patients are followed for five years. Also with the other release systems a follow-up is done of all patients (9) or only of the hospital and outpatient department attendances and fatal cases (28). There are more differences but these are not discussed here, mainly because these systems are currently not in use. Disadvantages are the costs and lack of controls. According to Stephens many pharmaceutical companies initiated monitored release schemes on new drugs but up till now with little success (9).

CASE-CONTROL STUDIES (29)

Case-control studies start with the disease. A group of patients with the disease ('cases') is compared to a group of matched controls. The exposure rate to (a) particular drug(s) in cases is compared to controls. This may be done retrospectively or prospectively, on an on-going basis. Advantages of case-control studies are the fact that they can be performed relatively quickly and are relatively inexpensive. Problems are the data collection which may be unreliable or incomplete, especially when the study is done retrospectively. An important disadvantage is formed by the potential biases (e.g.'selection' bias, 'recall' bias). Case-control studies are very suitable when adverse events are involved with an incidence which is too low for detection by cohort studies.

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Chapter 4

THE NETHERLANDS CENTRE FOR MONITORING OF ADVERSE REACTIONS TO DRUGS

Before changing to the subject of this doctoral thesis it is important to give a short review of the objectives and methods of the monitoring centre where the studies were performed. Indirect activities of the centre, such as functioning as a drug information centre, advising as regards data sheets, and educational activities, are not discussed here. The reason for this review is that the studies are based on the data generation by the Netherlands Centre for Monitoring of Adverse Reactions to Drugs (NARD). For more information the reader is referred to the reviews by Meyboom (1,56).

As a reaction to the thalidomide affair the NARD was founded in 1963 by a joint action of the Dutch Medical Association, the Inspectorate for Pharmaceuticals and the Medical Inspectorate (1,2). At the same time the evaluation of therapeutic and toxic effects of new drugs before marketing was started by the newly formed Committee for the Evaluation of Medicines (CEM). Functionally both the NARD and the CEM have been a part of the Ministry of Health and Environmental Hygiene, later the Ministry of Welfare, Health and Culture. The objective of the NARD has always been to discover unknown adverse reactions at an early stage in order to prevent an epidemic of drug-induced injury.

From all over the country the NARD receives reports of suspected adverse reactions to drugs, either by post or by telephone. The number of suspected adverse reactions is approximately 1000 per year. In every case a careful evaluation is made of essential data and in approximately one third of all reports additional data are requested. The reporting practitioner is often encouraged to publish all new and adequately documented and demonstrated adverse effects, either alone or in combination with other reporting practitioners or with one of the medical officers of the NARD. Reporting to the NARD is done voluntarily. Unlike in Sweden, reporting of serious adverse reactions is not mandatory in The Netherlands. No system can function without feedback. Basis of reporting suspected adverse reactions is the awareness that also the system of drug marketing is incomplete without feedback. Medical practitioners employing new drugs are in need of a central non-commercial office where they can report their unexpected experiences with these drugs.

The idea is that a cluster of reports of a particular adverse event in patients on a particular drug and originating from different parts of the country may reflect an adverse reaction to that drug. This is based on the assumption that the reporting practitioners are unbiased, i.e. not aware of each others reports. Since coincidence is not excluded, especially not with events which have a high

spontaneous incidence and which are non-specific (e.g. headache), all reports require careful evaluation and follow-up. Via this method it can be made likely that unexpected events are adverse effects, even when in each individual case a causal relationship with the intake of the suspected drug is not fully established. In exceptional cases an unknown adverse effect can be proven on the basis of a single case, e.g. because the adverse effect is very specific for a drug-induced cause or recurs on readministration. Of course functioning of the system depends on alert medical practitioners, who not only have to recognize the clinical event as a suspected adverse reaction to a drug, but also have to report their suspicions. Unfortunately this is done by a minority of medical practitioners in The Netherlands. Although especially in the English-speaking and in the Nordic countries the reporting rate is significantly higher, also there reporting is done by a minority (1). Apart from the reasons that a medical practitioner might not be willing to co-operate with a gouvernmentally-related monitoring centre or requires payment for his reports several reasons have been given for non-reporting, known as the "seven deadly sins" (3):

- Complacency - the mistaken belief that only safe drugs are allowed on the market.

- Fear - of involvement in litigation, especially in the USA.

- Guilt - because harm to the patient has been caused by the treatment the doctor has prescribed.

- Ambition - to collect and publish a personal series of cases, a common human failing that may lead to serious delays in recognition of a hazard.

- Ignorance - of the requirements for reporting, perhaps as a result of failure in communication between the reporting centre and the professions.

- Diffidence - about reporting mere suspicions which might perhaps lead to ridicule.

- Lethargy, an amalgam of procrastination, lack of interest or 'time', inability to find a report-card and other excuses.

Of course the doctor may not be aware of the fact that the patient used the drug because of an incomplete drug-history or use of 'over the counter' drugs. According to an enquiry, involving British general practitioners, there were several reasons for non-reporting. The fact that the adverse effect was well-known or trivial, insecurity about a causal relationship and a lack of feedback from the monitoring centres to practitioners were given as the most important reason for underreporting (4,5).

TABLE 5

ADVERSE REACTIONS AS PUBLISHED BY THE NETHERLANDS CENTRE FOR MONITORING OF ADVERSE REACTIONS TO DRUGS

amiodarone (Cordarone)	interaction with coumarines (6)
antiasthmatics	sudden death (7)
aprindine	agranulocytosis (8, 9)
benzydamine (Tantum)	psychic effects (10)
captopril (Capoten)	polymyositis, myocarditis (11)
camazepam (Albego)	rash (12)
cimetidine (Tagamet)	interstitial nephritis (13)
co-trimoxazole (Bactrim, Eusaprim)	interaction with coumarines (14)
dietary products (vit. K-containing)	interaction with coumarines (15)
dipyrone	bone marrow suppression (16)
doxycycline (Doxymycin, Vibramycin, Dagracycline)	oesophageal ulceration (17, 18)
emeproniumbromide (Cetiprin)	oesophageal ulceration (19)
ethambutol (Myambutol)	optic neuropathy (20)
flunarizine (Sibelium)	parkinsonism, depression (21)
flurbiprofen (Froben)	interaction coumarines (22, 23)
glafenine (Glifanan)	anaphylactic reactions (24), hepatic injury (25-27)
griseofulvin	interaction oral contraceptives (28)
ketamine (Ketalar)	apnoëa (29)
ketoconazole (Nizoral)	hepatic injury (30, 31), anaphylactic reactions (32)
labetalol (Trandate)	fever (33)
mazindol (Teronac)	testicular pain (34)
methyldopa (Aldomet, Sembrina, Mulfasin)	pancreatitis (35)
mianserin (Tolvon)	leucopenia, thrombocytopenia (36)
nalidixic acid (Negram)	thrombocytopenia (37)
nitrofurantoin (Furadantine, Furophen Tc, Ceduran)	several adverse effects (38), parotitis (39)
oral contraceptives	interaction other drugs (40)
paracetamol (Finimal, Hedex, Panadol)	hypersensitivity reactions (41)
phenprocoumon (Marcoumar)	hepatic injury (42)
pinaveriumbromide (Dicetel)	oesophageal ulceration (43)
pirenzepine (Abrinac, Gastrozepin)	agranulocytosis, thrombocytopenia (44)
pirprofen (Rengasil)	hepatic injury (45)
practolol	sclerosing peritonitis (46)
propylthiouracil	agranulocytosis (47)
pyrazinamide	fever (48)
scopolamine (Scopoderm TTS)	paradoxic effects (49)
spironolactone (Aldactone)	agranulocytosis (50)
terfenadine (Triludan)	skin reactions (51)
ticlopidine	thrombocytopenia (52, 53)
valproate (Depakine)	hepatic injury, hyperammonaemia (54)
thyreostatic agents	absence of congenital skin defects (55)

Underreporting not only means that an important new and unknown adverse effect may be missed. If underreporting was a constant factor (e.g.10%) it would be possible to obtain the absolute frequency by a ten times multiplication (1). Then drugs could be compared as regards their adverse effects related to sales figures. Unfortunately the reporting rate depends on the type of drug, on the age of the drug, the importance of the reason for use, on the type of adverse effect and probably on other unknown factors. Therefore it is difficult to estimate the incidence and it is generally accepted that voluntary reporting schemes are useful for the detection of unknown adverse effects (qualitative aspect) but not for establishing incidence figures (quantitative aspect).

In the past period the NARD has proved its usefulness as regards the former. Table 5 tabulates adverse effects which have been the subject of publication by the NARD, often as a so-called 'first report'.

Additional activities of the NARD include studies concerning mechanisms (e.g.testing of drug-dependent antibodies against granulocytes, thrombocytes and erythrocytes), etiologies (e.g. a case-control study concerning a possible relationship between intake of drugs and the Guillain-Barré syndrome) and in-depth studies of series of cases (e.g. hepatic injury by glafenine (chapter 8), ketoconazole (chapter 9), nitrofurantoin (chapter 10), valproic acid and other anticonvulsants, and salicylate-related Reye syndrome). If necessary the NARD contacts laboratories in other countries, e.g. for testing serum as regards halothane-dependent antibodies in patients with postoperative jaundice.

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PART 2

Chapter 5

OBJECTIVES OF THIS THESIS

The objectives of this thesis are:

- To investigate whether a voluntary reporting scheme can be used to study series of cases and, if so, whether it is possible to obtain reliable data concerning the clinicopathological pattern of a particular adverse reaction.

- To investigate whether "Intensive Case Monitoring" is also possible with reports from other countries.

- To investigate the clinicopathological patterns of hepatic injury to glafenine, ketoconazole, and nitrofuran derivatives.

What can be done with the data collected by a voluntary reporting system apart from the early detection of adverse reactions to drugs? In principle already one well-documented case-history might prove a causal relationship, although as a rule this is relatively uncommon. Are we able to do more with a series of such cases, in addition to the discovery of unknown adverse effects ?

It is generally accepted that drug-induced injury can mimic practically every disease, a fact which is attributed to the limited number of patterns with which the body reacts to an exogenous harmful stimulus (1). This means that a drug is only one of the alternatives in a differential diagnosis. If the study of a series of cases gives insight into the most frequently found patterns of injury it may help us to differentiate drug-induced injury from other causes of injury.

This study is focused on drug-induced hepatic injury. Drug-induced hepatic injury includes many different patterns varying from necrosis or cholestasis to vascular and neoplastic disorders. These may present acutely or insidiously after years of use of the suspected drug. Some drugs are the cause of a mainly hepatocellular pattern of injury (e.g.methyldopa), whereas the pattern caused by other drugs is predominantly cholestatic (e.g. contraceptive steroids) (2). The diagnosis 'drug-induced hepatic injury' is often made by exclusion of other potential factors. Unlike several forms of viral hepatitis where serologic investigation may confirm the diagnosis (e.g. hepatitis A and B, infectious mononucleosis), in cases of drug-induced hepatic injury a specific marker is usually absent. Knowledge about the pattern of hepatic injury which is most frequently caused by a particular drug would be a help in the differential diagnosis.

One of the problems is that most cases of drug-induced hepatic injury are secondary to either an immunoallergic reaction or an (metabolic ?) idiosyncratic reaction. Thus the reaction is rare and unpredictable and it is therefore

difficult to generate enough cases for studying the complete spectrum of hepatotoxicity of a particular drug. As a consequence most papers about drug-induced hepatic injury reported in the medical literature concern the medical histories of 1-3 cases. It is likely that this does not guarantee a reliable picture of the actual situation. Even review articles, including all cases reported in the medical literature, may yield a false picture because several unknown factors may cause the preferential reporting of a particular pattern. This is illustrated by the fact that most cases of nitrofurantoin-associated hepatic injury reported in the medical literature concerned chronic active hepatitis whereas acute hepatitis is probably more frequent (Chapter 10).

Hepatic injury is a serious adverse reaction, irrespective of whether it concerns the acute or chronic type, and it is unlikely that medical practitioners preferably report one type of hepatic injury. This means that, whereas a report in the medical literature of a particular type of hepatic injury may provoke publication of similar cases, it is more likely that a voluntary reporting scheme reflects the actual situation, i.e. a variety of patterns.

In Chapter 11 four cases of pirprofen-associated hepatic injury are described, which were guided to publication by the NARD from the early stages of patient care, thereby demonstrating the value of "Intensive Case Monitoring" on a European scale. One of the advantages of adverse reaction monitoring in The Netherlands is that - since it is a small and densely populated country - a relatively large number of adverse reactions will occur in a small area. This facilitates "Intensive Case Monitoring". This term is used at our centre when one of the medical officers actively participates in establishing the diagnosis "drug-induced disease", starting in the early phase of the disease. In consult with the reporting practitioner it is decided which diagnostic procedures can produce conclusive data. The advantage of a small and well-developed country is that a medical officer can receive an important report of an adverse effect and visit the reporting medical doctor and (if necessary) the patient on the same day. It will be clear that this may have an important impact on the quality of the report since the NARD can advise at an early stage which additional investigation could improve the documentation of the report. The obvious reason is that comprehensive documentation is indispensable, especially in the case of unknown adverse effects. Thanks to a well-developed motorway, railway and communication system it is easy to visit the reporting medical practitioner, make a telephone call to the local laboratory for additional data or obtain blood for assessment of drug-dependent antibodies or lymphocyte stimulation testing. This "Intensive Case Monitoring" may result in a joint publication of several well-documented cases. When at a particular moment there are too few cases in one country, the co-operation with other national monitoring centres may enlarge the covered population.

The covering of a large population by a voluntary reporting scheme has a second advantage. There are many rare adverse effects of which the appearance in a particular individual can not be predicted. The consequence is that it is very difficult to study the mechanisms of a particular adverse effect and to develop diagnostic methods. By covering a large population over several years enough cases may be gathered for further study. In a way the voluntary reporting system sorts out patient groups with certain characteristics.

Before discussion of the chapters on hepatic injury by glafenine, ketoconazole and nitrofuran derivatives it is essential to review in general the patterns of hepatic injury which have been associated with the use of drugs (Chapter 6) and to outline the guidelines for making the diagnosis 'drug-induced hepatic injury' (Chapter 7).

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Chapter 6

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I. PATTERNS OF DRUG–INDUCED HEPATIC INJURY

Generally speaking, patterns of drug–induced hepatic injury are not very specific, showing characteristics identical to those of non–drug–related injury. However, there are important exceptions to this rule (see Section III: 'Diagnosis and Analysis of Drug–Induced Hepatic Injury').

A short description of known patterns is given below. It is based on the assumption that the reader is familiar with the indications and interpretation of diagnostic procedures. Table 1 gives an overview of the patterns.

Mild and transient elevation of serial liver enzyme levels without

TABLE 1 *Patterns of drug-induced hepatic injury*

Acute		
	Hepatocellular	A. Steatosis
		B.1. Degeneration
		2. Necrosis
		C. Granulomas
	Cholestatic	A. Pure cholestasis
		B. Cholestatic hepatitis
Chronic		
	Hepatocellular	A. Steatosis and fibrosis
		B. Lipid storage disease
		C. Chronic persistent/active hepatitis
		D. Cirrhosis
	Cholestatic	A. Chronic intrahepatic cholestasis
		B. Biliary cirrhosis
Vascular disorders		
	A.1. Veno-occlusive disease	
	2. Occlusion of large hepatic veins	
	B. Sinusoidal dilatation/peliosis hepatis	
	C. Hepatoportal sclerosis, perisinusoidal fibrosis	
Tumors		
	A. Hepatocellular adenoma	
	B. Hepatocellular carcinoma	
	C. Cholangiocarcinoma	
	D. Angiosarcoma	

symptoms and histological signs of injury is not uncommon after starting drug therapy. It is uncertain whether this reflects minimal injury to the hepatocyte or enzyme leakage without injury. Although usually without clinical significance, a follow–up in such cases is advised since it may precede symptomatic hepatic injury in a number of patients.

ACUTE

HEPATOCELLULAR

A. Steatosis (e.g. methotrexate, tetracycline, alcohol, valproic acid etc.)

Fatty change of hepatocytes involves either small droplets without displacement of the nucleus (microvesicular, e.g. tetracycline, valproic acid) or large globules displacing the nucleus to the cell border (macrovesicular, e.g. methotrexate). Inflammatory cells are often scanty but may be present when necrosis coexists. Steatosis may predominate in the centrilobular (e.g. alcohol) or periportal (e.g. ethionine) region. Sometimes fatty cells coalesce to form fatty cysts, resulting in lipogranulomas.

Biochemical alterations depend on the degree of steatosis and (if present) necrosis. Aminotransferase and alkaline phosphatase levels may be normal but are mildly/moderately raised in most cases of acute toxic steatosis. Hypolipemia and hypocholesterolemia are present. Acute steatosis may present with acute hepatic failure (coagulation disorders etc.).

Symptoms may show the same spectrum of severity as in necrosis (see below). Immunoallergic manifestations are absent. Acute steatosis may have a rapidly fatal course (e.g. tetracycline).

B.1. Degeneration (e.g. many hepatotoxic drugs in low doses)

Hepatocellular unrest is seen with bi/trinucleation, mitotic figures, ballooning and acidophilic bodies; there is minimal infiltration, mainly by mononuclear cells.

Mild elevation of liver enzymes (ALAT/ASAT, alkaline phosphatase etc.) may occur.

Clinical symptoms are usually absent; sometimes there are vague, non–specific complaints.

B.2. Necrosis (many drugs, e.g. paracetamol, methyldopa, halothane, isoniazide etc.)

Necrosis varies in severity (focal/massive) and pathogenesis (toxic/idiosyncratic) determined by type of drug, status/localization of metabolizing enzymes and individual susceptibility. Necrosis is mainly zonal, but rarely massive in the toxic form. In the idiosyncratic form, necrosis is

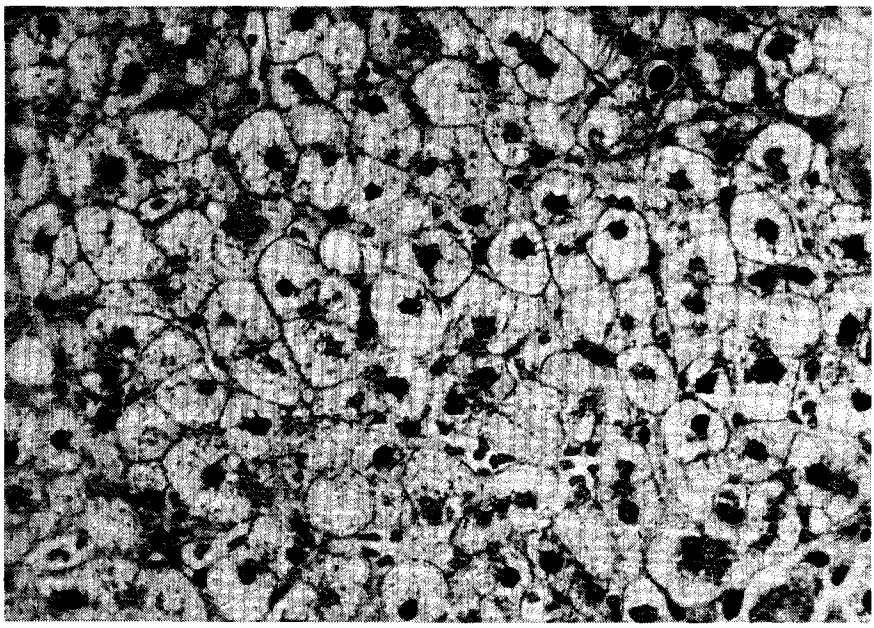

Fig.1: (HE 320x) NARD-case 86/916. Etretinate-associated microvesicular and mild macrovesicular steatosis

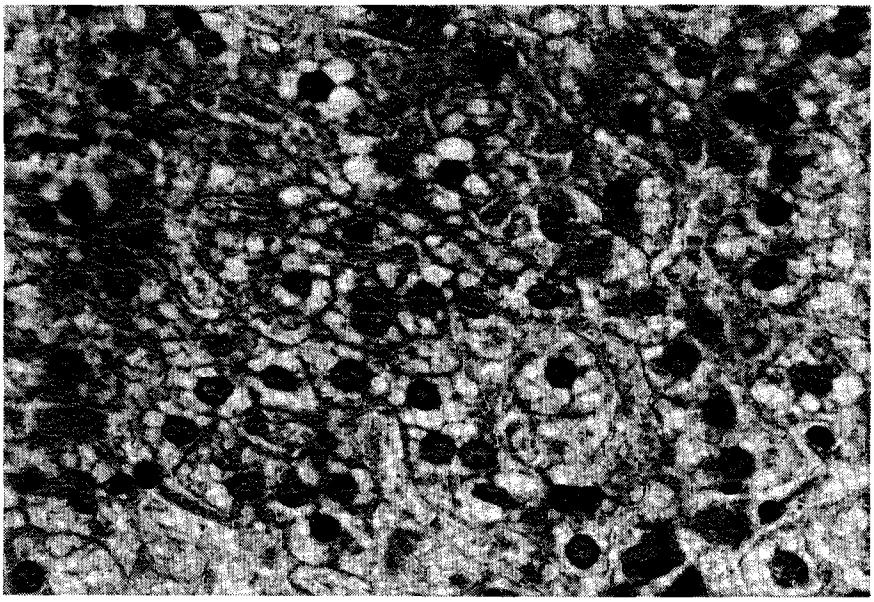

Fig.2: (HE 320x) NARD-case 83/109. Salicylate-associated Reye syndrome with panlobular microvesicular steatosis (by courtesy of Professor J.Huber)

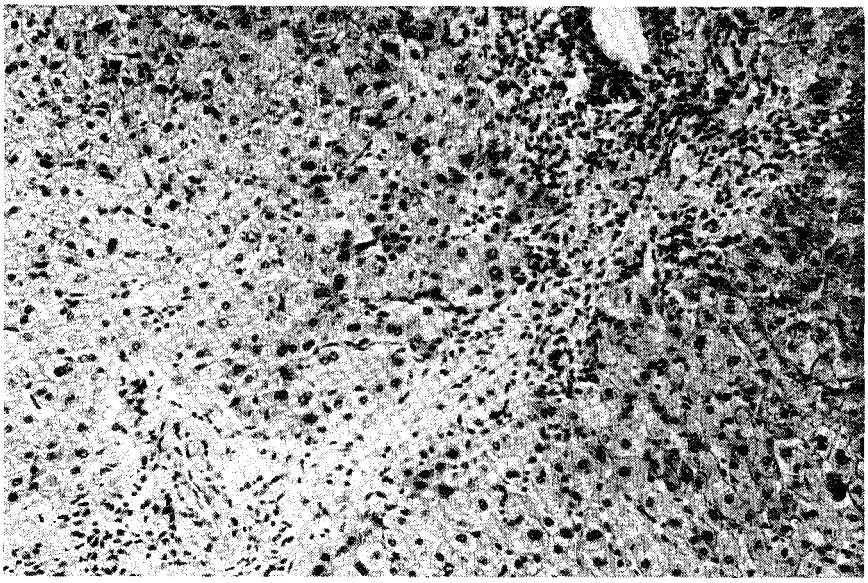

Fig.3: (HE 125x) NARD-case 83/1016. Allopurinol-associated bridging necrosis (by courtesy of Dr.M.M.van de Sandt)

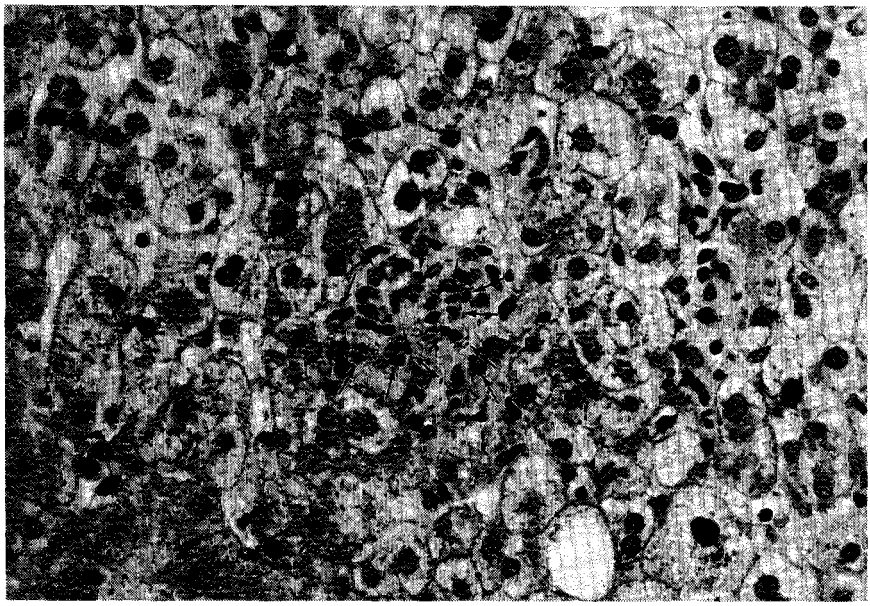

Fig.4: (HE 320x) NARD-case 83/1016. Same case as in fig.3; a pseudogranuloma consisting of focal accumulation of histiocytes and many eosinophils (arrows)

diffuse (immunoallergy?) or zonal (metabolic variant?). Necrosis may also vary in localization: although mostly centrilobular (Zone III, Rappaport; e.g. paracetamol), necrosis may predominate in the midzonal (furosemide overdose, rats) or periportal region (e.g. $FeSO_4$). Severe necrosis may cause bridging (central–central/central–portal/portal–portal) often with collapse of the reticular framework. Massive necrosis is caused mainly by idiosyncrasy, not toxicity. In immunoallergic hepatitis, necrotic sites and portal areas are infiltrated by mononuclear cells, which sometimes have a granulomatous appearance. Toxic injury may give a less densely, more neutrophilic infiltrate. Although eosinophilic infiltration is not highly specific, it is often associated with an immunoallergic pathogenesis. Necrosis around the central vein may result from severe hypotension or congestive heart failure, in the latter accompanied by edema, extravasation of blood and dilated sinusoids.

The extent of necrosis is usually paralleled by an equivalent rise in serum aminotransferase levels, although a sudden decline indicates hepatic failure when accompanied by a sharp rise in serum bilirubin and prolongation of the prothrombin time. Alkaline phosphatase and bilirubin levels in serum are less markedly elevated. Hypoprothrombinemia is frequent. Many other liver enzymes are elevated (e.g. γ–glutamyl transferase, 5–nucleotidase etc.). No markers of viral hepatitis are present. Eosinophilia in the blood may indicate an immunoallergic pathogenesis. In intoxication, blood levels of drug/metabolites confirm a causal relationship.

Symptoms vary depending on severity and susceptibility. They may be absent, mild (malaise, anorexia etc.) or severe (jaundice, bruising). Extrahepatic manifestations may be prominent, both toxic (e.g. renal, CCl_4) and immunoallergic (rash, fever, arthralgia). The latter can also occur as prodromal signs of viral hepatitis, which should be excluded. Sometimes minor hepatic injury is secondary to extrahepatic drug–induced hypersensitivity reactions (e.g. Stevens–Johnson syndrome, myocarditis, pneumonitis). In some cases a picture arises resembling that of infectious mononucleosis with fever, rash, generalized lymphadenopathy and atypical lymphocytes. Mortality of acute hepatocellular necrosis is high. It depends on which drug is responsible and is estimated at 50% for some drugs.

C. Granulomas (e.g. allopurinol, phenylbutazone, sulfonamides)

Drug–induced granulomas usually appear within the first 4 months of therapy, either with mild cellular swelling/cholestasis or without accompanying hepatocellular injury. Occasionally liver injury is more severe. Drug–induced granulomatous hepatitis is often pericholangitic. Granulomas may appear in portal, lobular and pericentral areas invariably accompanied by portal inflammation with lymphocytes, histiocytes, plasma cells and eosinophils; the eosinophils may be very numerous.

Portal granulomas are usually discrete with a surrounding mononuc-

lear infiltrate but may also be part of a diffuse portal inflammation expanding into the lobules. Granulomas are always non–caseating, although occasionally there is central nuclear fragmentation. Giant–cell formation may be marked. Eosinophilic infiltration may be prominent, especially in the early phase. There are no biochemical abnormalities unless hepatic injury is present. The diagnosis can only be made by biopsy.

Granulomas are often non-symptomatic. However, as part of a generalized hypersensitivity reaction, immunoallergic signs and symptoms (e.g. rash, eosinophilia, fever, arthralgia) may be prominent. Disappearance after discontinuation of therapy suggests a drug–induced cause. It is advisable to exclude at least sarcoidosis and infectious causes (especially tuberculosis, schistosomiasis).

CHOLESTATIC

A. Pure cholestasis (e.g. anabolic steroids)

B. Cholestatic hepatitis (e.g. chlorpromazine, erythromycin)

Predominantly centrilobular bile–staining of hepatocytes and bile casts is seen in (sometimes distended) canaliculi. Bile 'lakes' as seen in extrahepatic obstructive jaundice are absent. It may occur without (pure cholestasis) or with minor/moderate hepatocellular unrest/necrosis (cholestatic hepatitis). In the former, inflammatory cells are virtually absent; in the latter there is a mononuclear and eosinophilic infiltrate, rich in the portal zones, moderate at necrotic sites; some bile duct multiplication is often present. A pattern exists characteristic of both acute cholestatic and hepatocellular hepatitis (mixed pattern).

Serum bilirubin, alkaline phosphatase, 5–nucleotidase and γ–glutamyl transferase levels are high while aminotransferase levels are normal or moderately elevated (the latter especially in cases of cholestatic hepatitis). Imaging procedures (e.g. ultrasound, cholegraphic imaging, computed tomography) show normal extrahepatic bile ducts.

Jaundice and pruritus are outstanding features. Cholestatic hepatitis may be accompanied by rash, fever and arthralgia, manifestations that are usually absent in pure cholestasis. Mortality is low (estimated at less than 1%) especially in cases of pure cholestasis. Occasionally, recovery from cholestasis takes a long time ($> {}^1/_2$ year).

CHRONIC

HEPATOCELLULAR

A. Steatosis and fibrosis (e.g. methotrexate, alcohol etc.)

The same pattern is seen as for acute steatosis. If however accompanied

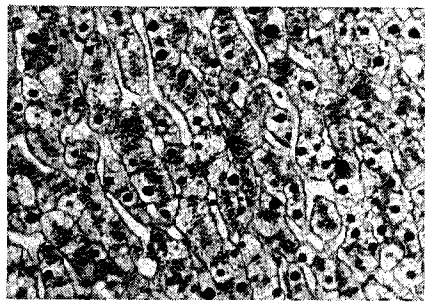

Fig.5: (HE 320x) NARD-case 74/249. Lipofuscin accumulation in a case of phenacetin abuse (by courtesy of Dr.M.van Wijhe)

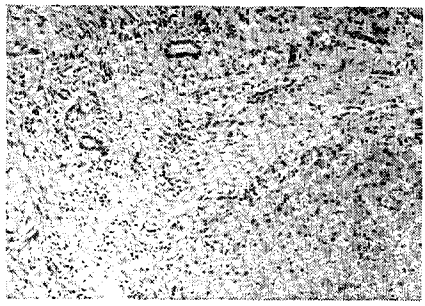

Fig.6: (HE 125x) NARD-case 84/681. Methyldopa-induced postnecrotic macronodular cirrhosis (by courtesy of Dr.A.T.Ariëns)

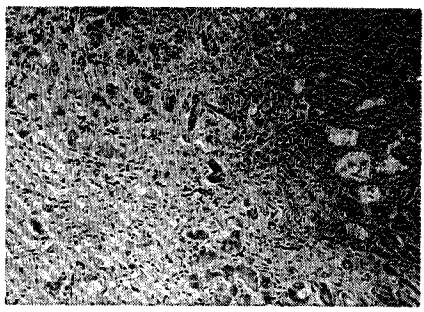

Fig.7: (HE 125x) alcoholic hepatitis and micronodular cirrhosis (by courtesy of Professor D.J.Ruiter)

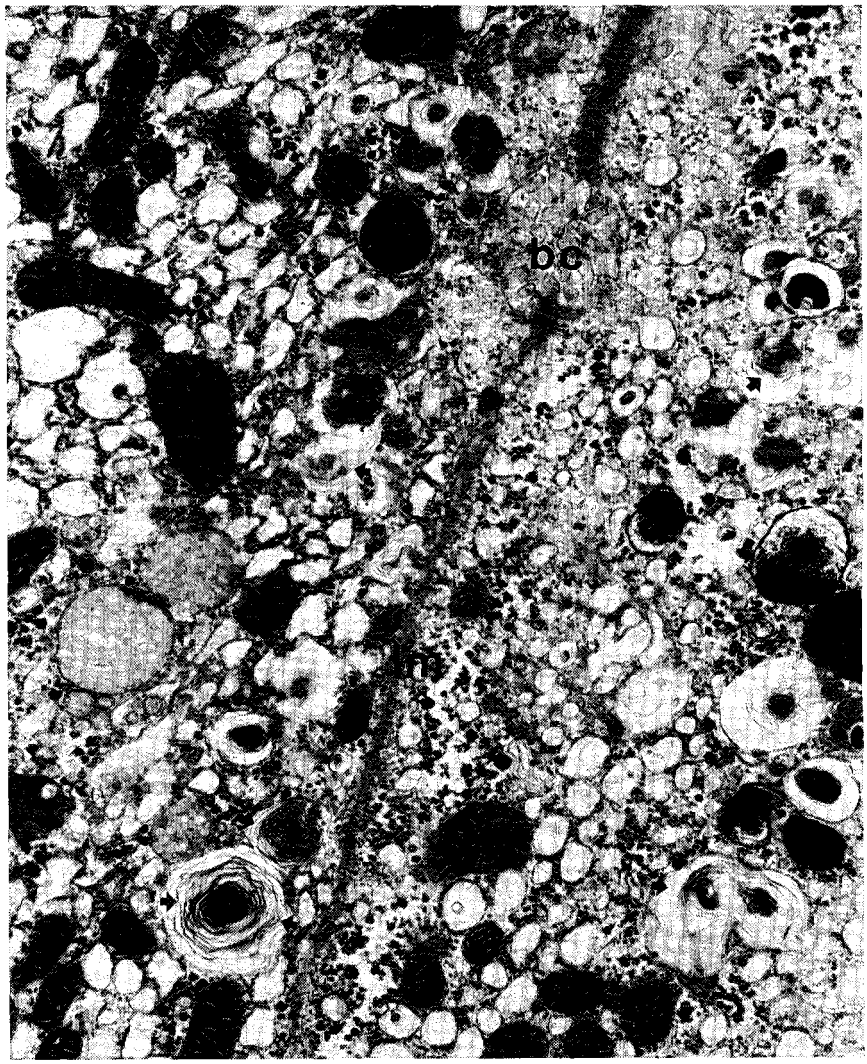

Fig.8: (19840x) NARD-case 84/845. Amiodarone-induced phospholipidosis of human liver. This picture shows parts of 2 hepatocytes separated by their cell membrane (lm) and a bile canaliculus (bc). Many lamellar lysosomal inclusion bodies (arrows) are seen in this pericanalicular area. Dilated smooth endoplasmic reticulum as well as small mitochondria with electron-dense matrix can also be observed (electron-micrograph supplied by Professor U.J.G.M.van Haelst)

by necrosis, prolonged administration of the responsible hepatotoxin will eventually lead to fibrosis and cirrhosis. In some patients, prolonged administration of a hepatotoxin causes a sudden exacerbation into an acute hepatitis (alcohol!) which may present either a hepatocellular or cholestatic pattern.

All liver enzyme levels may be elevated, mostly mildly; signs of metabolic dysfunction occur (e.g. hyperlipemia, hyperuricemia, hypoglycemia etc.). If necrosis starts early and fibrosis and cirrhosis develop, hepatic dysfunction (hypoalbuminemia, hyperammonemia etc.) may occur.

Often there are no symptoms or only non–specific complaints (malaise, anorexia etc.), unless acute necrosis or cholestasis is present.

B. Lipid storage disease (e.g. 4,4′–diethylaminoethoxyhexestrol, amiodarone, perhexiline)

Drug–induced lipid storage disease usually consists of phospholipidosis although ganglioside accumulation has also been mentioned in the literature (e.g. perhexiline). Hepatocytes, Kupffer cells, endothelial and biliary epithelial cells but also extrahepatic cells, e.g. blood and nerve cells and lung tissue, are involved.

Liver cells are enlarged and have a foamy appearance. Electron microscopy reveals multilamellated ('fingerprint') and crystalloid lysosomal inclusion bodies. In advanced cases, fibrosis and cirrhosis may ensue. The lysosomal inclusions resemble those seen in Niemann–Pick's and Fabry's disease, but the clinical setting differs.

Biochemical abnormalities are usually absent, but elevation of liver enzyme levels after prolonged treatment may reflect severe chronic hepatic injury. Hypertriglyceridemia and elevation of free fatty acids may present early. Lysosomal inclusion bodies in peripheral blood lymphocytes may be indicative and diminish the need for a biopsy.

Symptoms are usually absent but extrahepatic manifestations may indicate toxicity (e.g. neuropathy due to perhexiline or amiodarone, skin discoloration by amiodarone). Immunoallergic signs are absent. Malaise and hepatosplenomegaly, weight loss and hypoglycemia may precede or accompany liver damage.

C. Chronic persistent/active hepatitis (CPH/CAH) (e.g. oxyphenisatine, methyldopa, nitrofurantoin etc.)

Periportal rosette–formation and 'piece–meal' necrosis is seen with a rich portal and periportal infiltrate of lymphocytes and plasma cells. A more active state may be reflected by mild/moderate necrosis and infiltration around the central vein. Bridging necrosis occurs, connecting portal areas. Periportal fibrosis develops and may eventually progress to cirrhosis. In chronic persistent hepatitis (a less aggressive form), inflammatory cells in the portal area do not cross the limiting plate so that necrosis is absent.

Most liver enzyme levels are elevated, but are occasionally normal. Serum gammaglobulin levels are elevated, especially IgG. Immunological tests are positive, especially smooth muscle antibodies. If progressive, signs of severe hepatic dysfunction develop.

Symptoms may be absent or non–specific; they may arise suddenly or insidiously. In advanced cases, jaundice, portal hypertension with ascites and hepatic failure are present. Prognosis is good after discontinuation of the causative agent unless damage is too advanced and cirrhosis develops. Since immunoallergic symptoms usually lead to early discontinuation of the causative drug, these symptoms are uncommon in cases of chronic (> 6 months) drug–induced hepatic injury.

D. Cirrhosis (e.g. CCl_4, alcohol etc.)

Diffuse fibrosis occurs with nodules of hepatocytes, alteration of the normal lobular structure, regeneration and necrosis. Either small (micronodular) or large (macronodular) nodules are present. Drugs capable of producing chronic active hepatitis (see above) or any other chronic form of hepatic injury may eventually lead to cirrhosis. 'Cardiac cirrhosis' is not a usual type of cirrhosis but a pericentral fibrosis with 'reversed lobulation' caused by prolonged hepatic venous congestion. Theoretically, it may be induced by drugs aggravating congestive heart failure, but this is probably not of much practical significance.

Liver enzyme levels are elevated and amino acid patterns are changed. Hepatic failure occurs with hypoalbuminemia, hypoprothrombinemia, hyperammonemia etc.

Ascites, splenomegaly, jaundice etc. supervene. Defective coagulation, metabolic disturbances, encephalopathy, portal hypertension with esophageal varices and gastrointestinal blood loss present the main therapeutic problems. They are not present in every patient, however. Portal hypertension may be seen without cirrhosis in hepatoportal sclerosis (e.g. inorganic arsenicals).

CHOLESTATIC

A. Chronic intrahepatic cholestasis

B. Biliary cirrhosis (e.g. chlorpromazine, testosterone)

Centrilobular, midzonal and periportal cholestasis are seen with bile staining of hepatocytes, bile casts and duct dilatation, periductal inflammation and fibrosis. Interlobular and septal bile ducts finally disappear with periportal necrosis and fibrosis, ending in biliary cirrhosis. According to some authors, the drug–induced form of biliary cirrhosis has more scanty portal inflammation and bile duct destruction than primary biliary cirrhosis.

High levels of alkaline phosphatase and cholesterol are found in the

serum, but bilirubin and aminotransferase concentrations are moderately elevated. Antimitochondrial antibodies, present in almost every case of primary biliary cirrhosis, were often not assessed in the older reports of drug–induced cases of biliary cirrhosis so that few data are available. Serum IgM may be elevated.

Jaundice, pruritus etc. and dermal xanthomata are found. The prognosis is better than in primary biliary cirrhosis, since discontinuation of treatment in the early stages is often followed by improvement. Nevertheless, some fatal cases have been described. The data, however, are based on very few cases.

VASCULAR DISORDERS

Although the Budd–Chiari syndrome is often used as a synonym for thrombosis of the large hepatic veins, any obstruction of venous outflow, including veno–occlusive disease, causes this syndrome.

A.1. Veno–occlusive disease (e.g. urethane, pyrrolizidine alkaloids, tioguanine, dacarbazine)

Occlusion of the centrilobular and sublobular hepatic veins occurs due to disruption and edema of the venous wall and surrounding tissue (acute) or to fibrotic tissue (chronic). There are no lesions or thrombosis of larger veins. Congestion is found in surrounding dilated sinusoids with hemorrhagic necrosis of hepatocytes. There is collapse of the reticular framework around the central vein and fibrosis. Collateral vessels develop in the surrounding parenchyma.

There is elevation of bilirubin and liver enzyme levels in the serum depending on the extent and number of obliterated veins.

The onset of painful hepatomegaly and ascites is sudden, sometimes with circulatory shock. Collateral abdominal veins may be prominent. Occasionally there is mild jaundice. Mostly prompt clinical recovery occurs unless the drug is continued. Some cases develop recurring ascites, finally developing into cirrhosis.

A.2. Occlusion of large hepatic veins (e.g. contraceptive steroids)

This may lead to the Budd–Chiari syndrome resulting from occlusion of the main hepatic veins or their smaller branches (v. sushepatica) or of the inferior vena cava, mostly caused by thrombosis. There is centrilobular necrosis and congestion, sinusoidal dilatation and extravasation of blood; there are secondary thrombi in different stages of organization. Chronic cases show centrilobular fibrosis and periportal regeneration.

Serum albumin, alkaline phosphatase, bilirubin and aminotransferase levels are moderately elevated. Scintigraphy may show reduced uptake, with maximum uptake in the caudate lobe. Arteriography may show mul

tiple space–occupying areas resembling metastases. Venography shows obstruction and a lace–like pattern in the liver.

Abdominal pain, hepatomegaly, gross ascites and sometimes mild jaundice occur; collateral veins may be visible on the abdomen; the onset may often be insidious. The course may be slow with resistent ascites, portal hypertension, cirrhosis and death from liver failure/gastrointestinal hemorrhage in 3–4 years or rapid with acute hepatic failure in several months.

B. Sinusoidal dilatation/peliosis hepatis (e.g. contraceptive and anabolic steroids)

Dilatation of sinusoids occurs, sometimes resulting in blood pools. There is a phlebosclerotic form with endothelial or fibrous lining, draining into central veins/sinusoids, and a parenchymal type without a lining communicating with sinusoids. Especially the latter is associated with the use of anabolic and contraceptive steroids. Both forms may be present concurrently.

Peliosis hepatis is often secondary to other conditions (e.g. hepatic adenoma) which dominate the clinical and biochemical pattern.

C. Hepatoportal sclerosis, perisinusoidal fibrosis (e.g. arsenicals, vitamin A)

Idiopathic portal hypertension may originate from fibrous thickening of the portal veins and/or perisinusoidal fibrosis. There are varying degrees of portal fibrosis and intimal thickening and sclerosis of portal vein walls. There is often perisinusoidal fibrosis with collagen deposition in the space of Disse and fibers infiltrating between hepatocytes. In cases of vitamin A intoxication there is a marked increase in Ito cells, which may be the only histological feature.

Biochemical values are normal or slightly abnormal unless severe fibrosis and cirrhosis are present. In cases of intoxication, high levels of arsenic or vitamin A in liver tissue are diagnostic; vitamin A has a fluorescent appearance under the microscope.

Although initially without symptoms, prolonged portal hypertension will cause splenomegaly, ascites and collateral venous circulation, often clearly visible on the abdomen. For subsequent cirrhosis and hepatic failure the above–mentioned considerations apply. Extrahepatic manifestations of intoxication may facilitate the assessment of a causal relationship with the suspected agent.

TUMORS

A. Hepatocellular adenoma (e.g. anabolic and contraceptive steroids)

Hepatocellular adenoma is a non–malignant tumor of cells resembling

hepatocytes. These cells are arranged in tightly packed trabeculae, 2–3 cells thick, separated by compressed slit–like sinusoids. The cells are slightly larger than hepatocytes and are glycogen–rich, with uniform nuclei and little variation in size and shape; canaliculi are normal, bile ducts absent. The tumor is very vascular with dilated, thin–walled blood vessels which are randomly distributed.

α–Fetoprotein tests are negative. Diagnosis is only possible from the pathology. Liver scanning and ultrasound are helpful. Angiography shows a highly vascularized tumor with an arterial supply originating peripherally with multiple parallel vessels coursing towards the center of the tumor.

Anorexia, nausea and abdominal discomfort occur. The tumor is often palpable. Approximately $^1/_3$–$^1/_4$ of patients present with hemoperitoneum/rupture of the tumor.

B. Hepatocellular carcinoma (e.g. thorium dioxide, contraceptive steroids?)

Hepatocellular carcinoma is a malignant tumor composed of hepatocyte–like cells, often in combination with cirrhosis of the liver. Local vascular/lymphatic metastases may be present, having a typical appearance ('planet with satellites'). Since there are several variants of the basic trabecular pattern (pseudoglandular, solid, scirrhous, pleomorphic and clear cell), differentiation from other primary hepatic tumors may be difficult. In 'Thorotrast cases' brown–black particles are visible.

Mostly high levels of α–fetoprotein ($>$ 400 ng/ml) are found. High alkaline phosphatase or mildly elevated bilirubin levels in serum may occur but are non–specific. Elevated liver enzymes may reflect underlying cirrhosis. Angiography, ultrasound, isotopic scanning and computed tomography may be helpful by showing a rich arterial supply with clusters of bizarre tumor vessels and focal defects. Proline hydroxylase and chorionic gonadotropin levels may be elevated. Erythrocytosis and dysfibrinogenemia are rare. HbsAg is negative in drug–induced cases.

Abdominal pain and weight loss occur; symptoms may reflect cirrhosis with portal hypertension/(bloody) ascites. Hemoperitoneum by rupture may occur.

C. Cholangiocarcinoma (e.g. thorium dioxide)

Cholangiocarcinoma is a malignant tumor of cells resembling biliary epithelium. It is a firm grayish tumor growing along neighboring bile ducts in an otherwise normal liver. There are glandular mucus–secreting structures with much fibrous stroma or there is a papillary form. If a trabecular pattern is present, the tumor may be indistinguishable from hepatocellular carcinoma. However, α–fetoprotein is usually normal. In 'Thorotrast cases', brown–black particles are present.

Functional hepatic impairment occurs late in course. Diagnosis is

from the pathology. Angiography usually shows a less vascularized tumor than in cases of hepatocellular carcinoma, although intermediate forms are not uncommon. Ultrasound and liver scanning are helpful.

Patients may present with obstructive jaundice and weight loss or may have symptoms mimicking hepatocellular carcinoma.

D. Angiosarcoma (e.g. thorium dioxide, inorganic arsenicals)

Angiosarcoma is a malignant tumor of spindle–shaped cells lining vascular spaces. Multiple hemorrhagic nodules and blood–filled cysts are seen. Growth is tectorial on the surface of the liver cells. The tumor cells are elongated with ill–defined borders and with hyperchromatic nuclei varying in size and shape. There is an abundance of reticular fibers in the sinusoids. There is hematopoiesis. In 'Thorotrast cases', brown–black particles are demonstrable.

There are non–specific liver function abnormalities and hematological abnormalities. Angiography, ultrasound or computed tomography are useful for localization of the tumor.

There is progressive hepatomegaly, with hematological abnormalities (pancytopenia, hemolytic anemia, disseminated intravascular coagulation and terminal jaundice). Occasionally the tumor may rupture. The course is rapidly fatal.

Chapter 7

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III. DIAGNOSIS AND ANALYSIS OF DRUG–INDUCED HEPATIC INJURY

Roughly speaking, the evaluation of drug–induced hepatic injury occurs in two stages. Firstly, clinical, biochemical and other features of a particular case are gathered and a diagnosis is made. Such a procedure may resemble the algorithm shown in Figure 1. Several analogous diagnostic schemes, however, have been published in the past and since the reader of this book will probably be familiar with the more common diagnostic procedures, this algorithm is not discussed in detail.

Secondly, when several reports of suspected drug–induced hepatic injury incriminate a particular drug, monitoring centers will usually perform an in–depth analysis of such cases. To guarantee a consistent approach to the assessment of a causal relationship between hepatic injury and drug use, an analytical scheme may be used (see Fig. 2). Such an approach may be useful as a tentative strategy for decision–making and categorization. It should be realized, however, that such schemes are based on *current* knowledge and need regular updating. *Moreover, a scheme should never be so rigid or so rigidly interpreted that completely new patterns are discounted as unlikely when these do not conform to current knowledge.*

An explanation of the schema shown in Figure 2 is given below.

AN ANALYTICAL SCHEMA FOR DRUG–INDUCED HEPATIC INJURY

The analytical schema is based on 3 points:

1. *Specificity* of the clinicopathological pattern *and* its course
2. The *temporal relationship* between intake/discontinuation of the suspected drug and onset/disappearance of the hepatic injury
3. The *exclusion of other possible causes* for the observed patterns. This is complementary to the specificity. The less specific a pattern is, the more important does this factor become

The clinicopathological pattern is defined as the combination of clinical, histological, biochemical, immunological, toxicological, experimental and other variables by which the pattern may be specified.

The model assesses the degree of certainty of a causal relationship between hepatic injury and the intake of a drug. There are several levels. A and B (see Fig. 2) represent the highest degree of certainty because of a highly specific pattern/course and course/temporal relationship ('posi-

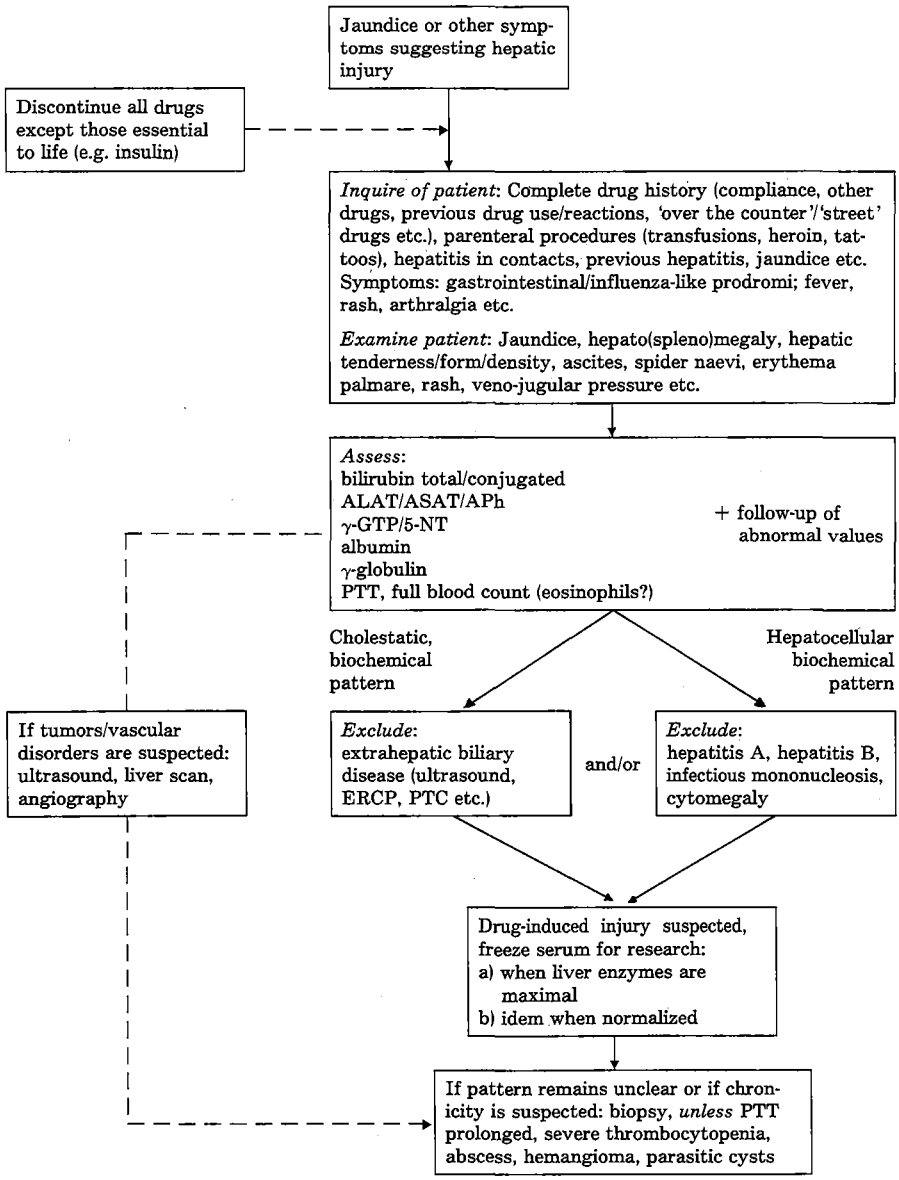
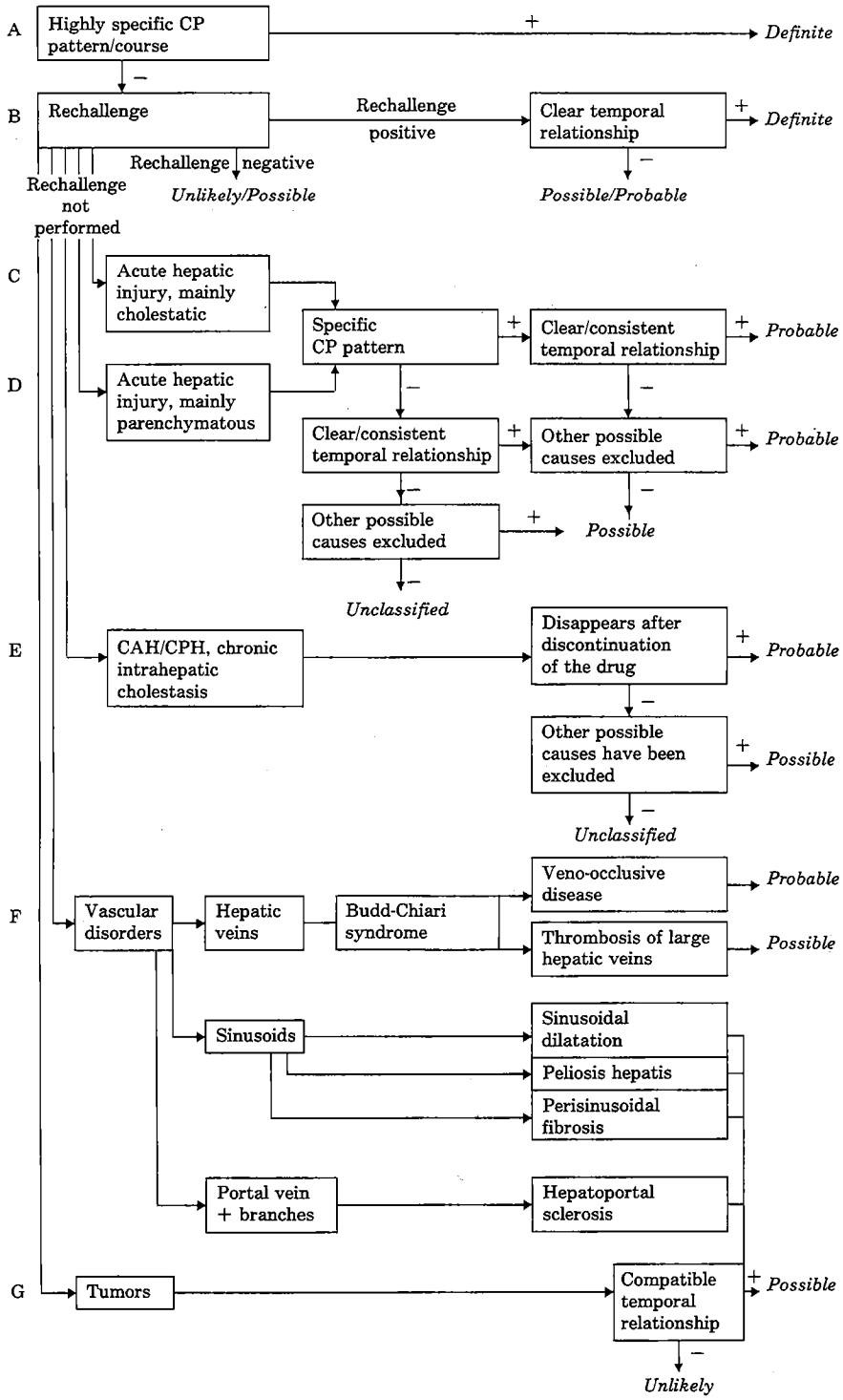


FIG. 1 *The diagnostic 'tree'.*

FIG. 2 *Analytical procedure for cases of suspected drug-induced hepatic injury. If hepatic injury is probably or definitely drug-induced but 2 or more drugs are equally suspected, a causal relationship* per drug *is possible.*


A
Highly specific CP pattern/course
+
Definite
–
B
Rechallenge
Rechallenge positive
Clear temporal relationship
+
Definite
–
Possible/Probable
Rechallenge negative
Unlikely/Possible
Rechallenge not performed
C
Acute hepatic injury, mainly cholestatic
Specific CP pattern
+
Clear/consistent temporal relationship
+
Probable
D
Acute hepatic injury, mainly parenchymatous
–
–
Clear/consistent temporal relationship
+
Other possible causes excluded
+
Probable
–
–
Other possible causes excluded
+
Possible
–
Unclassified
E
CAH/CPH, chronic intrahepatic cholestasis
Disappears after discontinuation of the drug
+
Probable
–
Other possible causes have been excluded
+
Possible
–
Unclassified
F
Vascular disorders
Hepatic veins
Budd-Chiari syndrome
Veno-occlusive disease
Probable
Thrombosis of large hepatic veins
Possible
Sinusoids
Sinusoidal dilatation
Peliosis hepatis
Perisinusoidal fibrosis
Portal vein + branches
Hepatoportal sclerosis
G
Tumors
Compatible temporal relationship
+
Possible
–
Unlikely

tive rechallenge') respectively. This makes it easy to differentiate from non–drug–related causes. C, D, E, F and G represent less certain situations in which the causal relationship is assessed on the basis of a combination of (relative) specificity, temporal relationship and the exclusion of other possible causes. These levels are discussed below.

LEVEL A

A clinicopathological pattern and/or course is highly specific when it has unique features which make it possible to differentiate it from other causes. This may be by demonstration of the responsible agent in toxic amounts or of a unique histological pattern, but also a characteristic course in combination with a proven history of chronic intoxication (Table 1).

LEVEL B

Renewed hepatic injury after (often unintentional) rechallenge. There is an obvious temporal relationship between the intake of the drug and the occurrence of hepatic injury, especially a prompt recrudescence after rechallenge. Between challenge and rechallenge, liver enzyme levels (especially serum aminotransferases) should have returned to normal. The latter is necessary to help differentiate it from fluctuating non–A, non–B viral hepatitis. When a rechallenge is positive but a clear temporal relationship is absent, the causal relationship may be termed 'probable', or 'possible' if it is suspected to be coincidental (e.g. with a flare–up of underlying illness).

A negative rechallenge suggests that a causal relationship is unlikely;

TABLE 1 *Highly specific pattern/course*

Histology	In a certain age-group	Phospholipidosis in adults (e.g. 4,4′-diethylaminoethoxyhexestrol) (Coralgil) (1), amiodarone (31,32)
	Deposits at site of injury	Thorium dioxide (Thorotrast) (33)
		Polyvinyl pyrrolidone (30)
	Specific combination with extrahepatic lesion	Phenacetin pigmentation (2) and renal papillary necrosis
Demonstrable agent		Toxic blood-levels of hepatotoxins (e.g. paracetamol) (3); residue in stomach of hepatotoxins in combination with a characteristic course (e.g. *Amanita phalloides,* phosphorus, ferrous sulfate poisoning) (4)
Unique course		Well-documented case of prolonged intoxication (e.g. alcoholic hepatitis/cirrhosis)

however, it may be a false–negative because of 'desensitization' (immunoallergic type), the use of a lower dose (toxic type) or too short a challenge period (metabolic idiosyncratic type). In these cases a causal relationship may still have been present.

LEVELS C AND D

Here, the clinicopathological pattern is called 'specific' when its most characteristic cause is an adverse reaction to a drug. Because a specific pattern does not constitute proof of a drug–induced cause (in which case it would be *highly* specific), it needs a clear or consistent temporal relationship to produce a 'probable' causal relationship. One should realize that the following clinical signs are not specific *per se*, but in combination with hepatic injury and drug use. Moreover, even when the observed pattern is specific for a drug–induced cause, it will remain necessary to exclude other – less likely – causes.

Specific patterns (Levels C and D)

Clinical

Rash and hepatic injury (e.g. allopurinol (5), para–aminosalicylic acid (6))

Rash should *accompany* hepatic injury. The latter is important since rash may be a prodromal sign of viral hepatitis and it subsides when the hepatic injury reaches full expression, i.e. jaundice. In drug–induced injury, however, the rash, when present, remains even after jaundice has appeared. The pattern of rash and serious hepatic injury without an abnormal blood picture and/or preceding pharyngitis is easy to differentiate from infectious mononucleosis. Rash may occur in the immunoallergic form of drug–induced hepatic injury in adults. In children, this form seems less frequent and, if present, has a wider differential diagnosis.

Peripheral eosinophilia and hepatic injury (e.g. phenytoin (7), erythromycins (8))

Significant eosinophilia of the blood ($\geqq$ 7% eosinophils) should *accompany* hepatic injury and drug intake and should have been demonstrated on at least 2 successive occasions. This is relatively rare in viral hepatitis and is characteristic of the immunoallergic form of drug–induced injury, especially in combination with a prominently eosinophilic infiltration of the portal area and parenchyma. Helminthic infections should be excluded.

Fever and hepatic injury (e.g. methyldopa (9), nomifensine (10))

Hepatic injury *in combination* with high fever ($>$ 39°C) in the absence

of sepsis is fairly specific of a drug–induced cause. An elevation in temperature may precede viral hepatitis but is usually mild and it subsides when jaundice appears. Fever should have a close temporal relationship to the intake of the suspected drug and it usually persists even after jaundice appears. There are, of course, other causes to be considered (e.g. acute cholangitis, alcoholic hepatitis).

Hepatic injury and other extrahepatic manifestations

Occasionally, hepatic injury is accompanied by blood disorders (e.g. hemolytic anemia – methyldopa (9), agranulocytosis – chlorpromazine (11)), renal disorders (e.g. methoxyflurane (12)) or pulmonary disorders (e.g. nitrofurantoin (13)), myocarditis (e.g. methyldopa (14)), parotitis (e.g. phenylbutazone (15)), vasculitis (e.g. allopurinol (5)) or Lyell syndrome/Stevens–Johnson syndrome (e.g. ibuprofen (16)).

N.B. A pattern with rash, fever, lymphadenopathy and atypical lymphocytosis seen with some drugs (e.g. sulfasalazine (17), phenytoin (7)) is less specific and should at least be differentiated from infectious mononucleosis.

Histological

Pure cholestasis (e.g. C–17 alkylated steroids (6))

Intrahepatic cholestasis is seen, with bile staining of hepatocytes and bile casts in the canaliculi without parenchymal necrosis/infiltration. There is no portal infiltrate. Cholestasis predominates in the centrizonal area but may extend into the midzonal and periportal areas when the drug is continued. This pattern strongly suggests a drug reaction but may be seen in other conditions (e.g. postoperative cholestasis).

Zonal necrosis

Necrosis predominates in the centrizonal (e.g. paracetamol, CCl_4 (4)), midzonal (e.g. furosemide (31)) or periportal area (e.g. ferrous sulfate (4)) often surrounded by steatosis. There is little (mainly neutrophilic) or no infiltration.

Cholestatic hepatitis with eosinophilic infiltration (e.g. chlorpromazine (18))

The pattern is the same as that of pure cholestasis but with a prominently eosinophilic portal infiltrate and minimal parenchymal inflammation and necrosis. Especially the combination with blood eosinophilia strongly suggests drug–induced cholestasis.

Granulomatous hepatitis with eosinophilic infiltration (19)

Portal and/or lobular non–caseating granulomas with a prominently eosinophilic infiltrate are fairly specific of a drug–induced cause, especially if combined with peripheral eosinophilia and portal eosinophilic infiltration. Parasitic diseases should however be excluded. Sarcoidosis and tuberculosis may easily be excluded.

Experimental

Hepatic injury + a positive lymphocyte stimulation test (LST) (20, 22)

The LST usually yields inconsistent results and is insignificant when negative because of many possible interfering factors. However, when a well–performed and controlled LST is positive only if the drug or a metabolite is added, it may be regarded as fairly specific. Although a positive LST in this situation only means that the drug is immunologically recognized (which may be compatible with almost any immunological form of drug–induced injury), it may be regarded as specific if it is combined with an intake–related course of hepatic injury, especially if the LST becomes negative after recovery. Analogous considerations apply to comparable in–vitro techniques (e.g. leukocyte–migration inhibition)(21).

Hepatic injury + miscellaneous techniques

Less experience has been gained with some newer techniques which promise to develop into specific assessment methods, such as the demonstration of drug/metabolite–dependent antibodies by indirect immunofluorescence, drug/metabolite–dependent cytotoxicity assay (23) or possibly the demonstration of drug/metabolite in circulating immune–complexes (24) in the serum–sickness–like form of hepatic injury.

Temporal relationship (Levels C and D)

The temporal relationship between the intake of a suspected drug and the onset of hepatic injury should be *obvious*, e.g. promptly after the intake of toxic agents in overdose and within 6 weeks after initial intake of immunoallergic agents. *Or* the reaction should be *consistent* with a well–known pattern and/or course of the particular drug. Isoniazid, for instance, usually produces hepatic injury after a longer latent period (1 to several months) (25) and the temporal relationship should be compatible with this characteristic. The usual onset will depend on the pathogenesis (see Table 2). It is important to emphasize that an usually long latent period does not mean that a causal relationship is unlikely, but merely that the probability is more difficult to establish. In that case, 'probable' may become 'possible' but certainly not 'unlikely'.

Very rapid recovery after discontinuation of the suspected drug strongly favors a drug–related cause. The sudden disappearance of anti-

TABLE 2 *Usual temporal relationship*

1–3 days	Acute toxicity
1–6 weeks	Immunoallergy, metabolic idiosyncrasy
>6 weeks	Metabolic idiosyncrasy, chronic toxicity

gen may even suggest a faster recovery than with non–drug–related causes. Of course, this is not always true since recovery also depends upon regenerative ability, the pharmacokinetics of the drug (some drugs remain in the body for a very long time) and the reversibility of the lesion. Especially jaundice due to prolonged cholestasis may take a long time to disappear.

Exclusion of other possible causes (Levels C and D)

If no specific pattern is present, other possible causes should be excluded. Attention should be paid to the criteria shown in Table 3.

LEVEL E

Disappearance of chronic inflammatory hepatic injury after discontinuation of the suspected drug is highly suggestive of a causal relationship. It constitutes *no proof* because spontaneous remissions may occur (e.g. chronic active hepatitis). No normalization after discontinuation does not prove *the absence* of a causal relationship because the process may be irreversible in some cases and at some stages.

TABLE 3 *Criteria for assessment of cholestatic and parenchymatous patterns of hepatic injury*

Mainly cholestatic pattern
Normal bile ducts, gallbladder and pancreas
No pregnancy, *no* recent operation
No history of cholestasis/jaundice without the use of drugs, *no* underlying liver disease
No malignancy (e.g. Hodgkin's lymphoma!), *no* sepsis, *no* alcohol abuse
Mainly parenchymatous pattern
Viral causes excluded. At least HAV, HBV, Epstein-Barr virus and cytomegalovirus
No lymphadenopathy/lymphocytosis
No recent operation, *no* transfusions, *no* parenteral exposure (e.g. tattoos, heroin), high-risk sexual practice or other suspected exposure to viral hepatitis
No preceding hypotension/cardiac failure
No sepsis, *no* malignancy, *no* underlying liver disease
No alcohol abuse, *no* professional or other exposure to toxic substances (e.g. paraquat, 'glue sniffing')

LEVELS F AND G

In cases of suspected drug–induced vascular disorders, the exclusion of other possible causes yields too little additional information to have a decisive effect on the causal relationship, mostly because there are too many unknown factors. Veno–occlusive disease is always chemically induced and/or iatrogenic (e.g. alcoholism (26), pyrrolizidine alkaloids (27), cancer chemotherapy (28), irradiation (29)), so that the causal relationship is always probable.

The causes of large hepatic vein thrombosis, like veno–occlusive disease leading to the Budd–Chiari syndrome, are often unknown and it is therefore impossible to exclude them all.

Both may present acutely or after a prolonged period. Accordingly, the temporal relationship plays a less decisive role in the causality assessment. The other vascular disorders arise largely after long–term use. The temporal relationship between initial intake and appearance should be compatible in these cases. Especially a latent period which is too short (e.g. 1 month) suggests that the abnormalities were already present before the intake of the suspected drug. The same applies to hepatic tumors.

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Chapter 8

Liver, 1986: 6, 63–72
Key words: *cirrhosis; glafenine; hepatic injury; hepatic necrosis*

Glafenine-associated hepatic injury

Analysis of 38 cases and review of the literature

BRUNO H. CH. STRICKER[1], A. P. ROELAND BLOK[2] AND FRANS B. BRONKHORST[3]

[1]Netherlands Centre for Monitoring of Adverse Reactions to Drugs, Leidschendam, and Department of Gastroenterology and Hepatology, University Medical Center, Leiden, [2]Department of Pathology, Westeinde Ziekenhuis, The Hague and [3]Department of Pathology, Gemeenteziekenhuis, Arnhem, The Netherlands

ABSTRACT – Glafenine was associated with hepatic injury in 38 cases. The causal relationship was assessed on the basis of the temporal relationship with drug use, course and exclusion of other causes. In 27 cases a causal relationship was considered likely, i.e. 'probable' (12 cases) or 'possible' (15 cases), whereas in 11 cases it was either unlikely or unclassifiable. In both the 'probable' and 'possible' groups 60–70% of individuals were women. Jaundice was present in three-quarters of cases in both groups. Eosinophilia was more frequent in the group of 'probable' cases, and this group had the highest case-fatality rate (42%). Onset varied from 2 days (after a rechallenge) to 8 months, but most cases appeared between 2 weeks and 4 months after starting therapy. Histology in 22 cases showed a predominantly hepatocellular pattern, varying from spotty panlobular necrosis, centrilobular and (sub)massive necrosis (acute pattern) to fibrosis and cirrhosis (chronic pattern). The chemical structure of glafenine and the clinicopathological pattern it induces resemble that of cinchophen. The incidence is unknown. Either metabolic idiosyncrasy or an immunoallergic mechanism seems to be responsible.

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Glafenine is a 4-aminoquinoline derivative and is structurally related to chloroquine but lacks anti-inflammatory properties. It has been in use in The Netherlands since 1967 as an analgesic agent. The drug is an important cause of allergic reactions, and of the several hundreds of reports of anaphylactic reactions, reported to the Netherlands Center for Monitoring of Adverse Reactions to Drugs (NARD), a significant number consists of shock. From 1976 up to and including 1984, the NARD received 38 reports of hepatic injury associated with the use of glafenine in therapeutic amounts (up to 800 mg/day). Of these 38 reports some have been reported in detail in the Dutch medical literature (1–4). Besides these articles, other case histories have been published, varying from mild liver enzyme elevations with predominantly extrahepatic adverse effects to severe and irreversible liver damage (6–12).

The following study was carried out to outline the nature of the liver injury produced by glafenine. It includes clinical, biochemical and histological details of the 38 cases and a review of the literature.

Material and methods

All reports of glafenine-associated hepatic injury, received by the NARD through its nationwide voluntary reporting scheme between January 1976 and January 1985, were included in the study. All reports came from general practitioners, specialist doctors or hospitals. Full details were requested for each reported case, either by telephone or by a personal visit by the first author in order to study the original medical record. This was mostly done within 1 month after receiving the report. Minimally requested were data about the age and sex of the patients, dose and duration of use, concomitant drug use, underlying illness (e.g. chronic liver disease, cardiac failure), preceding events (e.g. operation), clinical signs and symptoms, biochemical test results, other possible causes of hepatic injury (e.g. hepatitis A and B, infectious mononucleosis, cytomegaly, transfusions) and the course and duration of the disease.

To ensure that the original diagnosis of glafenine-induced hepatic injury was not at variance with subsequent patient data, a follow-up was made of all cases, sometimes up to 9 years after the original report had been received. For all 38 reports, the clinical, biochemical and histological data were carefully reviewed, and for each case the causal relationship between drug use and hepatic injury was assessed. The causal relationship was considered 'probable' when there was an adequately documented reaction to rechallenge or a compatible temporal relationship with drug use in combination with the well-documented exclusion of other possible causes. A causal relationship was considered 'possible' when there was a temporal relationship compatible with drug use but other potential causes were insufficiently excluded. A causal relationship was considered 'unclassifiable' or 'unlikely', respectively, when too few data were available or when more likely causes were found.

Results

Of the 38 cases a causal relationship was considered unclassifiable in six. In three of these six cases too few data were available; in fact, in one of these it was impossible to determine whether liver damage or hemolysis was the cause of jaundice, whereas in another case glafenine was used because of abdominal pain in a patient with liver metastases. In three other patients a causal role of glafenine was not impossible but was difficult to assess because of scanty data about intake and concurrent administration of other potentially hepatotoxic factors (other drugs, transfusions, etc.).

In five cases a causal relationship between hepatic injury and use of glafenine was considered unlikely. One patient had had liver enzyme elevations on two occasions preceding the use of glafenine. In another patient there was improvement despite continuation of intake of glafenine and later – after discontinuation – a relapse; also, in a third patient, follow-up revealed aggravation of injury despite discontinuation of glafenine. In the fourth and fifth cases glafenine was used because of abdominal pain, in the former possibly associated with preexistent haemochromatosis, whereas in the latter there were gallstones, a negative reaction to rechallenge with glafenine, and a biopsy which showed hepatic venous congestion.

Clinical and biochemical pattern

In 27 cases a causal relationship was considered probable or possible. Since some of the cases in the 'possible' group may have had other causes (which complicates detection of a consistent pattern), symptoms have been separated per 'causality-group'.

Table 1 shows the clinical features of the group of 'probable' cases. The incidence of jaundice was the same in both the 'probable' and the 'possible'

Table 1

Symptoms and biochemical pattern of 12 'probable' cases of glafenine-associated hepatic injury

Jaundice	9/12 (75%)	Hepatocellular pattern*	9/12 (75%)
Nausea/vomiting	7/12 (58%)	Mixed cholestatic-hepatocellular**	2/12 (17%)
Hepatomegaly	6/12 (50%)	Cholestatic pattern***	–
Anorexia/malaise	3/12 (25%)	Liver enzyme elevations****	1/12 (8%)
Eosinophilia	3/12 (25%)	Fatal course	5/12 (42%)
Fever	2/12 (17%)	ALT > AST	11/12 (92%)
Abdominal pain	2/12 (17%)		
Drowsiness	1/12 (8%)		

* AST/ALT > 7½ × normal, APh < 3 × normal; ** AST/ALT > 7½ × normal, APh > 3 × normal; *** AST/ALT < 7½ × normal, APh > 3 × normal; **** AST/ALT < 7½ × normal, APh < 3 × normal.

Table 2

Clinical and biochemical features of glafenine-associated hepatic injury: 12 (highly) probable cases

NARD case no.	Age/sex	Daily dose/ duration	Time to onset first signs/sympt.	Peak of assessed biochemical values (U/l)				Other drugs	Rechallenge (time to 1st sign)	Outcome (time)
				AST	ALT	APh	Bili. (tot.)			
2	33/F	600–800 mg/ 5 days	2 weeks	760 (N < 50)	1240 (N < 50)	Normal	21 µmol/l	–	+ (2 weeks)	Recovered (5 months)
4	30/F	600–800 mg/ 3 months	3 months	186 (N < 12)	225 (N < 12)	–	33 µmol/l	Oral contracept.	+ (2 ×) (< 5 days)	Recovered (4½ months)
5	74/F	Dose (?)/ 2 months	2 months	380 (N < 27)	425 (N < 27)	168 (N < 100)	151 µmol/l	Propranolol frusemide nitrazepam	Same reaction to glafenine 3 years before	Recovered (approx. 2½ months)
6	53/F	400–600 mg/ 2 weeks	24 days	145 (N < 27)	255 (N < 27)	272 (N < 100)	24 µmol/l	Methyldopa epitizide/ triamterene	–	Recovered (approx. 2 months)
9	33/F	Irregular/ 8 months	8 months	558 (N < 15)	606 (N < 18)	–	97 µmol/l	–	+ (< 3 days)	Recovered (3½ months)
13	32/F	400–600 mg/ 59 days	45 days	1030 (N < 50)	1130 (N < 50)	–	630 µmol/l	–	–	Death
15	66/M	600 mg/d for 1 week followed by irregular use	Approx. 2 months	2100 (N < 30)	3150 (N < 20)	–	635 µmol/l	–	–	Death
25	46/F	200 mg/ approx. 3 months	Approx. 3 months	985 (NS)	700 (NS)	–	1071 µmol/l	Lorazepam bromhexine	–	Death
28	66/M	Varying dose up to 800 mg/4 months	4 months	913 (N < 20)	1360 (N < 30)	128 (N < 90)	282 µmol/l	Thyroxine	–	Death
32	51/M	400 mg/2 days	2 days	474 (N < 20)	711 (N < 26)	714 (N < 70)	< 20 µmol/l	Phenprocoumon	+ (2 days)	Recovered (6 months)
36	47/M	600–800 mg/ approx. 4 months	Approx. 4 months	580 (N < 18)	825 (N < 25)	282 (N < 105)	680 µmol/l	–	–	Recovered (approx. 2½ months)
38	87/F	400 mg/3½ months	3½ months	410 (N < 18)	515 (N < 25)	394 (N < 105)	395 µmol/l	An unknown homeopathic medicine	–	Death

groups. There were differences in the incidence of abdominal pain, hepatomegaly and anorexia/malaise, but the figures were too small to produce conclusive results. Lymphadenopathy was absent in five 'probable' and six 'possible' cases; in the other cases its presence/absence was not mentioned. Eosinophilia was more frequently reported in the 'probable' group, although in one of these cases it was discovered when liver enzymes had almost normalized again. There was a clear difference in biochemical pattern, which was assessed largely according to Zimmerman's criteria (13).

The pattern was mainly hepatocellular in the group of 'probable' cases, whereas the group of 'possible' cases showed a variety of patterns. Since the former group is the more important one, details about these cases are given in Table 2.

In the 'probable' group 8 out of 12 patients were women (67%), whereas the 'possible'-group consisted of 9 women out of 15 patients (60%). The mean age in the 'probable' group was 51.5 year (range: 30–87) with a mean age for men of 57.5 year (range: 47–66) and for women of 48.5 year (range: 30–87). In the 'possible' group the mean age was 57 year (range: 25–91) with a mean age for men of 57.7 year (range: 30–91) and for women of 56.7 year (range: 27–79).

In the 'probable' group the daily dose ranged from 200 to 800 mg, and in the 'possible' group from 200 to 1600 mg. The latent period between first use and onset of first symptoms was mostly between 0.5 and 4 months (mean: 2.7; range: 2 days to 8 months) in the 'probable' group. This was also the case in the 'possible' group, although here data about duration of use were somewhat less well-documented.

In the 'probable' group, five cases had a fatal course (42%) as against two cases in the 'possible' group (13%). The overall case-fatality rate was 26%. All fatal cases exhibited hepatic failure, either isolated or in combination with renal failure, gastrointestinal haemorrhage or arrhythmia as the primary cause of death.

Most patients in both groups used glafenine because of dental pain, headache or neuralgic pain. Concurrent drugs were used in 8 of 12 patients in the 'probable' group, several of which have been associated with hepatic injury before. However in Case 2, oral contraceptives were not used during the rechallenges. In Cases 5 and 32, recovery followed discontinuation of glafenine despite continuation of other drugs. Patient 5 had also used ibuprofen during the first episode of hepatic injury but not during the rechallenge 3 years later. The drugs used in Case 6 had been used safely for over a year and this fact, especially in the absence of fever, makes methyldopa an unlikely cause (17). Patients 25 and 28 were on long-term treatment with drugs which are not (or perhaps very rarely) associated with hepatic injury. Whereas some non-orthodox medicines (especially herbal agents which contain pyrrolizidine alkaloids) are hepatotoxic, homeopathic medicines (Case 38) are highly diluted and therefore an unlikely cause of hepatic injury.

In the 'possible' group several patients used other drugs concurrently, but these were either unknown as a cause of hepatic injury or continued without problems. Only in Case 14 (diazepam,

Table 3
Histological patterns of hepatic injury in 22 'probable' and 'possible' cases studied by the authors**

Predominating pattern of injury	Probable	Possible	Total
Steatosis	–	26	1 (5%)
Cholestasis	6	21, 22	3 (13%)
'Spotty' necrosis	32	33	2 (9%)
Centrilobular/submassive	2, 5, 9, 36, 38	1, 3	7 (32%)
Massive	13 (?), 15	14, 37	4 (18%)
Fibrosis/cirrhosis	25, 28	16, 35	4 (18%)
Undetermined	4	–	1 (5%)
Total	12	10	22

** The underlined numerals refer to the NARD nos. of the study.

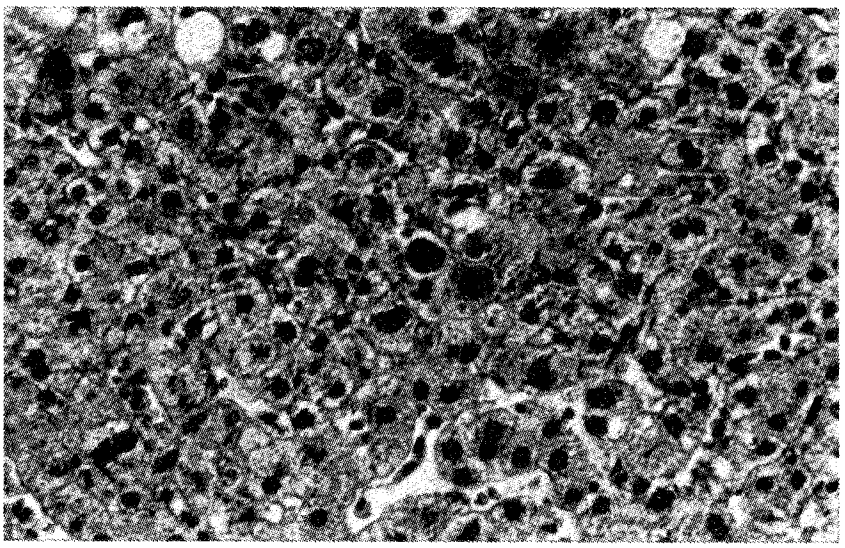

Fig. 1. (Case 32) Spotty necrosis. Varying degrees of ballooning and binucleation. Note acidophilic degeneration (arrow) (HE 250×) (by courtesy of A. H. Lely and H. Barrowclough).

pipotiazine, secobarbital), Case 21 (ibuprofen), Case 22 (paracetamol, diazepam, chlordiazepoxide, acetylsalicylic acid), Case 27 (chlortalidone) and Case 35 (ajmaline, phenprocoumon, halothane) may other drugs also have been responsible.

Histopathology

In 33 of the 38 cases a biopsy and/or autopsy was performed. The original slides were requested by us from the reporting medical practitioners and/or the involved pathologists, who kindly co-operated in all cases. However in three cases it was impossible to obtain the original slides. In two other cases a biopsy was not successfully performed. Of the remaining 28 biopsies/autopsies, 12 consisted of 'probable' and 10 of 'possible' cases (see Table 3). All material had been stained with hematoxylin-eosin, whereas most slides had been stained with Gomori or Gordon & Sweet's, Azan, periodic acid Schiff (PAS) with and without diastase and Perl's or Turnbull's iron reaction.

Cholestasis. One 'probable' and two 'possible' cases demonstrated a mainly cholestatic pattern, varying from almost pure cholestasis in Case 21 (however, with many ceroid-laden Kupffer cells, thereby suggesting past necrosis) to cholestatic hepatitis with bile staining and plugging, focal infiltration with lymphocytes and eosinophils, ballooning and acidophilic bodies in zone III, as seen in Case 6. In Case 22 cholestasis in zones II and III was accompanied by panlobular ballooning, spotty necrosis, infiltrated by a mixed lymphocytic/neutrophilic infiltrate, and moderate steatosis. There was no Mallory's hyaline or a pattern of alcoholic steatosis. Liver architecture was preserved in all cases.

Spotty necrosis. One 'probable' and one 'possible' case showed a pattern of panlobular spotty necrosis with occasional cell drop-out and acidophilic bodies, mostly surrounded by a predominantly lymphocytic infiltrate (Fig. 1). There was a varying degree of ballooning in all areas and a few foci of steatosis. Cholestasis was absent and

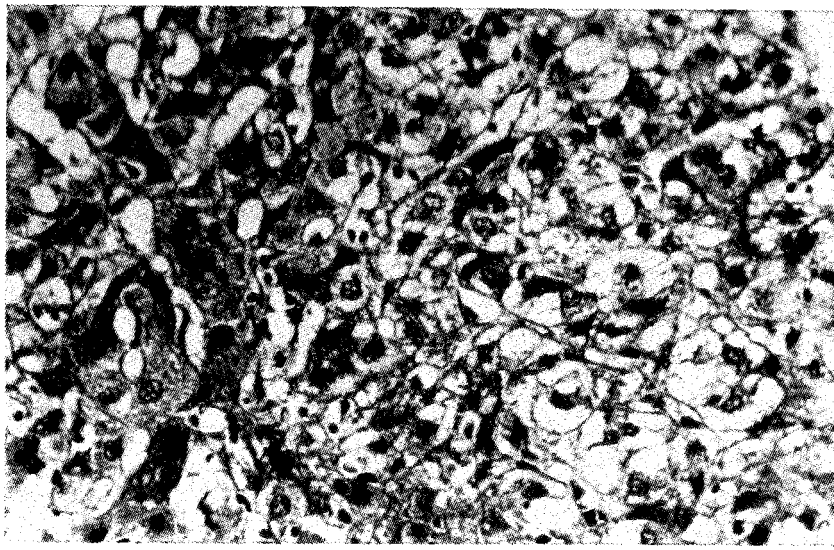

Fig. 2. (Case 38) Centrilobular area with much cell drop-out and ballooning. Predominantly mononuclear infiltration (HE 250×).

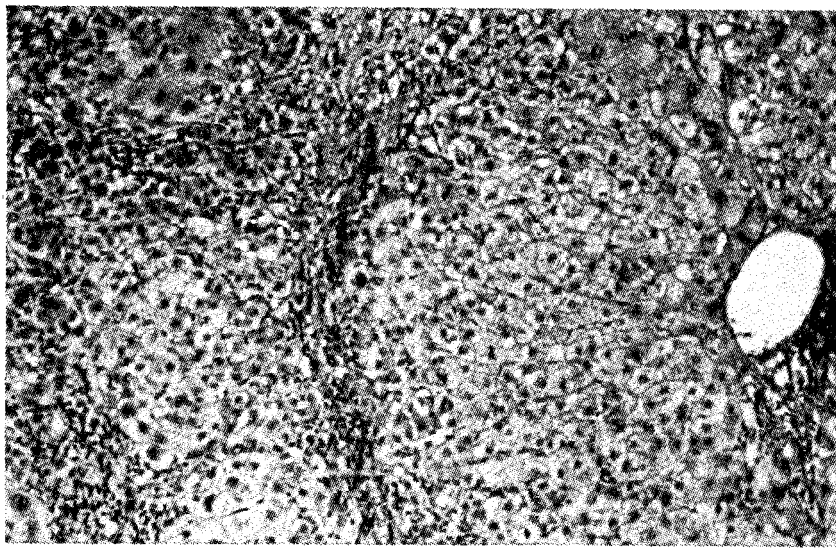

Fig. 3. (Case 5) Bridging necrosis. Cell drop-out and central-central bridging. Ballooning in zones I and II. Predominantly mononuclear infiltration. (HE 100×) (by courtesy of L. van Leeuwen and M. Th. M. Sindram).

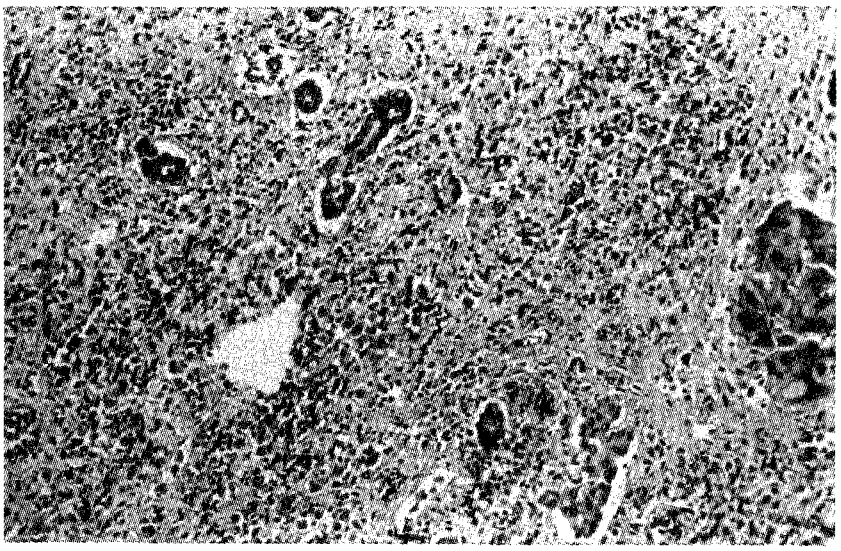

Fig. 4. (Case 15) Massive necrosis. Panlobular necrosis with haemorrhage and a mixed mononuclear/neutrophilic infiltrate. (HE 100×) On the right, a small island of relatively preserved hepatocytes.

the architecture was preserved. There was portal expansion and moderate infiltration mainly with mononuclear cells; Kupffer cell hyperplasia was moderate.

Centrilobular/submassive necrosis. In five 'probable' and two 'possible' cases there was centrilobular (zone III) to submassive (zones II and III) necrosis (Fig. 2 and 3), often with many erythrocytes in zone III. In three cases there was a sharp margin between complete cell drop-out in zone II and/or III and the relatively well-preserved zone I. Ballooning of varying severity, however, was present in most non-necrotic areas. In all cases there was collapse of the reticulin framework with passive septum formation, varying from a single bridge to extensive central-central and central-portal bridging. The infiltrate was predominantly mononuclear in all cases, mainly lymphocytic, but always mixed with a mild to moderate number of neutrophils and eosinophils. Cholestasis was present in Case 36 and 38, in the latter accompanied by marked bile duct proliferation. Steatosis was absent in all cases. In Case 2 a control biopsy 5 months later showed much improvement, however with persistence of some intralobular focal areas of necrosis, fibrotic bridges and mild piecemeal necrosis. Follow-up in Cases 3, 9 and 36 within 1 year after admission showed normalization but with rests of fibrotic bridging in all.

Massive necrosis. In four cases there was massive necrosis (zones I–III). In Case 13 the presence of a small and collapsed liver at autopsy suggested massive necrosis, but the slides obtained contained too little material for diagnosis. In the other cases no normal liver cells were visible between areas of cholangiolar proliferation. Cell debris and many erythrocytes were present in necrotic areas (Fig. 4). In Cases 14 and 15 there were also some areas of submassive necrosis with wide bridges of collapsed fibres and the beginning of fibrosis. In all cases infiltration was relatively moderate, predominantly lymphocytic but with many neutrophils and some eosinophils. In one case Kupffer cell hyperplasia was very prominent.

Fibrosis/cirrhosis. Two 'probable' and two 'possible' cases had marked fibrotic changes in their biopsies. In Case 35 there was cholangitis with bile duct destruction and proliferation with marked steatosis and periportal, perivenular and perisinusoidal fibrosis. There was a mixed mononuclear/eosinophilic infiltrate. In Cases 25 and 28 there was nodular regeneration separated by wide fibrotic bridges, but no micronodular cirrhosis at autopsy. Case 38 had a pattern of micronodular cirrhosis without signs of active necrosis at autopsy, performed 2 months after a biopsy showed submassive necrosis, portal fibrosis, bile duct proliferation and areas of perisinusoidal fibrosis. This patient, however, had no history of exposure to arsenicals, high-dose vitamin A, vinylchloride, thorium dioxide or cytostatic agents. In Case 16 the first half of the biopsy was fibrotic, whereas the 'deeper' part showed centrilobular necrosis and ballooning with formation of fibrotic bridges. A control biopsy, 4 years later, showed normal liver tissue with minimal fibrotic rests.

Discussion

It is clear from this study that glafenine may induce severe hepatic damage. Although the less consistent pattern in the group of 'possible' cases suggests that in some cases another cause may have been responsible, this is unlikely to have played a role in the group of 'probable' cases. In the latter group, cases were either 'rechallenge-proven', thereby suggesting a highly probable causal relationship, or no other causes such as hepatitis A, B, infectious mononucleosis, cytomegaly, cardiac failure, sepsis, malignancy or underlying liver disease were demonstrated. The high AST-values in combination with an ALT/AST-ratio greater than 1 and the absence of alcoholic liver disease on biopsy excludes alcohol as a cause in the 'probable' group. The absence of lymphadenopathy/lymphocytosis and the fact that no transfusions had been given, make any viruses other than the aforementioned hepatotropic viruses (e.g. NANB viral hepatitis) less likely causes. Only Patient 32 had received a transfusion preceding the second period of administration of glafenine, but liver enzymes were already elevated before the transfusion.

The pattern was predominantly hepatocellular, especially in the 'probable' group. This is of importance since the pattern was not used as a criterion for inclusion in one of the groups and therefore it seems that glafenine-induced hepatic injury is primarily hepatocellular. Unlike viral hepatitis, which often consists of spotty necrosis randomly distributed panlobularly, glafenine-induced hepatic injury is predominantly centrizonal and mostly severe with (sub)massive necrosis. The clinicopathological pattern may be distinguished from other drugs in the same pharmacotherapeutic group (Table 4).

The chemical structure of glafenine shows some resemblance to cinchophen, an uricosuric agent used as an analgesic in the early decades of this century and dropped from clinical use because of

Table 4
Comparison of characteristics of hepatic injury induced by some analgesics/antirheumatics

	Glafenine	Salicylates[15]	Phenylbutazone[16]	Cinchophen[14]
Jaundice	75%	rare	50%*	100%**
Rash/fever	17%	–	50%	11%
Eosinophilia	25%	–	(?)	(?)
Pattern	H	LE	M/H	H
Granulomas	–	–	+	–
Latent period	0.5–4 mo.	< 1 wk.	< 6 wk.	0.5–4 mo.
Dose dependency	–	+	–/+	–
Case-fatality rate	42%	low	22–28%	52%

* Approximation based on serum bilirubin levels (total) above 3 mg/dl (51 μmol/l).
** Overrepresented because of the absence of sensitive assessment methods. Therefore the number of fatal cases was probably overrepresented too.
Pattern hepatocellular (H), mixed cholestatic-hepatocellular (M) or non-symptomatic liver enzyme elevations. Salicylate-associated Reye-like syndrome is not included.

its ability to induce severe hepatic necrosis (13). The basis of both drugs is the quinoline structure, but their side-chains differ. Relatively little is known about their metabolism. Both agents cause the same clinical and histological pattern of hepatocellular injury with a fatality-rate of approximately 50%. Immunoallergic phenomena such as rash, fever or arthralgia occur in 10–15% of cases of hepatic injury associated with the use of both drugs.

In the (old) literature about cinchophen eosinophilia was not mentioned. Possibly at that time no special attention was paid to this clinical sign, since cinchophen-induced hepatic necrosis was the first recognized example of the idiosyncratic, non-dose-related (i.e. 'non-toxic') type of drug-induced hepatic injury. Most cases of cinchophen-associated hepatic injury appeared between 2 weeks and 4 months after starting therapy (14). This is analogous to the latent period in this study.

Besides the cases discussed in this study, eight cases of hepatic injury associated with glafenine have been described in the medical literature (6–12). They concerned six women (mean age: 69 year) and two men (mean age: 52 year). In three cases, hepatic injury consisted of mild liver enzyme elevations (7, 9, 12) and eosinophilia (7, 12), which both recurred after rechallenge (7, 12). In one of these three cases fever, pulmonary involvement and eosinophilia accompanied liver enzyme elevations (12). In another case AST/ALT-elevation was trivial when compared to severe intravascular coagulation and renal failure (9). In a fourth cases serum ALT was up to 37 times normal value and accompanied by hemolysis and renal failure; however, biopsy 1 day later showed bile staining of hepatocytes in the absence of necrosis (8). The remaining four cases comprised a pattern varying from a mixed cholestatic-hepatocellular to a hepatocellular pattern with the first symptoms appearing between 0.5 and 3 months after starting therapy (6, 10–12). A rechallenge, performed in three cases, was positive within a period of 7–9 days (6, 10, 12). In one case no biopsy was performed but biochemistry suggested recovery within 2 months (6). One patient exhibited a pattern of cirrhosis and fatal hepatic failure 1 month after starting the second period of administration of glafenine (10). In another case a biopsy, performed 5 months after discontinuation, suggested post-necrotic cirrhosis and showed chronic active hepatitis (12). With the exception of one case with a weakly positive basophil degranulation test (9), immunologic tests gave negative results.

The mechanism of glafenine-induced hepatic injury is unknown. The reaction is unpredictable, dose-independent and seems to be relatively rare. We are not aware of any animal toxicity studies suggesting a hepatotoxic effect. Therefore a direct toxic effect seems unlikely. Glafenine is a frequent cause of anaphylactic reactions, and it seems plausible that these, when accompanied by shock, may cause transient elevations of liver enzymes. In those cases where extrahepatic clinical features dominate, other immunological mechanisms may also play a role, as in the patient with hemolysis, renal failure, liver enzyme elevations and circulating immune-complexes (8). In the cases where hepatic injury is the only sign, however, other mechanisms may be responsible. The delayed onset, the infrequent presence of rash, arthralgia and fever, and the negative immunologic tests seem compatible with a metabolic type of idiosyncrasy. On the other hand, eosinophilia was fairly frequent and there was an accelerated reaction to rechallenge. Although, in our opinion, the latter may also be compatible with 'metabolic idiosyncrasy' when the period between dechallenge and rechallenge is short, it seems that an immunoallergic component cannot be excluded.

Our study like other studies suggests a higher incidence in women. Since we are not aware of any reliable consumption data, it is impossible to say whether this is related to a more frequent use of analgesics in this group, or to an increased vulnerability of females; the latter has been suggested in many other studies of drug-induced hepatic injury.

If we compare the 117 cinchophen-associated cases, collected by Weir et al. (14) from the U.S.A. (population in 1932: 125 million individuals) with the 32 likely and unclassified cases in this study (population of The Netherlands in 1984: 14.5 million individuals), we have the impression that glafenine-associated hepatic injury may be equally frequent. Weir et al., however, collected mostly cases reported in the medical literature. These

were almost always symptomatic since more sensitive methods of liver injury assessment (serum liver enzymes) were not available at that time. Even if we compare only the 12 symptomatic cases of glafenine-associated hepatic injury described or summarized in the Dutch medical literature (1–5, 12) with Weir's collection, it seems reasonable to assume that glafenine-associated hepatic injury may be as frequent as cinchophen-induced hepatic injury. According to Zimmerman (13), the latter had an incidence of up to 0.1%. It should be realized, however, that some cases may remain unrecognized whereas some others may be wrongly associated with other causes. Since underreporting of adverse effects to National Monitoring Centers is a widely recognized problem, and since sales figures are unknown to us, it is difficult to estimate the incidence of glafenine-induced hepatic injury.

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Address:
B. H. Ch. Stricker
P.O. Box 439
2260 AK Leidschendam
The Netherlands

Chapter 9

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Ketoconazole-Associated Hepatic Injury

A Clinicopathological Study of 55 Cases

B.H.Ch. Stricker[1], A.P.R. Blok[2], F.B. Bronkhorst[3], G.E. Van Parys[4] and V.J. Desmet[4]

[1]Netherlands Centre for Monitoring of Adverse Reactions to Drugs, Leidschendam; Departments of Histopathology of the [2]Westeindeziekenhuis, The Hague and [3]Gemeenteziekenhuis, Arnhem (The Netherlands) and of the [4]University Hospital Gasthuisberg, Leuven (Belgium)

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Summary

Fifty-five cases of ketoconazole-associated hepatic injury, reported to the Netherlands Centre for Monitoring of Adverse Reactions to Drugs, were analysed in detail. In 50 cases a causal relationship was considered likely, i.e. 'probable' (27 cases) or 'possible' (23 cases). Eight-four % of individuals were women. Forty-six % of patients were over 50 years of age which suggests that, considering the lower prescription rate in this age group, the elderly are more vulnerable to ketoconazole. In 60% of all cases hepatic injury appeared within the first 6 weeks of therapy but in the group of 'probable'-cases the onset was mostly later. Jaundice was present in 44% of all cases but in 63% of the group of 'probable'-cases. Eosinophilia (10%), fever (6%) and rash (2%) were uncommon. Biochemically the pattern was hepatocellular in 54%, cholestatic in 16% and mixed cholestatic-hepatocellular in 30%. Histology (14 cases) showed a predominantly hepatocellular pattern in 57% with extensive centrilobular necrosis and mild to moderate bridging. In 43% cholestasis predominated. None of the cases had a fatal course. The incidence of symptomatic hepatic injury may be estimated at approximately 1:2000 but is probably higher. The mechanism of ketoconazole-induced hepatic injury seems to be based on metabolic idiosyncrasy although it is not excluded that in some patients an immunoallergic mechanism is causative.

Introduction

Ketoconazole is an antimycotic agent introduced in 1981. Several case-reports have been published in the medical literature of mild to severe hepatic injury [1–21,23] and 2 studies of cases have been made [20,21]. These studies, however, partly cover the same cases and include only 2 biopsies and 1 autopsy. In addition to the first cases, reported to the Netherlands Centre for Monitoring of Adverse Reactions to

Correspondence address: B.H.Ch. Stricker, Medical Officer, Netherlands Centre for Monitoring of Adverse Reactions to Drugs, P.O. Box 439, 2260 AK Leidschendam, The Netherlands.

Drugs (NARD) and published [12], 50 cases have been reported. Since cases of hepatic injury are intensively monitored, the majority of cases of ketoconazole-associated hepatic injury are well-documented and include histopathology of 16 cases. Four of these 50 cases have been published before [10,11,13]. Here we outline the clinicopathological pattern of 50 'probable'- and 'possible'-cases of ketoconazole-associated hepatic injury and review the literature.

Material and Methods

The study includes all cases of ketoconazole-associated hepatic injury reported to the NARD between 1981 and April 1986. All reports came from medical doctors. Of each case full details were requested, mostly within 1 month after receiving the report: age/sex, dose/duration of use, concomitant drugs, preceding events (e.g. recent operations/transfusions, hepatitis in contacts), underlying illness (e.g. chronic liver disease, cardiac failure), signs/symptoms, physical examination, imaging procedures, liver enzyme assessments and exclusion of other possible causes (e.g. HAV, HBV, cytomegaly, infectious mononucleosis). A follow-up was made of all cases. All clinical, biochemical and available histological data were carefully evaluated and for each case the causal relationship between use of ketoconazole and hepatic injury was assessed. It was considered as 'definite' when there was a well-documented reaction to rechallenge and as 'probable' when there was a compatible temporal relationship between onset of hepatic

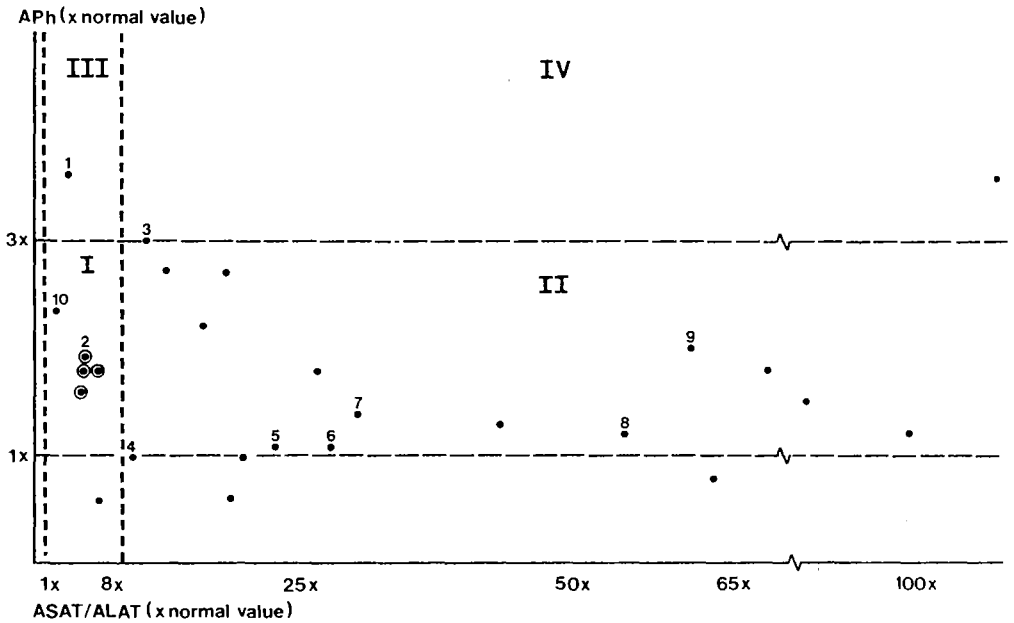

Fig. 1. Biochemical pattern in 26 probable cases (based on refs. [21,28] with a modification by the authors). Numericals refer to biopsy numbers in text. Cases of pure cholestasis encircled.

I = Liver enzyme elevation (alkaline phosphatase (APh) < 3× normal, aspartate/alanine aminotransferase (ASAT/ALAT) < 8× normal, bilirubin < 2× normal).
Pure cholestasis (as above but bilirubin > 2× normal).

II = Hepatocellular (APh < 3× normal, ASAT/ALAT > 8× normal).

III = Cholestatic hepatitis (APh > 3× normal, ASAT/ALAT < 8× normal).

IV = Mixed cholestatic-hepatocellular (APh > 3× normal, ASAT/ALAT > 8× normal).

injury and drug use in combination with adequate exclusion of other possible causes. When there was a compatible temporal relationship but insufficient exclusion of other potential causes the causal relationship was considered to be 'possible'. In 5 patients a more likely cause than ketoconazole was found and these cases were omitted.

Results

A causal relationship was considered as likely in 50 cases, i.e. 'definite' in 1 case (hereafter included in the group of 'probable'-cases), 'probable' in 26 cases and 'possible' in 23 cases. With the exception of abdominal pain signs/symptoms were less frequently reported in the group of 'possible'-cases. In none of the 50 likely cases lymphadenopathy was reported; indeed its absence was emphasized in 28 reports. One of the reports mentioned rash, starting 1 week after discontinuation of ketoconazole. Eosinophilia (≥7%) was present in 2 out of 22 'probable'-cases in which a differential count was mentioned. Eosinophilia was present in 3 'possible'-cases but clearly stated as absent in 6 cases. In 3 'probable'-cases hepatic injury was accompanied by fever, low grade in 2 and high (39.5 °C) in 1 case. The biochemical pattern (Fig. 1) was hepatocellular in 18 'probable'- (67%) and 9 'possible'-cases (39%). In the group of 'possible'-cases most reports concerned mild to moderate liver enzyme elevations (48%). In the 'probable'- and 'possible'-group 81.5% and 87% of patients respectively were female. The mean age in the 'probable'- and 'possible'-group was 48.4 years (range: 24–76) and 53 years (range: 34–99) respectively without a significant difference in age between both sexes. Twenty-seven patients (54%) used ketoconazole because of onychomycosis and 15 (30%) because of dermatomycosis. The daily dose was 200 mg in 90% of cases. The mean latent period between first intake of ketoconazole and onset of first symptoms in the 'probable'- and 'possible'-group was 68 days (range: 1 week–7½ months) and 52 days (range: 16 days–6½ months) respectively. In 44% and 79% respectively onset was within the first 6 weeks. None of the 50 cases had a fatal course. In the group of 'probable'-cases 13 individuals (48%) also used other drugs but these were either continued or reinstituted without aggravation or relapse of hepatic injury. We explicitly asked for ingestion of griseofulvin in the 6

TABLE 1

SYMPTOMS AND SIGNS IN 27 PROBABLE CASES OF KETOCONAZOLE-ASSOCIATED HEPATIC INJURY

Jaundice	63%	Abdominal pain	4%
Malaise	59%	Rash	4%
Nausea/vomiting	41%	Excessive perspiration	4%
'Influenza'-like	11%	Headache	4%
Fever	11%	Amenorrhoea	4%
Anorexia	11%	Hepatomegaly	33%
Eosinophilia	9%	ALAT > ASAT	89%
	No symptoms 11%		

TABLE 2

CLINICOPATHOLOGICAL FEATURES OF 99 CASES OF KETOCONAZOLE-ASSOCIATED LIVER INJURY

	Literature [1,3,6–9, 14–19] (n = 16)	Lewis et al. [21][a] (n = 33)	This study[b] (n = 50)
Female	50%	67%	84%
>50 year	44%	61%	46%
Latent period			
≤6 weeks	38%	66%	60%
>6 weeks	62%	34%	40%
Biochemical pattern			
hepatocellular	75%	55%	54%
cholestatic	–	15%	16%
LEE/mixed	25%	27%	30%
Histology (predominating pattern)	(n = 9)	(n = 3)	(n = 14)
necrosis	100%	100%	57%
cholestasis	–	–	43%
Clinical pattern			
jaundice	56%	82%	44%
eosinophilia	13%	–	10%
fever	–	–	6%
rash	–	–	2%
Fatal course	19%	3%	–

[a] Includes references [5,23]
[b] Includes references [10–13].

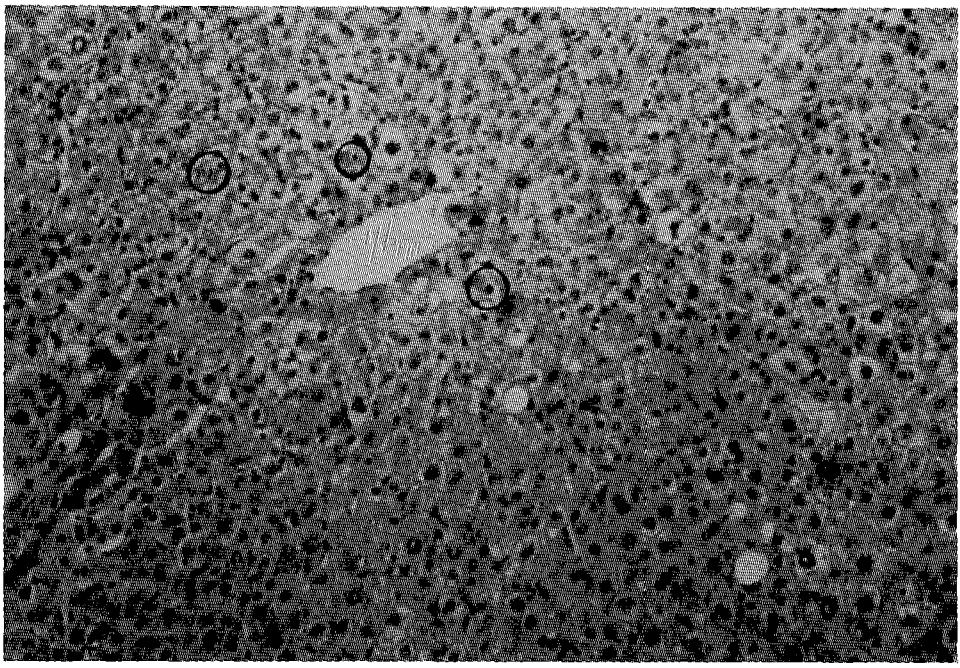

Fig. 2. Biopsy No. 1. Centrilobular cholestasis, no necrosis, no infiltration, bile plugs encircled. HE, ×100.

months preceding use of ketoconazole in all 'probable'-cases in which hepatic injury appeared in the first 6 weeks of therapy. In none of these cases griseofulvin had been used. Only 2 of 50 cases had previously developed an idiosyncratic reaction to an other drug (4%). Forty had not whereas in 8 cases a reaction to other drugs was not mentioned or unknown.

Histopathology

Two 'possible'- and 3 'probable'-cases exhibited a mainly cholestatic pattern. One 'probable'-case (No. 2) showed almost pure cholestasis, however, with some ceroid-laden Kupffer cells. Cases 1 (Fig. 2) and 3 showed an analogous pattern but with moderate portal and minimal lobular mononuclear infiltration. Another 'possible'-case showed, besides cholestasis, many acidophilic bodies and discrete cholangiolitis with many eosinophils. Biopsy 10 showed distinct cholangitis without significant cholestasis in the absence of necrosis or lobular infiltration.

Biopsy 4 showed a pattern varying from mild foci of necrosis to moderate, sharply demarcated, centrilobular necrosis surrounded by some acidophilic bodies. Infiltration was mainly mononuclear but mixed with some neutrophils. Zones I and II were well-preserved, however, with occasional ballooning. Biopsies 5–9 showed more extensive, but also sharply delineated, necrosis with confluence of necrotic zones and bridging by collapse of the reticulin framework. Bridging was relatively mild in biopsies 5 (Fig. 3), 7 and 9. In biopsies 6 (Fig. 4) and 8 signs of beginning fibrosis were present. Biopsies 5–9 exhibited moderate to dense portal infiltration, mainly with lymphocytes but often mixed with some neutrophils. Most biopsies showed a mild degree of bile duct proliferation. Occasional areas of 'piece-meal' necrosis were also present in most biopsies but in case 9

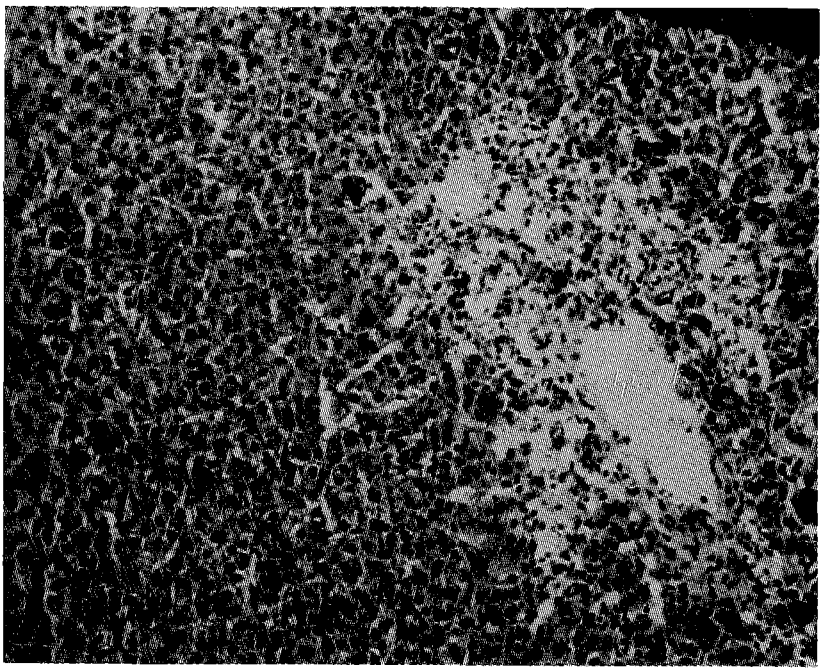

Fig. 3. Biopsy No. 5. Sharply demarcated centrilobular necrosis. HE, ×100.

this was accompanied by an increase in portal and periportal fibrosis. None of biopsies 4–9 showed bile plugging. Biopsy 6 showed minimal steatosis. In biopsy 9 there was hyperplasia of perisinusoidal lipocytes, especially in the parenchyma surrounding necrotic areas; an increase in perisinusoidal fibrotic fibres, however, was absent. Biopsies of 2 'possible'-cases showed a hepatocellular pattern. In one of these extensive, sharply demarcated, centrilobular necrosis and mild bridging were present. The second biopsy showed acidophilic bodies and focal areas of necrosis in zones II and III with some portal areas showing 'piece-meal' necrosis.

Discussion

It is important to distinguish 'probable'- from 'possible'-cases since it is especially the former group which gives insight into the clinicopathological pattern by a particular drug; 'possible'-cases are mostly less well-documented. The absence of a history of hepatitis in contacts, recent operations and parenteral procedures make NANB viral hepatitis a less likely cause. Although some mycotic infections may cause hepatic injury, this is rare and unlikely as a cause in these patients since ketoconazole was mostly used because of uncomplicated onycho- or dermatomycosis. Moreover, hepatic injury developed in these patients during use of ketoconazole instead of during the more active phase of the infection before ingestion.

The pattern was mostly hepatocellular but cholestatic hepatitis and even pure cholestasis may occur. Most cases in the medical literature [1–21] consisted of a mainly hepatocellular pattern and a biopsy in some of these cases showed predominantly necrosis, varying from mild to moderate [1,6,8,9,19,21] and

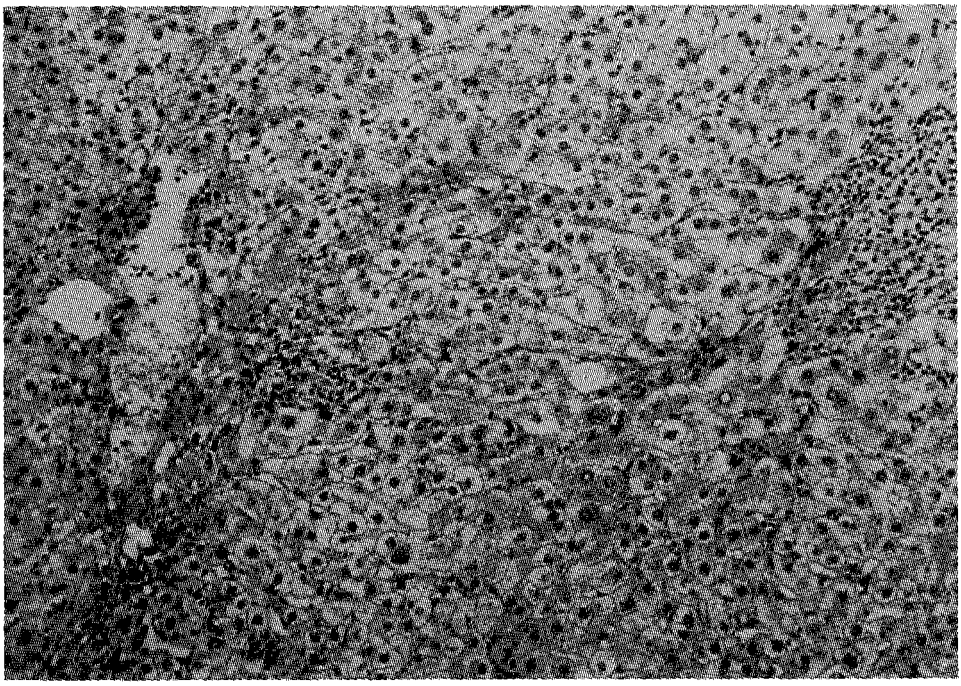

Fig. 4. Biopsy No. 6. Centrilobular necrosis with beginning central-portal bridging. HE, ×100.

more extensive bridging necrosis [7] to massive necrosis [15,17,21]. Immunoallergic signs such as high fever, rash and eosinophilia were uncommon. In The Netherlands (1982–1986) approximately 60% of individuals treated with ketoconazole were women [30]. Since this included treatment of mycotic vaginitis, the male to female ratio of patients treated for skin- or nailmycosis may be estimated at approximately 50/50. Even if we exclude mycotic vaginitis, more than 80% of patients was female. Since we are not aware of any other reason for this overrepresentation this suggests that females are more likely to develop ketoconazole-induced hepatic injury. Forty-six percent of individuals was over 50 years old. It seems likely that the elderly are more susceptible since in The Netherlands (1982–1986) only 14–19% of all tablets were prescribed to patients of 55 years and older [30]. Most of our cases appeared within the first 6 weeks. When we distinguished, however, between 'probable'- and 'possible'-cases, it came out that 'probable'-cases mostly appeared between 6 weeks and 7½ months after starting treatment. Most of our patients recovered within 3 months after discontinuation of ketoconazole but in 2 'possible'-cases there was still a progressive but temporary increase in liver enzymes after discontinuation. This was also noted in some other cases [6,7,9,16], in one of which administration of corticosteroids was followed by rapid recovery [9]. The low mortality in Lewis et al. [21] and our studies is remarkable, especially since there is no doubt that ketoconazole-associated hepatic injury may be fatal [15,17,21,22]. Moreover, the very high ASAT/ALAT-levels in several of our patients seem to indicate that the reaction is potentially lethal. The high number of patients with a history of hepatitis (26%) or idiosyncrasy to other drugs (29%) in the study by Janssen et al. [20] was not confirmed in our study and we do not think that it has any relationship

with the problem. The high number of patients with preceding use of griseofulvin (45%), mentioned by Janssen et al. [20], seems related to the underlying mycotic infection rather than being a predisposing factor since our specific enquiry revealed no cases in which use of griseofulvin preceded use of ketoconazole.

The incidence of ketoconazole-associated liver enzyme elevations has been varyingly reported as 6% [20] and approximately 12% [21] although some studies give higher figures [21] and some much lower [25,26]. The incidence of symptomatic hepatic injury was estimated at 1:10 000 [20] and later at 1:15 000 [22] by the manufacturer. No correction was made for the reporting-rate, however, which was estimated in the U.K. at 11–20% of serious and non-trivial adverse effects [29]. Moreover, the estimation was solely based on cases known to the manufacturer which excludes many cases reported to regulatory authorities. Hence the real incidence may be estimated at 1:1000–3000 but is probably higher. We realize that voluntary reporting schemes are not suitable for calculations of incidences. Nevertheless we cannot discard the fact that ketoconazole was the most frequently reported suspected cause of hepatic injury (NARD-period: 1982–1985: 22% of all reports of drug-induced hepatic injury) suggesting that ketoconazole-associated hepatic injury is a serious clinical problem.

The fact that mild liver enzyme elevations may disappear despite continuation of therapy has been explained as a Herxheimer-like reaction, reflecting release of hepatotoxic mycotoxins and accompanying disappearance of the mycotic organism [20]. In 2 cases from the literature [3,19] hepatic injury disappeared despite continuation of ketoconazole in the same, or a lower, dose but this may as well mean that another cause was responsible. Moreover a Jarisch–Herxheimer reaction develops very early after starting treatment but not after several weeks. High doses (121–139 mg/kg body weight) induced significant hepatic injury in several experimental species after 2–4 weeks administration [21]. This high-dose hepatotoxicity, however, is not relevant for the use of therapeutic amounts in man. Moreover acute overdosage (5.8 g) in an 18-year-old person had no measurable effect on the liver [24] and we know of 1 case treated for several months with 1200 mg daily without liver enzyme elevations. Moreover clinical trials with daily doses up to 800 mg did not reveal a higher incidence of liver enzyme elevations [25,26]. This absence of dose-dependency makes a direct toxic effect an unlikely mechanism in these cases. Lewis et al. [21] suggested metabolic idiosyncrasy as a more likely mechanism than hypersensitivity although they did not dismiss the possibility that the latter contributed in some cases. Indeed the uncommon hypersensitivity signs, variable latent period and absence of a more severe and immediate (<48 h) response to rechallenge are in favour of such a mechanism. Even the accelerated reaction to rechallenge as seen in one of our cases and in the literature [1,6] does not necessarily point at an immunoallergic reaction since it is likely that, if the time between two treatment-periods is too short, an accumulated toxic intermediate has not been removed from the body completely and may lead to a more rapid relapse after rechallenge. A positive lymphocyte stimulation test [6] and immunoallergic signs/symptoms in a small minority suggests that hypersensitivity may play an (additional?) role occasionally.

We advise monitoring of liver enzymes during treatment with ketoconazole at weekly intervals for the first 2 months, especially since mild liver enzyme elevation during this period may normalize spontaneously but may as well progress to symptomatic liver injury. Later, assessment every 2 weeks and after 6 months monthly assessment of liver enzymes seems justified.

Although ketoconazole is a very useful antimycotic agent re-evaluation of its need for long-term treatment of simple onychomycosis seems warranted.

Acknowledgements

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Chapter 10

HEPATIC INJURY ASSOCIATED WITH THE USE OF NITROFURANS

A CLINICOPATHOLOGICAL STUDY OF 52 REPORTED CASES

Bruno HCh Stricker (1), AP Roeland Blok (2), Frans HJ Claas (3), Geert E Van Parys (4), Valeer J Desmet (4)

(1) Netherlands Centre for Monitoring of Adverse Reactions to Drugs, Rijswijk, and Department of Internal Medicine II, University Hospital Dijkzigt, Rotterdam, The Netherlands

(2) Department of Histopathology, Westeindeziekenhuis, The Hague, The Netherlands

(3) Department of Immunohaematology, University Medical Centre, Leiden, The Netherlands

(4) Department of Histopathology, University Hospital Gasthuisberg, Leuven, Belgium

Correspondence address:
BHCh Stricker, Medical Officer, Netherlands Centre for Monitoring of Adverse Reactions to Drugs, P.O.Box 5406, 2280 HK Rijswijk, The Netherlands

SUMMARY

Fifty cases of nitrofurantoin-associated and two cases of nifurtoinol (hydroxymethylnitrofurantoin)-associated hepatic injury reported to the Netherlands Centre for Monitoring of Adverse Reactions to Drugs were analysed in detail. In 38 cases a causal relationship was considered likely, i.e.'highly probable' (n=4), 'probable' (n=23) or 'possible' (n=11). In 25 cases hepatic injury was of the acute type whereas 13 cases presented a chronic type of reaction. Both types were more common in the elderly. The female preponderance (89%) in this study largely reflected the higher incidence of urinary tract infections in this group. Eighty percent of the acute reactions appeared within the first 6 weeks of treatment and these were sometimes accompanied by fever (28%), rash (12%) and eosinophilia (16%). Biochemically the pattern was mainly hepatocellular (32%), whereas mixed cholestatic-hepatocellular (4%) and cholestatic (4%) patterns were uncommon. Although mild to moderate liver enzyme elevations (60%) were common these were mostly symptomatic. The reaction was fatal in one 'acute' and one 'chronic' case. In the chronic cases nuclear (82%) and smooth muscle (73%) antibodies and LE-cells (50%) were frequently present. HLA typing showed no increase of the HLA B8 or HLA DRw3 haplotype. HLA DR2 (56%) and HLA DRw6 (56%) were more frequent than in controls (both 29%), but this was not statistically significant. Histology showed mainly necrosis, varying from spotty to massive, in the acute cases and a pattern consistent with chronic active hepatitis in the chronic cases. The incidence of symptomatic nitrofurantoin-induced hepatic injury in The Netherlands may be estimated at approximately 1:3000-5000 (0.020-0.035 %). The mechanism of nitrofurantoin-induced hepatic injury seems to be immunoallergic.

Nitrofurantoin was introduced in the early fifties and proved to be an effective agent against infections of the lower urinary tract. Related furan derivatives, e.g. nitrofurazone, nifuroxime and furazolidone, have been employed as topical or gastrointestinal antibacterials. Nitrofurantoin may rarely cause idiosyncratic (e.g. hemolysis in patients with a deficiency of G6PD, enolase or glutathione peroxidase) and immunoallergic reactions (e.g. fever, rash or acute pneumonitis). Other adverse effects, e.g. polyneuropathy, are based on a toxic mechanism in which cases the incidence depends upon dose, tissue level and renal function (1). Several cases of acute and/or chronic hepatitis have been attributed to use of nitrofurantoin (2-42), nifurtoinol (43) and furazolidone (16,44-49). Since these reports mostly consisted of single cases we studied 52 cases of hepatic injury associated with the intake of nitrofurantoin (50 cases) or nifurtoinol (2 cases) as reported to the Netherlands Centre for Monitoring of Adverse Reactions to Drugs (NARD). Some of these cases have been described previously (9,29-31).

MATERIALS AND METHODS

Since 1963 the NARD receives reports of suspected adverse reactions to drugs from medical practitioners and sometimes from pharmacists through a nationwide voluntary reporting scheme. All reports of hepatic injury associated with the use of the urinary tract antibacterials nitrofurantoin and nifurtoinol (hydroxymethylnitrofurantoin), and received since 1963, were included in this study. All reports came from general practitioners or specialists. Of each report we requested full details about: age and sex of the patient, dose and duration of use, concomitantly used drugs, underlying illness (e.g.congestive heart failure, chronic liver disease), events preceding the onset of hepatic injury (e.g. operation, transfusions, hemodialysis), all clinical signs and symptoms, biochemical test results, course and duration of hepatic injury, and the exclusion of other possible causes of hepatic injury (e.g. gallstones, hepatitis A and B, cytomegalovirus infection, infectious mononucleosis). We reviewed the original liver slides of patients who had undergone biopsy or on whom autopsy had been performed. A follow-up of all cases was done for two reasons: firstly, to exclude the possibility that another causative factor was present and secondly, to determine whether irreversible damage occurred.

The clinical, biochemical and histological data were scrutinized and for each case the causal relationship between drug use and hepatic injury was assessed. The causal relationship was considered 'highly probable' when there was a well-documented reaction to (unintentional) rechallenge. If there was a compatible temporal relationship with drug use in combination with well-documented exclusion of other possible causes, the causal relationship was considered 'probable'. When the temporal relationship was compatible but other causes were insufficiently excluded the causal relationship was considered 'possible'. If too few data were available or if more likely causes were

found, the causal relationship was considered 'unclassifiable' or 'unlikely' respectively.

In 9 out of 13 chronic cases HLA typing was performed according to previously described methods (50,51).

RESULTS

In 10 cases the causal relationship was unclassifiable. Although it is not excluded that nitrofurantoin played a causative role in these cases, they were omitted because of a lack of data. In 4 cases a causal relationship was unlikely. In one patient serum liver enzymes were already abnormal before use of nitrofurantoin. In two of these 4 patients liver enzymes did not normalize after discontinuation of nitrofurantoin (one patient had ulcerative colitis). The fourth patient probably had autoimmune chronic active hepatitis (CAH). Table 1 presents the demographics, symptoms, biochemical patterns, immunological and protein test results, and temporal relationships of drug use to hepatic reactions in the 38 remaining cases in which a causal relationship was found to be 'highly probable', 'probable' or 'possible'. Also included in this table is parallel information from 53 cases reported in the literature.

CLINICAL AND BIOCHEMICAL PATTERN

In 4 cases a causal relationship was considered 'highly probable' and in 23 'probable'. In 11 cases a causal relationship was 'possible'. Since some of the cases of the latter group may have had other causes we have separated the symptoms per group.

The 27 'highly probable' and 'probable' cases (hereafter referred to as probable-group) were used to outline the clinical pattern (table 2). Most patients were female. The mean age in the probable-group was 64 years (range: 34-79). All patients used nitrofurantoin or nifurtoinol because of urinary tract infections, often of a recurring or chronic type, but none of them had an impaired renal function as judged by serum urea or creatinine levels. In all cases the daily dose ranged from 100 to 400 mg. The majority (85%) of the chronic cases in the 'probable' group had a delay period of approximately 6 months or more of use before onset of symptoms. In the cases of acute hepatitis in this group, onset of symptoms occurred within the first 6 weeks of nitrofurantoin treatment in 86 %, and in approximately half, symptoms appeared within the first week of treatment. Jaundice was the most common symptom, followed by abdominal pain, malaise, nausea and anorexia (table 2). Hepatomegaly was present in 13 of the 23 cases in which presence or absence of this feature was clearly stated. None of the reports mentioned lymphadenopathy as a clinical feature and its absence was emphasized in 20 of 27 cases. Eosinophilia was present in five (20%) of 25 cases in which this sign was specified. Rash and fever were relatively uncommon. However, when only the acute cases in the 'probable' group were examined, eosinophilia was present in only 14%, rash

TABLE 1

COMPARISON OF NARD-CASES WITH CASES FROM THE LITERATURE

2-8, 11, 12, 14-18, 20-28, 32-43

	Literature			This study*			
	Acute (n = 17)		Chronic (n = 36)	Acute (n = 25)		Chronic (n = 13)	
AGE							
⩽ 20 years	12 %		3 % (n = 33)	8 %		0 %	
21-40	18 %		42 %	20 %		0 %	
41-60	58 %		49 %	20 %		15 %	
61-80	12 %		6 %	52 %		85 %	
SEX							
Female/Male	10/7		29/4	22/3		12/1	
SYMPTOMS/SIGNS							
Fever	65 %		24 % (n = 33)	28 %		0 %	
Rash	59 %		3 %	12 %		0 %	
Eosinophilia	47 %		9 %	16 %		23 %	
Jaundice	47 %		64 %	44 %		77 %	
Hepatomegaly	47 %		36 %	16 %		62 %	
Malaise	35 %		52 %	32 %		69 %	
Anorexia/weight loss	24 %		27 %	12 %		46 %	
Myalgia/arthralgia	18 %		18 %	4 %		0 %	
Abdominal discomfort	18 %		18 %	24 %		31 %	
Pulmonary signs	18 %		21 %	12 %		23 %	
Nausea/vomiting	18 %		9 %	32 %		31 %	
Lymphadenopathy	12 %		0 %	0 %		0 %	
Fatal course	12 %		12 % (n = 36)	4 %		8 %	
BIOCHEMICAL PATTERN**							
Liver enz. elevation	35 %		27 % (n = 33)	60 %		8 %	
Cholestatic	41 %		3 %	4 %		0 %	
Hepatocellular	18 %		45 %	32 %		62 %	
Mixed chol. -hepatoc.	6 %		18 %	4 %		23 %	
IMMUNOLOGY + PROTEINS							
ANF	0 %	(n = 2)	81 % (n = 32)	25 %	(n = 4)	82 %	(n = 11)
ASMA	–		71 % (n = 24)	0 %	(n = 2)	73 %	(n = 11)
LE-cells	0 %	(n = 1)	14 % (n = 7)	0 %	(n = 3)	50 %	(n = 6)
Hypoalbuminemia	60 %	(n = 10)	72 % (n = 25)	18 %	(n = 11)	67 %	(n = 12)
Hypergammaglobulin.	29 %	(n = 7)	88 % (n = 26)	0 %	(n = 12)	100 %	(n = 12)
TIME-INTERVAL							
⩽ 1 week	41 %		0 %	52 %		0 %	
1-6 weeks	47 %		0 %	28 %		0 %	
6 weeks-6 months	12 %		8 %	0 %		15 %	
> 6 months	0 %		92 %	4 %		85 %	
Undetermined	0 %		0 %	12 %		0 %	

* Includes references 9, 29-31

** See legends figure

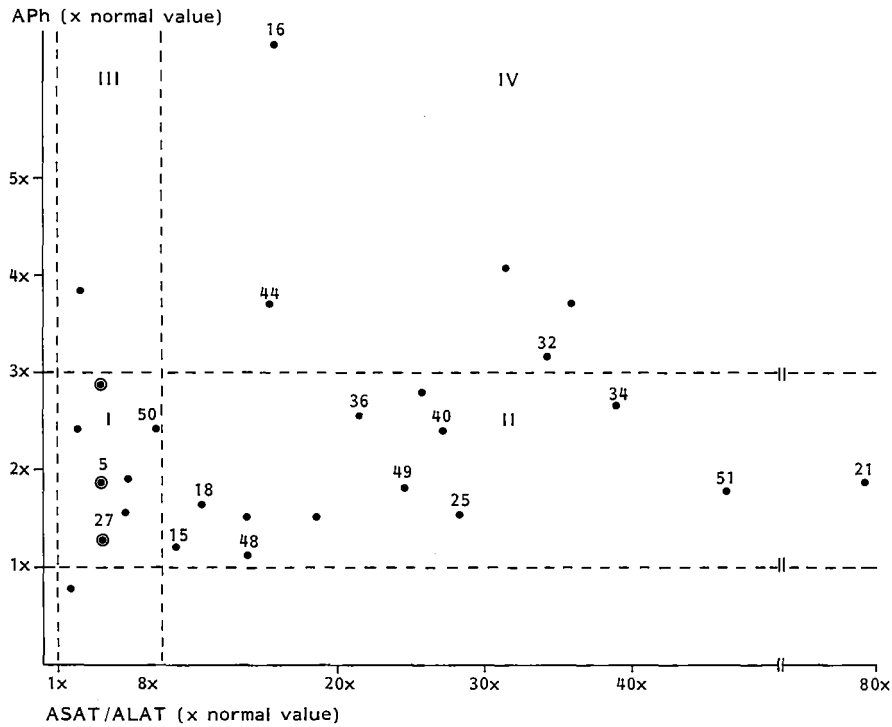

Fig. 1: Biochemical pattern in 27 probable cases. Numericals refer to biopsy numbers in text. Cases of pure cholestasis encircled.

I = Liver enzyme elevation (alkaline phosphatase (APh) < 3 × normal, asparate/alanine aminotransferase (ASAT/ALAT) < 8 × normal, bilirubin < 2 × normal).

II = Hepatocellular (APh < 3 × normal, ASAT/ALAT > 8 × normal).

III = Cholestatic hepatitis (APh > 3 × normal, ASAT/ALAT < 8 × normal).

IV = Mixed cholestatic-hepatocellular (APh > 3 × normal, ASAT/ALAT > 8 × normal).

TABLE 2

SYMPTOMS/SIGNS of 27 'HIGHLY PROBABLE' and 'PROBABLE' cases of NITROFURANTOIN-ASSOCIATED HEPATIC INJURY

Jaundice	16/27 (59 %)
Abdominal pain	11/27 (41 %)
Malaise	10/27 (37 %)
Nausea/vomiting	10/27 (37 %)
Anorexia/weight loss	9/27 (33 %)
Dyspnoea/coughing	5/27 (19 %)
Pruritus	4/27 (15 %)
Fever	4/27 (15 %)
Rash	3/27 (11 %)
Myalgia	1/27 (4 %)
Headache	1/27 (4 %)
Excessive perspiration	1/27 (4 %)
Tongue pain	1/27 (4 %)
No symptoms	3/27 (11 %)
Hepatomegaly	13/23 (57 %)
Lymphadenopathy	–/20
Eosinophilia	5/25 (20 %)
Fatal course	1/27 (4 %)
ALAT > ASAT	61/97 (63 %)

TABLE 3

HISTOLOGICAL PATTERNS of HEPATIC INJURY in 19 'PROBABLE' and 'POSSIBLE' CASES STUDIED by the AUTHORS and COMPARED to CASES in the LITERATURE

Predominating pattern*	Probable	Possible	Total	
			This study (n = 19)	Literature (n = 44)
ACUTE				
Cholestasis	**5, 27**	**20**	16 %	7 %
Necrosis			47 %	18 %
'spotty'	**36**	**6**		
centrilobular/bridging	**15, 32, 34, 40, 48, 51**	–		
massive	–	**13**		
CHRONIC				
Chronic active hepatitis		–	32 %	57 %
without early cirrhosis	**16, 25, 44**			
with early cirrhosis	**21, 49, 50**			
Cirrhosis	–	–	–	2 %
MISCELLANEOUS				
Steatosis	–	–	–	2 %
Granulomas	–	–	–	7 %
Undetermined	**18**	–	5 %	–
Normal	–	–	–	7 %
Total	16	3	100 %	100 %

*) Numericals in bold printing refer to the NARD no. of the cases in the study

in 21%, and fever in 29 percent. The biochemical pattern of the probable cases was hepatocellular in almost 50 percent (figure 1). One patient died secondary to hepatic failure, after prolonged treatment with nitrofurantoin. A follow-up revealed that 4 other patients had died due to causes unrelated to liver disease.

Clinical features in the possible-group were largely similar to those in the probable-group but mild to moderate liver enzyme elevations were more common in the possible-group (73 %). Only one patient in the possible-group had no symptoms. One patient in this group died secondary to massive necrosis.

In 63 percent of probable-cases other drugs had been used, mostly cardiovascular agents. None of the other drugs, however, was causative. Either the intake of these drugs was temporarily unrelated to the onset of hepatic injury or they were continued without problems. In one patient with cholestasis (no.27), both nitrofurantoin and oral contraceptives were discontinued; a rechallenge with nitrofurantoin alone was positive. In the possible-group other drugs may have played a causative role in three cases (methyldopa, sulfamethizole and an analgesic mixture).

HLA Typing

With the exception of two antigen types no differences were found between these patients (n=9) and a control group of healthy donors of blood from The Netherlands (n=505). HLA DR2 and HLA DRw6 were each present in 5 of our 9 patients (56%) with chronic liver damage whereas the incidence was 29 percent in the group of controls. The combination of HLA DR2 and HLA DRw6 was present in 3 of 9 patients (33%) as against 8 percent in the control-group. These differences, however, were not statistically significant.

HISTOPATHOLOGY

A biopsy or autopsy had been performed in 21 patients. On our request slides of liver tissue from these patients were submitted for review. In 2 cases a causal relationship with use of nitrofurantoin was considered 'unlikely' and these are not discussed here. Of the remaining 19 cases, 16 consisted of a 'probable' and 3 of a 'possible' causal relationship (see table 3). All slides had been stained with hematoxylin-eosin and most series included periodic acid Schiff (PAS) with and without diastase, staining for iron (Perls or Turnbull) and trichrome staining (Azan or van Gieson).

Cholestasis

In two 'probable' and one 'possible' case the pattern was predominantly cholestatic. Whereas in the latter case the pattern was that of 'pure' cholestasis, the two 'probable' cases showed some portal infiltration and an occasional area of intralobular spotty infiltration without or with minimal necrosis. In both cases there was marked chole-

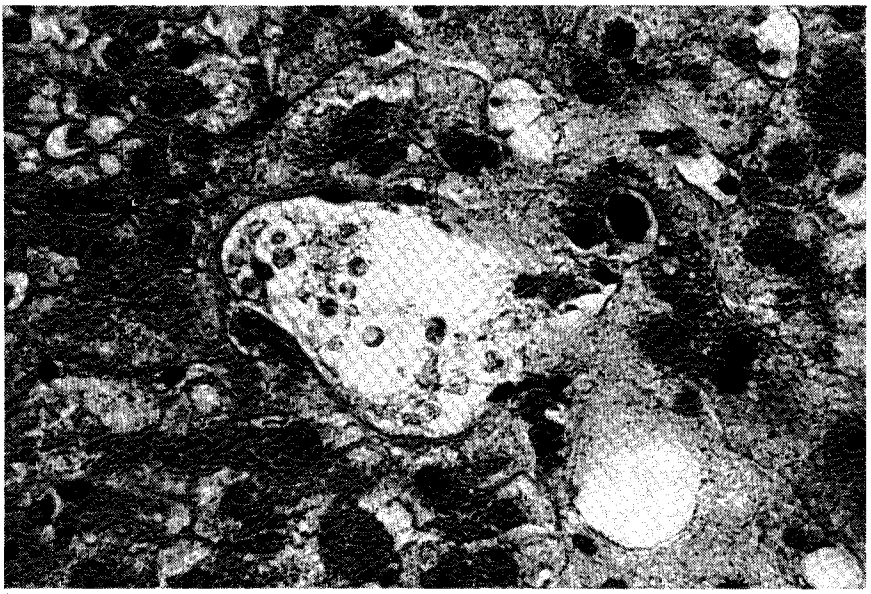

Fig.2: (HE 500x) Case 27. Cholestasis; centrilobular area with bile plugging (arrows) and cellular degeneration (by courtesy of Dr.Elias)

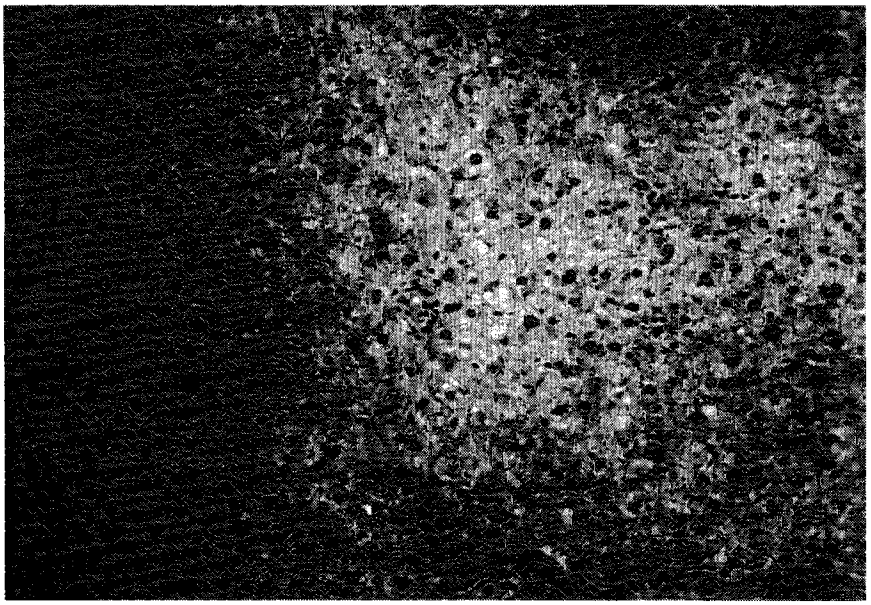

Fig.3: (HE 125x) Case 36. 'Spotty' necrosis with areas of ballooning and marked anisokaryosis (by courtesy of Professor D.J.Ruiter)

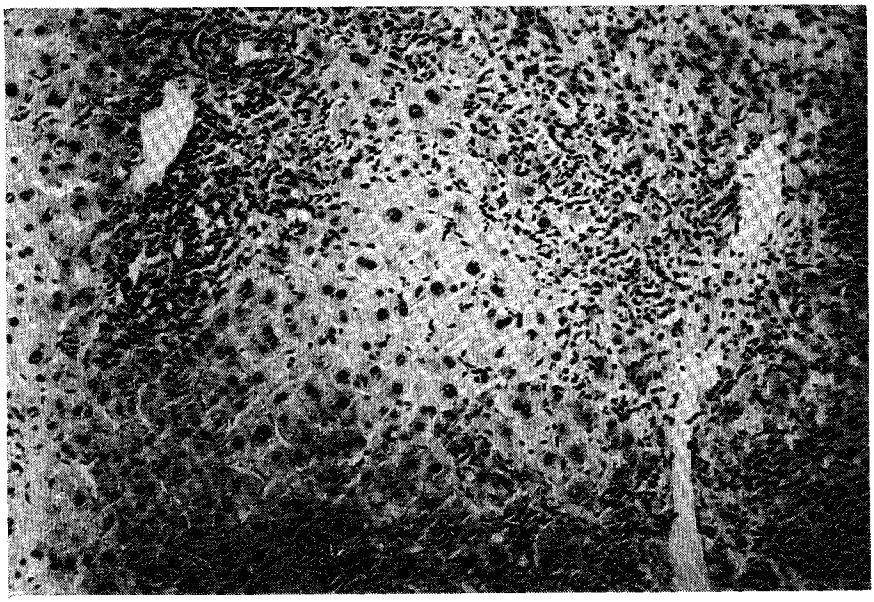

Fig.4: (HE 125x) Case 34. Bridging necrosis (by courtesy of Dr.B.Kazzaz)

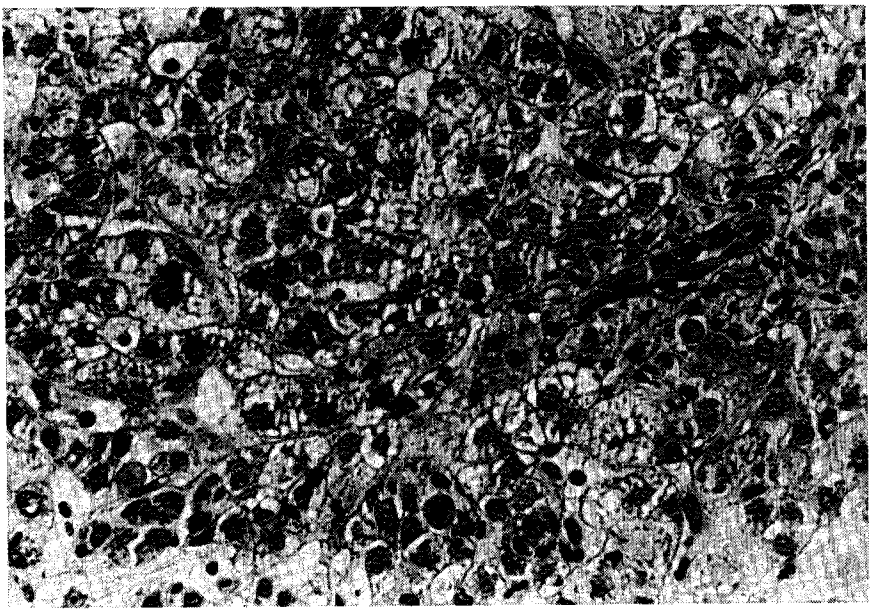

Fig.5: (HE 320x) Case 51. Ballooning and acidophilic degeneration. On the right a bile duct surrounded by mononuclear cells and neutrophils

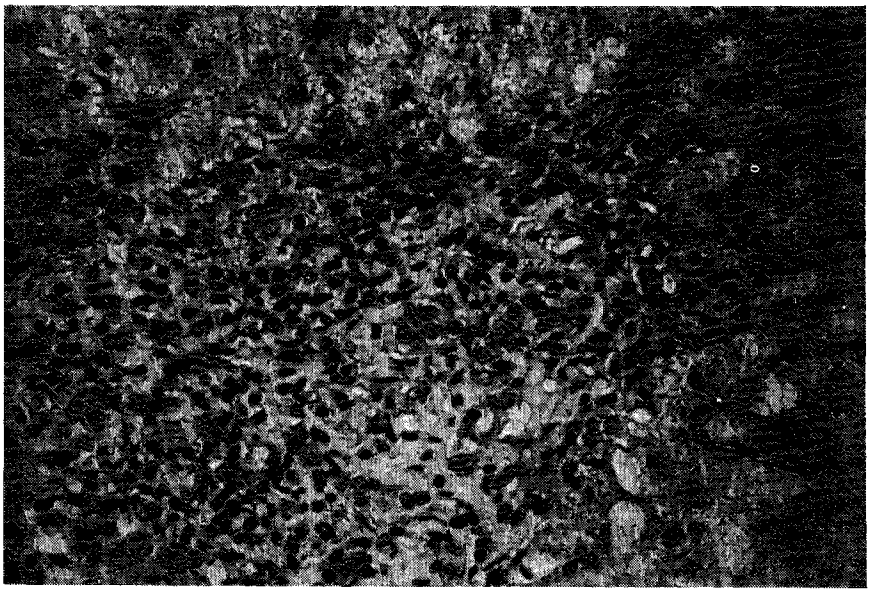

Fig.6: (HE 320x) Case 50. Chronic active hepatitis; expanded portal area with piecemeal necrosis (by courtesy of Dr.J.Grond)

TABLE 4

COMPARISON of CASES of HEPATIC INJURY to FURADANTINE® (n = 34) with PRESCRIPTION FIGURES as regards AGE and SEX

	FEMALE		MALE	
	Incidence	Prescription	Incidence	Prescription
AGE				
0-19 years	2.9 %	13.5 %	-	2.3 %
20-39 years	8.8 %	20.8 %	-	6.2 %
40-64 years	29.4 %	20.9 %	-	10.2 %
> 64 years	47 %	18.4 %	11.8 %	7.2 %
	88.1 %	73.6 %	11.8 %	25.9 %

stasis with, often large, bile plugs in zones II and III. Infiltration was predominantly lymphocytic. The lobular architecture was preserved.

Spotty necrosis

Case 6 showed a pattern of non-specific reactive hepatitis with minimal changes, with some Kupffer cell hyperplasia and an occasional spotty area of infiltration. More extensive, but nevertheless relatively mild, changes were present in case no.36 with several foci of necrosis surrounded by lymphocytes and Kupffer cells and minimal cholestasis. Lobular architecture was preserved in this case.

Centrilobular/bridging/massive necrosis

In 4 'probable' cases necrosis was not diffusely panlobular but predominantly centrilobular, mostly with bridging (no.15, 32, 40 and 48). The presence of periportal fibrosis - without piecemeal necrosis - in three of these (no.15, 40 and 48) suggested that there had been a prolonged course or that these patients had had several relapses. High serum levels of gamma-globulin and low levels of albumin in two of these 3 patients were suggestive of some form of chronic hepatitis. Case 34 showed both areas of spotty necrosis and areas with mainly centrilobular necrosis with central-central bridging and case 51 combined many acidophilic bodies in zones I-III with a few areas of bridge formation. Most biopsies showed minimal to mild cholestasis with some bilirubin in hepatocytes and occasional bile plugs. Four biopsies showed bile duct multiplication with signs of cholangiolitis. Although portal and lobular infiltration was mostly mononuclear with a predominance of lymphocytes, infiltration in areas surrounding bile ducts included many neutrophils. In patient 34 there were many eosinophils in the portal areas. Remarkable was a pattern of extensive steatosis in the control biopsy of case 32. This biopsy had been taken 2 months after a first biopsy which showed only minimal fatty degeneration. This patient had not been treated with corticosteroids, denied abuse of alcohol and had no underlying conditions which could explain this feature (e.g.diabetes mellitus, obesity). In one 'possible' case, necrosis varied from submassive with surviving hepatocytes in zone I, to massive with large areas of collapse, filled with cell debris and erythrocytes.

Chronic active hepatitis

Six 'probable' cases demonstrated either a pattern of chronic active hepatitis or a pattern suggesting that chronic active hepatitis had been present before discontinuation of nitrofurantoin, several weeks earlier. In cases 16 and 21, besides expanded densily infiltrated portal tracts with a predominantly mononuclear infiltrate and conspicuous piecemeal necrosis, there was ballooning degeneration of hepatocytes in zones II and III. There was cell-dropout and acidophilic degeneration with bridging necrosis in case 16. In case 21 this was also present but only during the second episode after accidental rechal-

lenge. In cases 21,49 and 50 the biopsy was suggestive of beginning cirrhosis. In case 49 this was confirmed by laparoscopy. In cases 25 and 44 biopsy was performed 1.5-3 months after discontinuation of nitrofurantoin. At that time piecemeal necrosis was absent but expanded portal tracts with star-shaped periportal fibrosis suggested healed periportal necrosis. Most biopsies showed varying degrees of bile duct multiplication with infiltration of neutrophils. In case 44 there were relatively many eosinophils in the portal tracts.

DISCUSSION

Several histological types of hepatic injury have been attributed to the intake of nitrofurantoin. An association with focal nodular hyperplasia has been suggested (52) but this has not been confirmed and remains somewhat speculative. Sometimes symptoms may falsely suggest liver disease. Nitrofurantoin may cause jaundice by hemolysis (1). Some patients demonstrate brownish discolouration of urine which is caused by high urinary concentrations of the drug (53) and a history of dark urine may falsely suggest bilirubinuria and severe liver damage. This was indeed the case in two of our patients in whom mild liver enzyme elevations were accompanied by normal or near-normal serum bilirubin levels. Occasionally acute pancreatitis due to nitrofurantoin may cause extrahepatic bile duct obstruction by edema of the head of the pancreas and mimic gallstone disease (54). These causes, however, are usually easy to exclude by laboratory assessment and imaging procedures.

In this study of 52 reports of hepatic injury associated with use of nitrofurantoin or nifurtoinol, 38 cases were considered to be 'possibly' or 'probably' related to the intake of the suspected drug. In order to outline the clinicopathological pattern these two groups were presented separately since in the former other causes may have been responsible. In the 'probable'-group, cases were either proven by a positive reaction to rechallenge or other causes, such as hepatitis A, hepatitis B, cytomegalovirus infection, infectious mononucleosis and gallstones were excluded. In none of these patients was cardiac failure, sepsis or malignancy demonstrated and no transfusions or operations had preceded the onset of hepatic injury. Underlying liver disease (e.g.alcoholic liver disease, primary biliary cirrhosis, autoimmune or viral chronic active hepatitis) was unlikely considering the uneventful follow-up after discontinuation of nitrofurantoin in these cases for periods up to 10 years. We compared the 38 'possible' and 'probable' cases with cases reported in the medical literature distinguishing between acute and chronic cases (Table 1). The existence of an intermediate form is suggested by the abnormal values for serum proteins in some of the acute cases in our study and in the literature (4,14).

Table 1 shows some interesting features. Going by the literature, nitrofurantoin-associated chronic liver disease seems to be far more common than acute liver injury. Cases of nitrofurantoin-associated hepatic injury have randomly

been reported in the literature from several countries all over the world. Since acceptance for publication in a medical journal depends on several unknown factors, these numbers may give an unreliable picture of the relative incidence of acute versus chronic liver reactions. This problem is probably less serious with a national voluntary reporting system since it is unlikely that medical doctors in a particular country prefer to report acute instead of chronic liver reactions. Despite the fact that chronic liver injury may have an insidious course and remain unrecognized, we believe that our figures indicate that the acute type is more common.

Our study shows a female preponderance of 89 percent. This is higher than the sex-related prescription rate in women in The Netherlands (female/male: 74/26 percent) (Dr T Stempels, Dr Brandt Rowles, Norwich Eaton Pharmaceuticals Inc,Personal Communication 1987). Although this difference is relatively small it possibly indicates that women are more likely to develop this adverse effect since we are not aware of any convincing reason for a preference to diagnose and report cases of drug-induced hepatic injury in this group. Sixty-three percent of our patients were 61 years or older as against 40 percent in the literature. We compared the incidence of hepatic injury in our study with the age-related prescription data in The Netherlands (table 4). Under the age of forty years the reporting rate of hepatic injury to nitrofurantoin was lower than the prescription rate whereas there was a sharp increase in the number of cases in the elderly, especially those older than 64 years. Of diagnostic importance is the fact that whereas in our chronic cases (mean age 69 years; range:54-79) the clinicopathological pattern was very similar to autoimmune CAH, the latter is mostly diagnosed in young female (mean age ranging in several studies from 30-46 years (55)).

The high incidence of fever (65 %), rash (59 %) and eosinophilia (47 %) in the acute cases, described in the medical literature, is at variance with the incidences in our study of 28 %, 12 % and 16 % respectively. We have no explanation for this difference but think that the high figures are an overrepresentation. Perhaps it is caused by a preference to publish cases accompanied by hypersensitivity signs, for instance because the first cases - which were accompanied by rash, eosinophilia and fever - stimulated the description of similar cases or because concomitant signs of hypersensitivity leave less doubt about a causal relationship.

The prognosis of nitrofurantoin-associated chronic hepatic injury seems to be good unless the intake of nitrofurantoin is continued despite the appearance of symptomatic hepatic injury. In that case the course may be fatal (27,40). Occasionally, however, hepatic injury may have a fatal course despite discontinuation of nitrofurantoin as is demonstrated by our case no.14 and by one case from the literature (26). In both cases hepatic failure developed in one to three years after discontinuation of nitrofurantoin. In the acute cases the patients usually recovered within 1-3 months following discontinuation. In the chronic cases

recovery usually took longer but the majority of patients recovered without use of corticosteroids. In 2 of our cases, however, there were signs of irreversible - albeit not life-threatening - damage and also several other reports describe irreversible damage (25-28). Moreover, since a control biopsy was performed in a minority of chronic cases, it is possible that even in the cases with normal liver enzymes mild post-necrotic scarring may be present. One of the weak points in the establishment of a diagnosis is that hepatic injury is considered to have another cause when discontinuation of the suspected drug is not followed by recovery. In one of our 52 cases we came to the conclusion that autoimmune CAH was the more likely diagnosis since a pattern of chronic active hepatitis, accompanied by autoantibodies, was still present 5 years after discontinuation. Nevertheless it seems possible (but difficult to prove) that some drugs induce a pattern of continuing chronic active hepatitis despite discontinuation of their intake.

Most literature cases concern either acute cholestatic hepatitis or CAH, whereas our study suggest that acute symptomatic hepatocellular injury and mild to moderate liver enzyme elevations are more common. In the chronic cases antinuclear and smooth muscle antibodies were mostly present. Antimitochondrial antibodies were absent in all assessed chronic cases in the literature (n=9) and in our study (n=9). One of four assessed chronic cases in our study had antibodies against double-stranded DNA but this was not reported in the cases from the literature. Remarkable is the high incidence of LE-cells in the chronic cases of our study (50%), which is much higher than the incidence reported in the literature (14%). It should be emphasized, however, that only 6 patients have been tested.

It has been suggested that nitrofurantoin-induced CAH is associated with the HLA B8 haplotype (25,26). In order to test this hypothesis we obtained blood from 9 of our 13 patients with chronic liver injury. In our patients HLA B8 was not more frequent than in a control population (33% vs 23%). Although in our cases HLA DR2 and HLA DRw6 were - separately (56 %) as well as in combination (33%) - more frequent than in a control group (29% vs 8%), this difference was not significant when a correction was made for the number of assessed HLA groups. Mackay et al. found a marked increase of the HLA B8 and HLA DRw3 haplotype in cases of autoimmune CAH (56). The fact that these haplotypes were not increased in our cases suggests that HLA typing may help in differentiating autoimmune from nitrofurantoin-induced CAH.

Some histological features are of interest. Many of our cases showed cholangiolitis. Berry et al.(36) reported invasion of bile ducts by neutrophils and in a case described by Jokela the second biopsy showed bile duct proliferation and cholangitis (4). In the case described by Gonzalez et al.(3) cholangiolitis predominated. Some other cases with bile duct proliferation and portal infiltration with neutrophils suggest that perhaps (mild ?) cholangiolitis may have been present in some cases (2,21,24,27).

Interesting is the fatty change of hepatocytes in some of our patients during the recovery phase. It is known that corticosteroids may induce steatosis but none of our patients had received such agents. Several literature reports also refer to steatosis (2,4,6,14,22,24,37,45) and in some of these fatty change is especially reported in the second (control) biopsy (2,14). Burns et al (13,14) reported cytoplasmic crystalline regions in hepatocytes but emphasized that this feature is not specific.

The incidence of hepatic injury due to nitrofurantoin is difficult to estimate. In a study of 15000 patients admitted to hospital 55 were found to have drug-induced hepatic injury. Twenty percent of them had used nitrofurantoin (incidence: 0.00073 %) but mostly in combination with other potential hepatotoxins (10). Another study in outpatients found an incidence of drug-induced liver disease of 1:100000 person-years but no nitrofurantoin-induced cases were reported in this series (57). In a recent survey of manufacturers data, D'Arcy estimated the incidence of hepatic adverse effects at 0.0003 % of courses of therapy with nitrofurantoin (58). Based on the estimated sales figures in The Netherlands during a ten-year period (1977-1986) (Dr T Stempels, Dr Brandt Rowles, Norwich Eaton Pharmaceuticals Inc, Personal Communication 1987), we think that the incidence may be higher. With an approximated ratio of 9:1 for the incidence of acute versus chronic infections of the lower urinary tract (Dr JAP Hooykaas, Personal Communication 1987), a mean treatment period of 10 days (400 mg/day) and 6 months (100 mg/day) respectively, and an estimated mean of 3-5 acute urinary tract infections during a ten-year period (based on ref 59), we come to a denominator varying between 889160 and 1114445 treated individuals and a numerator varying between 23 (probable) and 32 (probable+possible) symptomatic cases during 1977-1986. We have to correct, however, for the large underreporting of adverse reactions which has been estimated in the United Kingdom at approximately 80-90 percent of serious reactions (60). This would suggest that the incidence of symptomatic nitrofurantoin-induced hepatic injury may be approximately 0.020-0.035 percent. We emphasize, however, that our calculation is based on estimated figures and may not reflect the actual situation. Moreover the reporting rate will differ per drug and per adverse effect (61). On the other hand it is important to realize that the reporting rate in The Netherlands is much lower than in the United Kingdom (62).

The mechanism of nitrofurantoin-induced hepatic injury is either immunoallergic or secondary to metabolic idiosyncrasy. Direct toxicity is unlikely since the reaction does not seem to be dose-dependent and appears to be relatively rare and unpredictable. According to a review (63) nitrofurantoin caused fatty change in cats and necrosis in hamsters but no liver damage in mice, rats and dogs. Mice on 40 mg/kg/day during 6 months, however, showed hepatocellular degeneration, vacuolated hepatocytes and portal lymphocytic infiltration, mainly surrounding bile ducts (64). In turkeys on furazolidone there was bile duct hyperplasia,

portal fibrosis and hepatocellular vacuolisation (65). Experiments in rats with furfural, which is a related furan-derivative, showed cirrhosis (66). These data are, however, difficult to extrapolate to the human situation because of species-related differences and the use of high doses in animal toxicity studies. With one exception none of our cases had renal function impairment and - unlike peripheral neuropathy, which is clearly a toxic adverse effect of nitrofurantoin - we do not think that kidney function has any consequences as regards liver injury. In our opinion immunoallergy is a more likely mechanism than metabolic idiosyncrasy. The acute reactions appear almost invariably within the first six weeks of therapy, and there is an accelerated reaction to rechallenge in our acute cases as well as in the literature (2,4,14). In those cases where hepatic injury emerged after 2-3 days of therapy the drug had been used before without apparent complaints. That low amounts of nitrofurantoin may cause hepatic injury in hypersensitive individuals is suggested by a case in which ingestion of milk from a nitrofurantoin-treated cow was followed by liver damage (36). In support of an immunologically-mediated reaction is a case with apparent cross-sensitivity to furazolidone and nifuroxime (16). Also the simultaneous occurrence of rash, fever and eosinophilia may be regarded as circumstantial evidence of a hypersensitivity reaction. These signs are less frequent in cases of chronic liver injury but here the high incidence of autoantibodies suggests an immunoallergic mechanism. This is compatible with the clinical and histological similarity to autoimmune chronic active hepatitis. Sensitization by repeated use of nitrofurantoin as therapeutic agent or as a veterinary contaminant (36) is a likely explanation of a higher incidence in the elderly although a higher vulnerability of an ageing liver can not be excluded (67). Lymphocyte stimulation testing performed in six cases was negative in two (28,31) but positive in four cases (11,15,28). In rat mitochondria nitrofurantoin is reduced to nitro anion radicals followed by autoxidation to superoxide anions (68) and inhibition of mitochondrial respiration (69). Possibly an immunological reaction is involved, directed against cellular components which are structurally altered by these radicals either covalently or by lipid peroxidation. If so, it seems plausible that either acute hepatitis or chronic active hepatitis ensues, depending on the ferocity of the immunological reaction. In chronic cases, however, it is not excluded that nitrofurantoin - by direct interference with the immunesystem - facilitates the appearance of autoimmune CAH in susceptible persons.

When compared to the chronic pulmonary and chronic hepatic adverse effects of nitrofurantoin the acute reactions seem to be relatively innocuous. These are usually easily recognized and may lead to discontinuation in an early stage. Longterm use of nitrofurantoin should be discouraged unless the patient is kept under regular medical supervision.

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Chapter 11

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Case Report

Pirprofen-associated hepatic injury

W.W. De Herder[1], P. Schröder[2], A. Purnode[3], A.C.M. Van Vliet[1] and B.H.Ch. Stricker[4]

[1]Diakonessenhuis Refaja, Dordrecht (The Netherlands); [2]Evangelisches Krankenhaus, Lengerich (F.R.G.); [3]Clinique Saint-Michel, Brussels (Belgium); and [4]Netherlands Centre for Monitoring of Adverse Reactions to Drugs, Rijswijk (The Netherlands)

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Summary

Four cases of hepatic injury attributed to the use of pirprofen (Rengasil) were reported to the Monitoring Centres for Adverse Reactions of Belgium, The Netherlands and the German Medical Association. One man and three women developed severe hepatic injury between 3½ and 6½ months after starting treatment with 400–1200 mg pirprofen daily.

Histology showed acute hepatocellular damage, often with bridging necrosis. Two patients died. The other two patients made an incomplete recovery. It is possible that this severe type of hepatic injury is due to a metabolic idiosyncrasy to pirprofen, as this reaction seems to be rare and unpredictable but is not associated with immunoallergic signs.

Introduction

Pirprofen, a phenylpropionic acid derivative, is a new non-steroidal antiinflammatory drug. The incidence of raised liver enzymes in patients using pirprofen is low for this type of drug and ranges from 0.6% [1] to 2.8% [2–4].

Symptomatic liver injury attributed to pirprofen has, however, recently been reported [5,6,10]. We describe four additional patients with severe liver injury probably caused by pirprofen, two of whom died.

Case Reports

Case 1

A 75-year-old nun, living in a convent, was admitted on 1 August, 1985 because of weakness and jaundice. She complained of anorexia and weight loss of two weeks duration, without fever or abdominal pain. There was no history of alcohol abuse, hepatitis contact, operations, transfusions or travel to tropical areas. Since 1983 she had been taken 50 mg of triamterene and 4 mg of epitizide on alternate days and occasionally 10 mg of oxazepam as a sedative.

Correspondence address: B.H.Ch. Stricker, Medical Officer, Netherlands Centre for Monitoring of Adverse Reactions to Drugs, P.O. Box 439, Leidschendam, The Netherlands.

From 27 March, 1985 she had taken 400–1200 mg of pirprofen daily because of osteoarthritis. Physical examination revealed jaundice. There was no fever, lymphadenopathy or hepatosplenomegaly. Erythema palmare, spider naevi, ascites or other signs of chronic liver disease were absent.

Laboratory results included a normal blood and differential count, normal renal function and normal values for serum amylase and lipase. On repeated testing, abnormal biochemical liver tests were obtained with the following maximal values (normal values in brackets): aspartate aminotransferase (ASAT) 435 U/l (< 30), alanine aminotransferase (ALAT) 655 U/l (< 30), alkaline phosphatase (APh) 167 U/l (< 100), total bilirubin 226.3 μmol/l (< 17), conjugated bilirubin 146.2 μmol/l (< 5), total protein 54 g/l (65–80) and albumin 54% (50–65). The partial thromboplastin time was 71 s (30–40) and the Thrombotest (Nyegaard) was 14% (80–100%). Serologic tests for hepatitis A and B, infectious mononucleosis, cytomegalovirus, toxoplasmosis, herpes simplex, Q fever and syphilis were negative. Slightly dilated intrahepatic bile ducts were seen by ultrasonography but endoscopic retrograde cholangiopancreatography showed normal bile ducts, a normal pancreatic duct and a normal gall-bladder. Esophageal varices were not seen during endoscopy. All drugs were discontinued on admission.

She gradually developed a bleeding tendency and bilateral leg oedema, and ascites despite treatment with diuretics. Peritoneoscopy showed ascites and a pale liver with a granular surface was seen. A liver biopsy showed extensive bridging necrosis and hepatocyte ballooning, the latter mainly in zones I and II. Early fibrosis was present, especially in the expanded portal areas. Infiltration was mixed lymphocytic-neutrophilic, with a predominance of the mononuclear cells. Cholestasis was absent (Fig. 1). The pa-

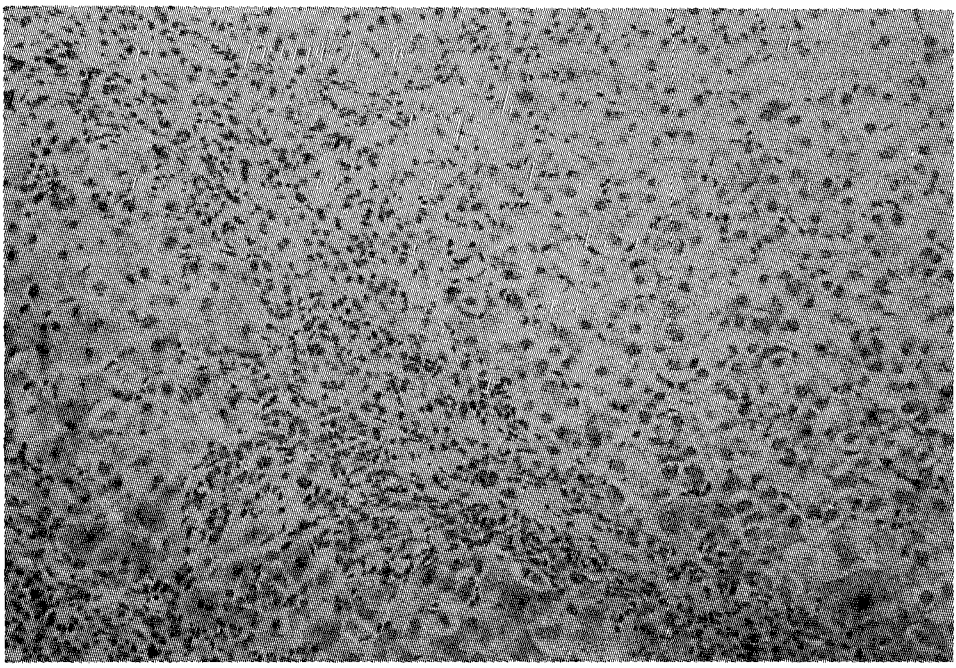

Fig. 1. Portal-central and central-central bridging necrosis with ballooning and acidophilic degeneration in zones I and II; mainly lymphocytic infiltration. HE, ×100.

tient's condition deteriorated. She became comatose and died 44 days after admission. Permission for postmortem examination was not given.

Case 2

A 62-year-old woman was admitted on 17 September, 1985. For three weeks she had had abdominal discomfort, nausea and low-grade fever. One day before admission she became jaundiced. She had been treated since February 1985 with 400 mg of pirprofen daily because of osteoarthritis. Four days before admission this medication had been changed to 10 mg of piroxicam daily because of the abdominal discomfort. No other drug had been used. There was no history of transfusions, operation, chronic liver disease, alcohol abuse or hepatitis contact. Physical examination was unremarkable except for jaundice and hepatomegaly. There was no ascites, erythema palmare or other signs of chronic liver disease and no lymphadenopathy or splenomegaly. Laboratory data included a normal blood and differential count and a normal renal function. Abnormal serum values were ASAT 571 U/l (< 15), ALAT 645 U/l (< 19), APh 376 U/l (< 170), gamma-GT 288 U/l (< 18), bilirubin total/conjugated 16.5/12.4 mg/dl (< 1/0.25) and prothrombin level: 50% (70–130%). Serologic tests for hepatitis A and B, infectious mononucleosis and cytomegalovirus were repeatedly negative. Antinuclear and antimitochondrial antibodies were absent. Piroxicam was discontinued on admission. Repeated ultrasonographic imaging showed cholecystolithiasis without signs of biliary obstruction.

Follow-up examinations showed a decrease in liver size.

Whereas serum values for ALAT and ASAT slowly decreased, total bilirubin increased to a peak of 19.4 mg/dl on 1 October. The serum prothrombin level decreased to 20% of normal despite parenteral administration of vitamin K. The arterial ammonia was 110 μg/dl (< 82) and she was treated with lactu-

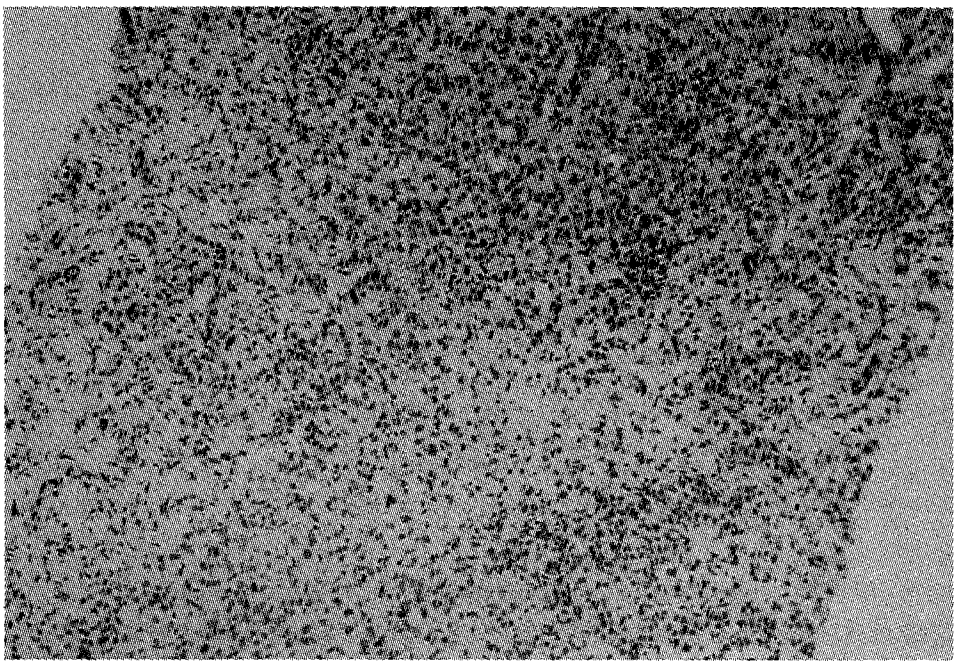

Fig. 2. Massive necrosis with some areas of bile duct proliferation; lymphocytic infiltration. HE, ×100.

lose and parenteral nutrition. Diuretics were given for peripheral oedema. A liver biopsy on 19 November showed submassive to massive necrosis with extensive bridging and occasional small islands of intact hepatocytes. Infiltration was mononuclear. There was early diffuse fibrosis and periportal bile duct proliferation (Fig. 2). She made a slow but incomplete recovery and was discharged on 20 December. At that time serum aminotransferases were normal. Total bilirubin was 2.3 mg/dl and total protein 63 g/l. The serum prothrombin level was still abnormal on 25 March (60%).

Case 3

A 58-year-old woman was admitted in February 1985 because of jaundice. She had been treated with 0.2 mg of thyroxine daily for primary hypothyroidism since February 1984 and with 10 mg of flunarizine daily since June 1979 because of peripheral cold hypersensitivity. Because of osteoarthritis she had taken 800 mg of pirprofen daily since July 1984. There was no history of transfusions, recent operation, hepatitis contacts, alcohol abuse or chronic liver disease. On examination she was found to be jaundiced. There was no lymphadenopathy or splenomegaly. Signs of chronic liver disease such as erythema palmare or spider naevi were absent. Laboratory data, including a blood and differential count, were normal except for serum ASAT 916 U/l (< 40), ALAT 431 U/l (< 40), APh 876 U/l (< 115) and total bilirubin 30.8 mg/dl (< 1). Serology was negative for hepatitis A and B, coxsackie B, chickenpox, herpes- and cytomegalovirus. Antimitochondrial and smooth muscle antibodies were absent. Antinuclear factor was present in low titre (1/40 immunofluorescence) as were antibodies to DNA: 39 U/ml (normal value < 25). Endoscopic retrograde cholangiopancreatography demonstrated normal bile ducts, gall-bladder and pancreatic ducts. Liver biopsy showed expanded portal areas with inflammatory infiltration. The infiltrate was mostly mononuclear. Periportal 'piecemeal' necrosis and mild cholestasis were present. In the lobules, signs of regeneration were found with mitosis in the hepatocytes.

Initially the cause of this hepatocellular type of liver injury was considered to be viral but later pirprofen was suspected. This drug had been discontinued on admission. There was a progressive but slow normalization of serum bilirubin and aminotransferases after discontinuation of pirprofen. One year later laboratory values had almost normalized.

Thyroxine was continued. Flunarizine was considered unlikely to be the cause of hepatitis as the laboratory values were already significantly improving by the time flunarizine was discontinued on 1 March.

Case 4

A 26-year-old male student was admitted in March 1985 with nausea and jaundice. He had ingested pirprofen irregularly and in unknown quantities since November 1984 because of psoriatic arthritis. In the first days of March he had discontinued pirprofen, presumably because of nausea. On admission serum aminotransferases and bilirubin were markedly elevated. Hepatitis A and B, cytomegalovirus disease and infectious mononucleosis were excluded serologically. The patient developed hepatic coma and died at the end of April 1985.

Discussion

The absence of other demonstrable causes and a compatible temporal relationship make pirprofen a probable cause of the liver damage in these four patients [7]. There was no history of excessive alcohol intake or signs of underlying liver disease and histology confirmed the acute, non-alcoholic type of liver injury. Hepatitis A, hepatitis B and cytomegalovirus infection were serologically excluded in four and infectious mononucleosis in three of our patients. Since no transfusions, injections or recent operation preceded the illness and since there was no hepatitis contact, non A non B viral hepatitis seems unlikely as a cause. Also concomitant medication does not seem to have played a causative role. Of the other drugs used, only piroxicam has been associated with liver injury [7–9] but patient 2 had changed to this drug a few days before admission, at a time when she already had symptoms of acute hepatitis. In patient 3

flunarizine was discontinued when laboratory values were already improving. Oxazepam and epitizide belong to therapeutic classes which rarely have been associated with hepatic injury [7]. These drugs had moreover already been used by patient 1 for two years without adverse effects. The rate of recovery was slow in the two surviving patients described by us, and it is possible that progression to post-necrotic scarring or even cirrhosis has occurred, since even after one year of follow-up liver enzymes were still mildly abnormal.

The temporal relationship between starting treatment with pirprofen and the appearance of the first symptoms varied from 3½ to 6½ months, which is comparable with the 3–9 months found in other reports [5,6,10]. Only in one case-report [10] onset was within 6 weeks after starting treatment with pirprofen but in that case ajmaline had been used concurrently since 1 week. Three of our patients were women over 50 years of age, as were most patients in the other reports [5,6,10]. A more frequent use of non-steroidal antiinflammatory drugs by this age group possibly explains this preponderance. In our opinion the number of cases is too small to decide whether women are more likely to develop pirprofen-associated hepatic injury than men.

Biochemically and histologically, the pattern of liver damage was mainly hepatocellular and was severe in all 14 reported cases. Four patients died. Hepatocellular necrosis, predominating in zone III and often with collapse, was prominent in all patients in whom a biopsy was done [5,6,10] and may be extensive [6]. In one patient the pattern resembled chronic active hepatitis [5]. We did not find microvesicular steatosis, as described by Danan et al. [6], in any of our patients. Although oil red O staining was not performed in any of our cases, also their description was based on hematoxylin–eosin [6]. The mechanism of pirprofen-induced hepatic injury is unknown. Intrinsic dose-dependent hepatoxicity is unlikely since non-steroidal antiinflammatory drugs with this effect usually fail to pass animal toxicity tests and clinical trials. The absence of symptoms of a hypersensitivity reaction (with the exception of one patient with peripheral eosinophilia [5], the delayed onset and the delayed reaction to rechallenge [5], are compatible with metabolic idiosyncrasy rather than an immunoallergic mechanism [7]. At present there is insufficient evidence to suggest that pirprofen causes more frequent or more serious adverse reactions of the liver than other non-steroidal antiinflammatory drugs. However, these case-reports suggest that patients using this drug should be monitored and use of pirprofen stopped if symptoms or biochemical signs of hepatitis develop. Administration of pirprofen for short periods (< 3 months) may be relatively safe for the liver.

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Chapter 12

SUMMARIZING DISCUSSION

There were three reasons to perform these studies. Firstly, the aim was to demonstrate that, apart from the generation of signals concerning unknown adverse reactions to drugs, a voluntary reporting scheme may be used for in-depth studies. Although the former remains the most important aim of the scheme, it is clear that important additional information can be obtained with series of cases. A good example is the study of Inman and Mushin of 170 reports to the Committee on Safety of Medicines of halothane-associated jaundice (1). They elegantly demonstrated that most cases of halothane-associated jaundice occurred after multiple exposure and that in these cases the delay period between administration of halothane and onset of hepatic injury was reduced. Another successful example of studying series of cases, as reported to a national adverse reaction monitoring centre, is described in the paper on 57 cases of glibenclamide-associated hypoglycaemia (2). If only the alerting function of a voluntary reporting scheme would be of importance only a few well-documented cases of a particular adverse effect would be enough. Because of under-reporting and the absence of sales data the only use of subsequent cases would be to get a rough estimate of the incidence. Secondly, one of the objectives was to show that "Intensive Case Monitoring" can also be performed with cases from other countries. Thirdly, the clinical and histological patterns of hepatic injury induced by glafenine (Glifanan), ketoconazole (Nizoral), and nitrofurantoin (Furadantine, Ceduran, Furophen Tc)/nifurtoinol (Urfadyn, Uridurine) were characterized. Starting with the third reason for performing the studies, discussion here will be restricted to common features since the clinicopathological patterns of these drugs have been reviewed extensively in Chapters 8-11.

It is impossible to outline the clinicopathological pattern of a particular adverse effect without making a selection of cases in which a causal relationship with drug use is probable. The reason for this is that cases in which a causal relationship is 'unclassified' or 'possible' may have had an other cause. Especially the 'unclassified' cases can not be used because of scanty documentation. In the 'possible' cases documentation is usually relatively good but in these cases either another concomitantly used drug may have been responsible or another common cause has not been excluded.

Whereas the literature on glafenine-associated hepatic injury suggested a pattern varying from mild/moderate liver enzyme elevations to a mixed cholestatic-hepatocellular pattern of injury, the study in this thesis (Chapter 8) showed a predominance of hepatocellular damage with a high case-fatality rate. The clinicopathological pattern showed a remarkable resemblance to hepatic injury by cinchophen. Immunoallergic phenomena accompany the adverse reaction

of the liver to both drugs in a minority of cases and the latent period between first intake and onset of the reaction is the same. As the chemical structures show some resemblance it seems possible that a metabolic idiosyncratic reaction is the cause.

Unlike the cases reported in the medical literature, hepatic injury induced by ketoconazole was not always found to be hepatocellular. The study of 'probable' cases included several patients in whom cholestasis predominated. Besides the study presented in Chapter 9, an earlier study performed by Lewis et al.(3), gave comparable results. The biochemical pattern they found was similar to the study in this thesis. In the study by Lewis et al., however, cholestatis was not confirmed by biopsy and histology was available in only three cases. Their study also showed a female preponderance, albeit less strongly than that reported in this thesis, and liver damage also appeared mostly within the first six weeks of therapy. The fatality rate in both studies was low. Lewis et al. found a higher incidence of jaundice, however, probably because the study in this thesis included a number of patients with mild to moderate liver enzyme elevations. Most medical practitioners in The Netherlands are now aware of the potential hepatotoxicity of ketoconazole, which means that they regularly assess liver enzymes during therapy and discontinue the drug at an early stage. The latter explains the low fatality rate in the study in this thesis. A recently published paper on 78 cases of ketoconazole-associated hepatic injury gave similar results (4). Despite the fact that the criteria in the study of Lake-Bakaar et al. were less strict than in this study only 16 'probable'-cases were reported. Either the cases in their study were less well-documented or medical practitioners in the United Kingdom rarely exclude other possible causes of hepatic injury. The latter seems to be unlikely. Details of the 78 cases originated from reports to the Committee on Safety of Medicines. It seems plausible that careful documentation at the moment of reporting would have led to a higher number of 'probable'-cases in the study of Lake-Bakaar et al.(4). This underlines the importance of adequate documentation and evaluation at the moment of receiving the report and confirms the value of "Intensive Case Monitoring".

The literature about nitrofurantoin suggests that most patients with hepatic injury to this drug have chronic active hepatitis. The study in Chapter 10 suggests, however, that acute hepatic injury is more common. Unfortunately it was impossible to relate the acute/chronic cases to the ratio of short-term/long-term treatment so that the exact figures about the incidence remain unknown. Nevertheless it was possible to approximate the incidence with the help of an estimation of the prescription data. It should be realized, however, that this approximation is based on a number of assumptions, which may mean that it does not reflect the real incidence. The study revealed some interesting characteristics in that the frequency of nitrofurantoin-induced hepatic injury shows a clear increase in the elderly. Especially for the differentiation between chronic

cases of nitrofurantoin-induced hepatic injury and autoimmune chronic active hepatitis this is of importance since the latter usually appears in a younger age-group. Comparison of the sex-related sales figures with our cases suggest that women are somewhat more susceptible. Since the data of voluntary reporting schemes are not suitable for calculation of incidences, however, it was not possible to assess the statistical significance of a sex-related difference in susceptibility. Interesting is the fact that the chronic cases differ from autoimmune chronic active hepatitis as regards HLA-typing. Whereas the latter is mainly associated with HLA-DRw3 the cases in this study showed no such association.

The small study about hepatic injury by pirprofen and also the other cases reported in the medical literature suggest that the type of injury induced by this drug is mainly hepatocellular. It should be emphasized, however, that the number of reported cases is small. The study demonstrated that "Intensive Case Monitoring" is a method which is not restricted to the national area of monitoring. The term "Intensive Case Monitoring" is used when, after receiving information suggestive of an unknown adverse effect, the reporting medical practitioner is contacted at an early stage and advised on the studies which should be done for adequate documentation. The aim of all medical practitioners is to treat their patients, if possible at low expenses, rather than to prove the causal relationship of adverse effects. For this justified reason they sometimes have to be convinced of the need for additional, often expensive, investigation. Most of them, however, are willing to co-operate, especially when the importance of early detection of unknown adverse effects is reiterated. Contact in the early phase of the disease is highly important since it facilitates extensive documentation. On an international basis exchange of information by telephone about the results of additional investigation and the exchange of liver slides made it possible to document these cases adequately. Communicating with reporting medical practitioners from other countries on the results of physical examination and laboratory investigation in a particular case and consultation about additional investigation are usually not a problem in Europe since most of them are familiar with the English language.

The first and most important objective of this thesis - the question whether voluntary reporting schemes can be used for more than merely generating alerts about unknown adverse effects - has several aspects.

These studies demonstrated that not only careful investigation but also follow-up of each individual case is very important. This concept of "Intensive Case Monitoring" is especially successful when the reporting doctor contacts the monitoring centre at an early stage. A clear example is the case of jaundice attributed to glafenine in which hemolysis was not excluded because of insufficient documentation and late presentation. As discussed above for pirprofen-associated hepatic injury, it was often possible to improve the quality of documentation of a particular case

by advising on additional investigation. In many cases of follow-up the general practitioner was asked to assess liver enzymes when this had not been done already in the preceding year. A drug monitoring centre should not absorb data passively but should actively endeavour to improve the documentation of signals. By strongly advising on additional investigations, e.g. ultrasonography and the exclusion of hepatitis A and B, cytomegalovirus infection and infectious mononucleosis (when it concerns hepatic injury) the quality of the report can be enhanced significantly. When the report is received in an early phase of the illness it is possible to have an important influence on the quality of the report. This means that medical practitioners encountering adverse effects should contact the adverse reaction monitoring centre as early as possible. This type of "Intensive Case Monitoring" requires, however, much time and can not be done with all reports of adverse effects received by a drug monitoring centre without an increase in the number of medical assessors. Especially the follow-up may prove that a case is not drug-related at all and this means that it is not only an indispensible part of these studies but that it should be done with every important report of drug-induced disease. The patient with cholestatic hepatitis attributed to the intake of nitrofurantoin who later proved to have a deficiency of al-antitrypsin is a convincing example of the importance of a follow-up. An other example is the patient who had all features, which are considered characteristic of nitrofurantoin-induced chronic active hepatitis, but in whom a follow-up over several years made it more likely that the actual diagnosis was autoimmune chronic active hepatitis. It is clear that studies of series of cases require extensive documentation and follow-up of all cases for several years. A prerequisite is that enough medical doctors are willing to co-operate. All reports came from medical practitioners, mostly general practitioners and specialists in internal medicine. In most cases several medical specialties were involved, mostly general practitioners, internists and pathologists. None of the medical doctors refused to give additional data and in all cases where biopsy or autopsy was available it was possible to review the original liver slides. Most data could be obtained by copies of lists of laboratory data and specialists letters. Additional information was easily obtained by telephone. Especially the follow-up was done by telephone.

It is obvious that - even when a particular adverse reaction is relatively rare - over a period of several years enough case-reports may be received to facilitate further study. No single medical practitioner or department of a large hospital can collect the numbers of patients which have been covered in the studies in this thesis. Although manufacturers also receive many reports about patients developing adverse reactions to their products, they are not always willing to publish these series, presumably because they fear negative advertising. There are, however, exceptions since in some cases external experts are given the opportunity to use the manufacturers data base as has

been done, for instance, with ketoconazole (3) and valproate (5).

Voluntary reporting schemes probably give a more realistic picture of the spectrum of hepatotoxicity of a drug than individual case-histories in the literature. Acute hepatocellular necrosis, cholestatic hepatitis and chronic active hepatitis are all severe adverse effects. It is not very likely that the average general practitioner or internist preferentially reports only one of these patterns. It may be assumed that the relative incidence of these patterns is fairly well reflected by their distribution in a study of 30-50 cases as reported to an adverse reaction monitoring centre. The estimation of the distribution of these patterns with the help of the literature seems to be less reliable, possibly because many subsequent publications tend to follow previous ones by reporting analogous cases. Moreover the decision about publication of a case also depends on the opinion of the editors and referees of medical journals.

There are no reasons to suggest that an in-depth study of the clinicopathological pattern is only possible with drug-induced hepatic injury. Although the studies in this thesis were concentrated on this subject any organ- or bodily system-related adverse effect may be investigated. It should be admitted that there are some aspects, which make drug-induced hepatic injury an attractive issue for in-depth studies: the adverse reaction is objective and may be assessed easily by noninvasive methods (liver enzyme assessment, serology, ultrasonography). Collection of affected tissue samples by liver biopsy is relatively easy and not associated with significant morbidity and mortality, especially when compared to other tissues (e.g. kidney). On the other hand it must be clear that in-depth studies by careful documentation and follow-up of every case-history will reveal important characteristics of any type of adverse effect. The wide range of central and peripheral nervous system disorders, for example, may be investigated with the help of many methods, e.g. physical examination, electroencephalography, electromyography and investigation of visual or auditive evoked potentials. Every objective and careful study of patients will help to outline the clinicopathological characteristics of a particular type of adverse reaction irrespective of the involved organ or bodily system.

Well-documented series of a particular adverse reaction can also be used for studies about mechanisms and diagnostic methods. As has been outlined in previous chapters, the frequent and dose-dependent adverse effects are almost invariably discovered during pre-marketing studies. The dose-independent adverse effects have a low incidence and are usually discovered with one of the postmarketing studies. Since patients developing a particular type of reaction may have a common characteristic, such groups can be used for further study. When it concerns mechanisms, a cohort of these patients may be investigated, e.g. for certain metabolic features. Examples are the study of acetylator status in patients with hepatic injury to isoniazid (6) and of

oxidative capacity in patients with hepatic injury to perhexiline (7). Another example is the study of the effect of terfenadine, a new antihistamine, on the driving performance of patients with drowsiness to this drug, reported to the Netherlands Centre for Monitoring of Adverse Reactions to Drugs (NARD), which will be published soon. For an example of the possibility to study diagnostic features in a particular cohort the reader is referred to the aforementioned study of patients with chronic active hepatitis (CAH) to nitrofurantoin (Chapter 10) who did not exhibit the same HLA-haplotype as patients with autoimmune CAH. The voluntary reporting scheme also facilitates case-control studies. An example is the prospective case-control study on a possible association between the Guillain-Barré syndrome and drug use, as is currently performed by the NARD.

Underreporting of suspected adverse reactions to national monitoring centres remains a problem. Unfortunately there is not much known about the reasons for non-reporting. An enquiry among 348 British general practitioners revealed that 97 percent of them claimed that they would be likely to report severe adverse reactions to the national adverse reaction monitoring centre, whereas reasons for non-reporting were the fact that the adverse reaction was in the data sheet or well-documented (47 percent), that the adverse effect was trivial (24 percent), or that the doctor was unsure about a causal relationship (15 percent) (8). It is very important to emphasize again and again that also known adverse effects should be reported, especially the severe ones.

It may be concluded that it is highly important that every report of an important adverse effect received via the voluntary reporting scheme of an adverse reaction monitoring centre should be documented carefully and that in all cases a follow-up should be performed. Active participation at an early stage may significantly improve the quality of the report. This is not necessarily restricted to the area which is covered by a national monitoring centre: "Intensive Case Monitoring" may be done on an international basis. It is also concluded that through voluntary reporting schemes enough cases may be collected to perform in-depth studies and that these studies give a reliable picture of the clinicopathological patterns.

Moreover the collected cases can be used for further study as demonstrated by the assessment of HLA-status in 13 cases of chronic hepatitis by nitrofurantoin. Another important conclusion is that medical practitioners should not stop reporting adverse effects which are already known. Especially the severe adverse effects should be used for further study.

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SAMENVATTING

Dit proefschrift omvat 12 hoofdstukken en bestaat uit twee gedeelten. In het eerste gedeelte - de hoofdstukken 1 tot en met 4 - worden achtereenvolgens besproken de historische ontwikkeling met betrekking tot de herkenning van bijwerkingen van geneesmiddelen (hoofdstuk 1), de verschillende soorten bijwerkingen en het ontdekken van deze bijwerkingen gedurende de verschillende fasen van toxicologisch en klinisch onderzoek die voorafgaan aan het registreren en in de handel brengen van een geneesmiddel (hoofdstuk 2). In de hoofdstukken 3 en 4 komen respectievelijk aan de orde de verschillende types van studie na registratie van het geneesmiddel en de methodes en resultaten van het Bureau Bijwerkingen Geneesmiddelen.

In het tweede gedeelte - de hoofdstukken 5 tot en met 12 - worden de doelstellingen van het promotie-onderzoek geformuleerd (hoofdstuk 5) en methodes en resultaten van de studies gepresenteerd en bediscussieerd. Als onderwerp van studie werd gekozen voor 'leverbeschadiging door geneesmiddelen'. Ter verduidelijking worden in hoofdstuk 6 de verschillende histologische beelden van leverbeschadiging door geneesmiddelen beschreven en wordt in hoofdstuk 7 aangegeven hoe men tot de diagnose 'leverbeschadiging door geneesmiddelen' kan komen. De hoofdstukken 8, 9 en 10 betreffen descriptieve studies van 3 series patienten met leverbeschadiging, toegeschreven aan het gebruik van respectievelijk glafenine en ketoconazole en aan het gebruik van nitrofuraanderivaten. Hoofdstuk 11 geeft een internationaal verrichte studie weer van een aantal gevallen van leverbeschadiging, toegeschreven aan het gebruik van pirprofen. In hoofdstuk 12 worden de resultaten besproken.

Er waren drie redenen om de studies te verrichten, die tot dit proefschrift hebben geleid. Beoogd werd om:

1. Aan te tonen dat het nut van een systeem voor het vrijwillig melden van bijwerkingen niet beperkt is tot het opsporen van onbekende bijwerkingen, maar dat de meldingen ook bruikbaar zijn voor verder onderzoek.

2. Vast te stellen of het systeem van "Intensive Case Monitoring" ook internationaal kan worden toegepast.

3. De klinisch-histologische patronen van leverbeschadiging door glafenine (Glifanan), ketoconazol (Nizoral) en nitrofurantoïne (Furadantine, Ceduran, Furophen Tc)/ nifurtoïnol (Urfadyn, Uridurine) te bestuderen.

Deze drie punten worden hier achtereenvolgens toegelicht.

ad.1.

Alvorens een geneesmiddel in de handel mag worden gebracht, dient experimenteel onderzoek te zijn verricht teneinde werkzaamheid en (relatieve) onschadelijkheid aan te tonen. Dit meestal zeer uitvoerig onderzoek (een onderzoekperiode van 10 jaar is niet ongewoon) vindt plaats in verschillende diersoorten (toxicologisch), vrijwilligers (farmacokinetiek) en geselecteerde groepen patiënten (farmacokinetiek en -dynamiek). Gedurende deze verschillende fasen worden het gewenste farmacologische effect (therapeutische werking), de ongewenste effecten (bijwerkingen) en de optimale dosering vastgesteld. Een geneesmiddel wordt "geregistreerd" - mag in de handel worden gebracht - wanneer een afweging van werkzaamheid en potentiële schadelijkheid in het voordeel van de werkzaamheid uitvalt. Ondanks uitvoerig onderzoek echter, zijn meestal op het moment van registratie van een geneesmiddel een aantal bijwerkingen nog onbekend. Vaak betreft dit ongewenste effecten die ôfwel niet ontdekt werden tijdens het aan registratie voorafgaande onderzoek, ôfwel relatief zeldzame reacties, die tijdens de experimentele behandeling niet zijn opgetreden. Om deze onbekende bijwerkingen op het spoor te komen, beschikken de meeste ontwikkelde landen over een nationaal centrum waar gegevens over vermoedelijke bijwerkingen van geneesmiddelen verzameld, onderzocht en geïnterpreteerd worden. Deze centra werken internationaal samen. Artsen die tijdens de behandeling van een of meerdere patienten een hen onbekende bijwerking menen te signaleren, worden geacht deze verdenking ter kennis te brengen van het centrum. Daar beschikt men veelal over de meeste gegevens en heeft men de kennis om een signaal verder uit te zoeken. Met behulp van een dergelijk systeem kunnen dus onbekende bijwerkingen ontdekt worden. Helaas kan men op deze wijze geen nauwkeurige kennis verzamelen over de frequentie waarmee een bepaalde bijwerking vôôrkomt. Deze frequentie kan men namelijk slechts bepalen wanneer men alle bijwerkingen gedurende een bepaalde periode zou delen door gegevens over het gebruik van alle geneesmiddelen gedurende dezelfde periode. Om verschillende redenen is dit niet mogelijk. Ten eerste worden niet alle - door een geneesmiddel veroorzaakte - ongewenste effecten, die bij een bepaalde patiënt optreden, herkend. Ten tweede worden niet alle ongewenste effecten, die wel herkend zijn, ook aan het geneesmiddel toegeschreven en ten derde worden niet alle herkende bijwerkingen gemeld. Bovendien ontbreken vaak gegevens over het aantal gebruikers van een bepaald geneesmiddel. Als complicerende factor geldt voorts dat tenminste een aantal van de - wel gemelde - vermoedelijke bijwerkingen niet werkelijk door een geneesmiddel veroorzaakt is, en dat vaak niet kan worden vastgesteld in welke gevallen het wel en in welke gevallen het geen bijwerking betreft.

Betekent dit dat meldingen alleen bruikbaar zijn voor de ontdekking van onbekende bijwerkingen ?

Indien alleen dit laatste van belang was, zou men kunnen volstaan met enkele goed gedocumenteerde gevallen van een nieuwe bijwerking. Verdere gevallen zouden dan nog slechts

gebruikt kunnen worden om een zeer grove schatting te maken van de frequentie van vóórkomen van die bepaalde bijwerking. Alvorens deze vraag te beantwoorden, is het belangrijk om kort in te gaan op het begrip "klinisch-histologisch patroon".

Het menselijk lichaam heeft slechts een beperkt aantal reactiemogelijkheden op schadelijke effecten. Dat betekent dat bepaalde ziektebeelden meerdere oorzaken kunnen hebben, zoals bijvoorbeeld bacteriële of virale infecties, maar ook dat deze het ongewenste gevolg van het gebruik van een geneesmiddel kunnen zijn. Omdat kennis over de ontstaanswijze van een ziekte zeer belangrijk is, bijvoorbeeld omdat dit consequenties kan hebben voor de preventie en behandeling, is het gewenst om de verschillende oorzaken te kunnen onderscheiden. In het geval van bijwerkingen betekent dit dat het van belang kan zijn om door grondige bestudering van alle bekende gevallen van een bepaalde bijwerking kennis te verwerven over de kenmerken. Op basis van deze kennis zou men dan bij volgende patiënten met een bepaald ziektebeeld kunnen uitmaken of men met een bijwerking of met een andere oorzaak te maken heeft. Wat verstaan wij nu onder het klinisch-histologisch patroon van een bepaalde bijwerking ? "Klinisch" verwijst naar de verschijnselen, die de patiënt met de bijwerking vertoont. Dat kan variëren van buikpijn en geelzucht tot huiduitslag of lymfklierzwelling. Daarnaast zijn er nog bepaalde laboratoriumbepalingen te verrichten, die de bestaande beschadiging objectiveren. Een voorbeeld van dit laatste vormt de bepaling van serum aminotransferasen. Dit zijn enzymen die in de levercel betrokken zijn bij bepaalde stofwisselingsprocessen en die normaliter slechts in geringe hoeveelheden in het bloed voorkomen. Bij beschadiging van de levercellen komen deze enzymen in het bloed en vormen de, dan tot abnormale waarden gestegen, hoeveelheden van deze enzymen een objectieve graadmeter. "Histologisch" verwijst naar het beeld dat wij onder de microscoop waarnemen. Zo kan bij leverbeschadiging het beeld voornamelijk "hepatocellulair" (levercelbeschadiging domineert) of voornamelijk "cholestatisch" (galstuwing domineert) zijn, maar komen ook mengvormen frequent voor. Het klinisch-histologisch patroon, dat dus de combinatie vormt van klinische en histologische kenmerken van een bepaalde bijwerking, zal per bijwerking verschillen. Het klinisch-histologisch patroon kan echter ook bij eenzelfde soort bijwerking (het proefschrift is geconcentreerd op leverbeschadiging als bijwerking) per geneesmiddel verschillen. Bovendien kan het aspect van eenzelfde soort bijwerking tijdens gebruik van hetzelfde geneesmiddel per individu verschillen. Tenslotte werd zelfs in zeldzame gevallen waargenomen dat een bepaald geneesmiddel in een en hetzelfde individu op verschillende tijdstippen verschillende vormen van leverbeschadiging veroorzaakte. Door een serie gevallen van een bepaalde (verdachte) bijwerking te onderzoeken, kan men meer te weten komen over de meest frequent voorkomende of over de meest kenmerkende vorm. Met deze kennis kan men bij volgende ziektegevallen gemakkelijker tot een diagnose komen. Indien een geneesmiddel bij voorkeur een bepaald klinisch-histologisch patroon veroorzaakt

zal de bijwerking gemakkelijker als zodanig herkend kunnen worden. Wanneer men nu het klinisch-histologisch patroon van een bepaalde bijwerking aan de hand van een serie ziektegeschiedenissen wil uitzoeken, zal men dit vanzelfsprekend op de 'waarschijnlijke' en 'hoogst waarschijnlijke' gevallen moeten baseren. In de gevallen waarin een causaal verband 'onwaarschijnlijk', 'niet te classificeren' of 'mogelijk' is, zal namelijk een andere oorzaak aanwezig zijn, respectievelijk aanwezig kunnen zijn. Dit betekent dat elke ziektegeschiedenis apart bestudeerd moet worden, waarna met behulp van de beschikbare gegevens beslist moet worden over de mate van waarschijnlijkheid dat het verdachte geneesmiddel verantwoordelijk was voor de leverbeschadiging van een bepaalde patiënt. Vanzelfsprekend moet dit op consequente wijze gedaan worden met behulp van tevoren vastgelegde criteria. Alle klinische en histologische kenmerken van de op deze wijze verzamelde en goed gedocumenteerde gevallen kunnen zo geïnventariseerd worden (voor een nadere toelichting op de aangehaalde causaliteitsbegrippen wordt verwezen naar de "Material and Methods" van de hoofdstukken 8 tot en met 10, en naar hoofdstuk 7).

Grondige bestudering van drie series van patienten met leverbeschadiging, toegeschreven aan respectievelijk glafenine, ketoconazole en nitrofuraanderivaten, toonde aan dat de meldingen hiervoor bruikbaar zijn.

ad.2.

De kleine studie van gevallen van leverbeschadiging, toegeschreven aan het gebruik van pirprofen, toont dat de pogingen om onbekende bijwerkingen te ontdekken niet tot het eigen land beperkt behoeven te zijn. "Intensive Case Monitoring" bleek ook buiten de grenzen van Nederland te werken. Deze benadering bestaat uit de, in een vroeg stadium startende, begeleiding van de meldend arts ten aanzien van het stellen van de diagnose. In een dergelijk vroege fase kan men adviseren over eventueel aanvullend onderzoek zodat de ziektegeschiedenis optimaal gedocumenteerd is. Dit is vooral bij nieuwe bijwerkingen zeer belangrijk omdat op deze wijze de bewijsvoering in belangrijke mate ondersteund kan worden. De procedure is hetzelfde als in ons land. De Duitse en de Belgische arts, die bij de studie (hoofdstuk 11) betrokken waren, hadden ieder een patiënt behandeld met - aan pirprofen toegeschreven - leverbeschadiging. Zij werden telefonisch benaderd en de ziektegeschiedenissen van de patiënten werden doorgesproken. Daarna werd overlegd welk aanvullend onderzoek van belang was en werden de leverpreparaten ter inzage gevraagd. Tenslotte werd in alle gevallen een "follow-up" gedaan. Het is duidelijk dat men, door vergroting van het gebied dat bestreken wordt, sneller een behoorlijk aantal goed gedocumenteerde ziektegeschiedenissen verzamelen kan. Dit kan betekenen dat een onbekende bijwerking eerder ontdekt kan worden maar ook dat aanvullend onderzoek sneller kan plaatsvinden.

ad.3

Afgaand op de studie van leverbeschadiging, toegeschreven aan het gebruik van glafenine, is het klinisch-histologisch

beeld voornamelijk hepatocellulair. Bovendien blijkt de reactie vaak een ernstig beloop te hebben. Het beeld vertoont gelijkenis met het beeld dat gezien werd bij leverbeschadiging door cinchophen. Het laatste geneesmiddel werd in de U.S.A. reeds in de dertiger jaren uit de handel genomen vanwege ernstige leverbeschadiging in daarvoor gevoelige individuen.

In tegenstelling tot hetgeen de medische literatuur suggereert, kan ketoconazole ook cholestatische hepatitis veroorzaken. Opmerkelijk is het geringe aantal gevallen van leverbeschadiging met een fataal beloop in onze studie, ondanks het feit dat ketoconazolé ernstige leverbeschadiging kan veroorzaken. Dit zou verklaard kunnen worden door de ervaring dat een aantal artsen, gezien de bekendheid van deze bijwerking, frequent leverenzymen in het serum bepalen en het gebruik door hun patiënten meestal laten staken bij de eerste tekenen van leverbeschadiging.

De meeste, in de medische literatuur beschreven, gevallen van leverbeschadiging door nitrofurantoïne betreffen chronisch actieve hepatitis. Onze studie suggereert echter dat acute hepatitis, hetzij cholestatisch of hepatocellulair, frequenter voorkomt. De kans op leverbeschadiging door nitrofurantoïne lijkt toe te nemen met de leeftijd van de gebruiker. Dit komt het meest duidelijk naar voren bij vrouwen. Dit leeftijdsaspect maakt het gemakkelijker om onderscheid te maken tussen chronisch actieve hepatitis door nitrofurantoïne en chronisch actieve hepatitis op autoimmuun basis. Bovendien bleken de, bij chronisch actieve hepatitis op autoimmuun basis frequent voorkomende, haplotypes HLA B8 en HLA DRw3 niet vaker voor te komen in de patienten met chronisch actieve hepatitis door nitrofurantoïne dan in een controlegroep.

De belangrijkste conclusie die uit de studies in dit proefschrift getrokken kan worden, is dat naast de belangrijke signaalfunctie, meldingen van bijwerkingen van geneesmiddelen ook bruikbaar zijn voor verder onderzoek. Dit betekent dat het ook belangrijk is om bekende bijwerkingen te melden. Over een langere periode kan zo een serie worden opgebouwd, die een grondige studie van de verschillende klinische en histologische aspecten van een bepaalde bijwerking mogelijk maakt. Aangezien veel bijwerkingen een relatief lage frequentie van vóórkomen hebben, zal het voor een individuele arts meestal niet mogelijk zijn om een dergelijke serie op te bouwen. Dit laatste blijkt ook uit de medische literatuur, die veel beschrijvingen van ziektegeschiedenissen telt van een of enkele patiënten, maar weinig studies van enige omvang.

Bovendien wordt op deze wijze een bestand van gegevens opgebouwd over individuen die op een bepaalde wijze op een geneesmiddel reageren. Vaak betreft dit overgevoeligheidsreacties, waarvan het optreden onvoorspelbaar is zodat, voordat de reactie zich ontwikkelt, niet bekend is bij wie zich deze zal voordoen. Eventueel onderzoek naar ontstaanswijze en diagnostische methodes (bijvoorbeeld immunologisch onderzoek naar geneesmiddel-afhankelijke antilichamen) zal slechts kunnen worden verricht met behulp van de gegevens

van een groep van dergelijke individuen. Zo kunnen de meldingen van vermoedelijke bijwerkingen, zoals het Bureau Bijwerkingen Geneesmiddelen deze ontvangt, tevens dienen als basis voor verder onderzoek.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 25 augustus 1952 geboren te Rotterdam. Na het behalen van het eindexamen HBS-b, studeerde hij Geneeskunde vanaf september 1971 aan de Rijksuniversiteit te Leiden. Hier behaalde hij in juni 1972 het propaedeutisch examen. Na een duikongeval verbleef hij tussen september 1972 en juni 1974 in het revalidatiecentrum "de Hoogstraat" te Leersum, van waaruit hij in september 1973 zijn studie hervatte. Na het behalen van het kandidaatsexamen in 1975 en het doctoraal examen in 1977, werkt hij sedert maart 1978 bij het Bureau Bijwerkingen Geneesmiddelen te Rijswijk als inspecteur van Volksgezondheid in algemene dienst. Hij is tevens als gastmedewerker verbonden aan de afdeling Interne Geneeskunde II van het Academisch Ziekenhuis Dijkzigt te Rotterdam en aan de afdeling Pathologische Anatomie van het Westeinde Ziekenhuis te 's-Gravenhage.