

Self-induction of rifampicin metabolism in man

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Self-induction of rifampicin metabolism caused by daily administration of the drug was studied in 7 healthy subjects. Rifampicin 600 mg was administered daily for 10 days and additional doses were administered on the 4th and 8th days after drug administration ceased. The mean serum half-life of rifampicin decreased from 4.9 h on the 1st day to 3.5 h on the 4th day ($P < 0.01$), to 2.7 h on the 7th day ($P < 0.001$), and 2.5 h on the 10th day ($P < 0.001$). The difference between the mean values on the 7th and the 10th days was not significant. The mean value on the 8th day after stopping drug administration (3.8 h), was significantly higher than that on the last day of daily administration ($P = 0.02$), but was still lower than that on the 1st day ($P = 0.05$). There was a decrease in the excretion of both rifampicin and desacetyl-rifampicin in urine on induction, followed by a gradual return to normal when drug administration was stopped.

Rifampicin is known to induce its own metabolism¹⁻⁴ in addition to that of a number of other drugs^{5,6}. Administration of this drug has been shown to be accompanied by functional and morphological evidence of proliferation of smooth endoplasmic reticulum and activation of microsomal enzyme systems in the hepatocyte^{1,7,8}. Self-induction of rifampicin metabolism could be part of the generalised induction of the hepatic microsomal enzyme system, and it should be possible to study the phenomenon of the activation of these enzymes by following the changes in the serum half-life of this drug.

An investigation was undertaken in healthy subjects to determine the minimum number of daily doses of rifampicin needed to attain maximal induction of the hepatic microsomal enzyme system,

the stability of the maximal induction so attained with continued treatment, and the time necessary for the induced enzyme levels to return to normal after treatment is discontinued. The urinary excretion pattern of rifampicin and its principal metabolite, desacetyl-rifampicin, was also studied. A simple method to determine the concentrations of these two compounds in urine involving separation by thin-layer chromatography (TLC) was developed, and we present here the findings of these investigations.

Material & Methods

Design of the study : Rifampicin 600 mg was administered daily on an empty stomach to 7 healthy volunteers (mean body-weight : 60 kg; range 43 to 81 kg) for 10 days and then discontinued. Additional single doses of the drug were then adminis-

tered on the 4th and 8th days after the daily administration was discontinued (*i.e.*, on the 14th and the 18th day after start of rifampicin administration). On days 1, 4, 7, 10, 14 and 18, blood was collected at 3, 4½ and 6 h and urine excreted over the period 0-6 h following drug administration (on an empty stomach) was also collected. Concentrations of rifampicin in serum and those of rifampicin and desacetyl rifampicin in urine were determined after randomising and coding the samples.

Serum concentrations of rifampicin : Serum concentrations of rifampicin were determined according to the plate diffusion method of Dickinson and others⁹, employing a strain of *Staphylococcus aureus* (subgroup I, NCTC 10702) resistant to streptomycin and other antibiotics. Rifampicin standards ranging from 0.04 to 1.28 µg/ml were set up in quadruplicate in pooled human serum, and concentrations of the drug in the samples (set up in quadruplicate in dilutions of 1 : 10 and 1 : 20) were obtained from the regression line of the log concentration of the standard on the diameter of the zone of inhibition.

Assuming first order kinetics, the disposition rate constant (K) was calculated from the regression line of log rifampicin concentrations in serum on time, and the half-life ($t_{1/2}$) was then obtained from the equation $t_{1/2} = 0.693/K$.

The geometric mean concentration has been employed to describe changes in the serum rifampicin concentrations on the different test-days. The geometric mean of the concentrations at 3, 4½ and 6 h was calculated for each volunteer and the geometric mean of these mean values

was then computed for the group of 7 volunteers on each of the different test-days.

Rifampicin and desacetyl rifampicin in urine : The urine samples (3 ml) were mixed with 1.5 ml of a citrate-phosphate buffer (1.5 M, pH 7) and extracted with 3 ml of chloroform by shaking for 1 min on a Vortex test-tube mixer. The contents were centrifuged and the aqueous layer was discarded. To the chloroform extract was then added 0.5 ml of a saturated solution of sodium chloride, the contents shaken, centrifuged, and the aqueous layer discarded again. The extinction of the chloroform extract was read at 475 nm using microcells of 1 cm path-length, and the 'total' rifampicin concentration (free rifampicin plus desacetyl rifampicin) was calculated using rifampicin standards 10 and 50 µg/ml set up in normal urine and processed similarly. The chloroform extract was then placed in a water bath at 80-90°C and the volume concentrated to about 0.2 to 0.5 ml. Rifampicin and desacetyl rifampicin were separated from each other and from the other metabolites of the drug by TLC on silica gel G. About 100 µl of the concentrated chloroform extract was applied to the activated plate, and the plates were developed with a solvent system of chloroform and methanol (95 : 5). Portions of the adsorbent containing rifampicin and desacetyl rifampicin were identified by reference to standards, scraped off immediately, and the compounds eluted with 1 ml of methanol. The extinctions of the methanolic extracts of rifampicin and desacetyl rifampicin were recorded at 335 nm in 1 cm microcells, and the respective concentrations calculated on the basis of the ratio of the extinctions and the 'total'

rifampicin concentration determined previously after chloroform extraction.

Pure rifampicin powder was a Ciba-Geigy product, nutrient agar and nutrient broth were Difco products, silica-gel G (BDH) was obtained from Glaxo Laboratories (India) Ltd., and all the other chemicals used were of analytical grade. Desacetyl rifampicin was a gift from Dr G.A. Ellard, and the strain of *Staph. aureus* (sub-group I, NCTC 10702), resistant to streptomycin and other antibiotics, was kindly supplied by Prof. D.A. Mitchison.

Results

Recovery of rifampicin and desacetyl rifampicin from urine : On each of 4 occasions, mixtures of rifampicin and desacetyl rifampicin were set up in duplicate and in varying proportions in concentrations ranging from 2.5 to 100 µg/ml each. After randomisation, the samples were processed by the method described earlier in this communication. The mean recovery of 'total' rifampicin (rifampicin plus desacetyl rifampicin) was 103 per cent (range: 102-109%) following extraction of urine with chloroform. After separation by TLC on silica gel and elution with methanol, the mean recovery of rifampicin was 100 per cent (range : 98-102%), and that of desacetyl rifampicin was 108 per cent (range : 104-115%). The co-efficient of variation for replicate estimations ranged from 2-17 per cent for both the compounds. In a typical experiment, the R_f values for rifampicin and desacetyl rifampicin were 0.58 and 0.40, respectively.

Serum concentrations of rifampicin : The mean serum concentrations of rifampicin at 3, 4½ and 6 h, the overall mean concen-

tration (i.e., the geometric mean of the concentrations at 3, 4½ and 6 h), and the mean half-life of the drug on the different test days are presented in Table I.

A progressive and significant decrease was observed in the serum concentrations of rifampicin when the drug was administered daily for 7 days. The over-all mean concentration on the 4th day was 15 per cent lower ($P=0.03$), and that on the 7th day was 35 per cent lower ($P<0.001$) than the mean value on the 1st day. The mean concentrations on the 7th and the 10th days were the same, and the mean value on the 18th day, while 21 per cent higher than that on the 10th day ($P=0.2$), was 22 per cent lower than that on the 1st day ($P=0.03$).

The mean serum half-life of rifampicin was 29 per cent lower on the 4th day ($P<0.01$), 45 per cent lower on the 7th day ($P<0.001$), and 49 per cent lower on the 10th day ($P<0.001$) than the mean value on the 1st day. The difference between the mean values on the 7th and the 10th days was not significant. The mean values on the 14th and 18th days were the same, and higher than that on the 10th day ($P=0.02$), but lower than that on the 1st day ($P=0.05$).

Urinary excretion of rifampicin and desacetyl rifampicin : There was a slight but non-significant increase in the proportion of the dose of rifampicin excreted in the unchanged form in urine collected over the period 0-6 h from the 1st to the 4th day, followed by a significant decrease ($P<0.05$) when drug administration was continued for 3 more days (Table II). The proportion of dose excreted as desacetyl rifampicin was, however, less on the 4th as well as the 7th day in compari-

Table I. Serum concentrations and half-life of rifampicin

Day	Mean serum concentrations ($\mu\text{g/ml}$)* at the following times (h)			Over-all mean Serum concentration* ($\mu\text{g/ml}$)	Serum half-life* (h)
	3	4½	6		
1	10.1 (8.0-12.5)	8.3 (6.4-11.8)	6.5 (4.7-9.0)	8.2 (6.3-11.0)	4.9 (3.6-6.8)
4	9.4 (8.3-11.7)	6.9 (5.7-7.9)	5.3 (3.4-7.0)	7.0 (5.6-7.9)	3.5 (2.1-5.9)
7	7.8 (6.7-9.2)	5.2 (3.5-6.6)	3.6 (2.1-4.7)	5.3 (3.7-6.2)	2.7 (1.7-3.9)
10	7.9 (6.6-10.0)	5.7 (4.1-7.3)	3.4 (1.7-4.4)	5.3 (4.3-6.7)	2.5 (1.3-3.4)
14	8.6 (5.9-12.2)	6.1 (4.7-8.8)	4.9 (3.3-7.5)	6.3 (4.9-9.3)	3.8 (2.5-6.3)
18	8.4 (6.8-11.9)	6.6 (5.0-8.4)	4.7 (3.1-6.6)	6.4 (4.8-7.5)	3.8 (2.6-6.7)

*Based on the geometric mean of individual estimates for 7 volunteers. Rifampicin (600 mg) was administered daily for 10 days; single doses were then administered on the 14th and 18th days after start of the investigation. Figures in parentheses denote the range

Table II. Excretion of rifampicin and desacetylri-fampicin in urine collected over the period 0-6 h (Data are mean \pm SD based on estimates for 7 volunteers)

Day	Proportion of dose (%) excreted as the following	
	Rifampicin	Desacetylri-fampicin
1	7.1 \pm 1.6	2.8 \pm 0.7
4	7.4 \pm 1.1	2.3 \pm 0.7
7	6.7 \pm 1.0	1.7 \pm 0.3
10	6.5 \pm 1.6	2.0 \pm 0.7
14	6.9 \pm 2.0	2.4 \pm 0.7
18	6.8 \pm 2.0	2.4 \pm 0.6

Rifampicin 600 mg was administered daily for 10 days; single doses were then administered on the 14th and 18th days after start of treatment

son with that on the 1st day ($P < 0.05$). The differences between the 7th and 10th days in the excretion of both the compounds were not significant. The mean values on the 14th and 18th days were slightly higher than those on the 10th day, and the excretions of both the compounds on the 18th day were lower than those on the 1st day; the differences, however, were not significant.

The geometric mean ratios of desacetylri-fampicin to rifampicin in urine (not shown in the Table) were 0.35, 0.28, 0.22, 0.28, 0.31 and 0.32 respectively on the 6 different test-days. The decrease in the ratio from the 1st to the 7th day and the increase from the 7th to the 18th day were significant ($P < 0.01$), whereas the difference between the mean ratios on

the 1st and the 18th days was not significant ($P > 0.2$).

Discussion

Rifampicin is metabolised in the body into compounds such as desacetyl-rifampicin, rifampicin quinone, desacetyl-rifampicin quinone, 3-formylrifampicin, 3-formyldesacetyl-rifampicin and a few others which have not been characterized so far. These compounds are eliminated from the system through the bile and also the urine. Sunahara and Nagakawale have described a solvent-extraction procedure to separate rifampicin from desacetyl-rifampicin prior to their estimation in urine. Substantial interference from desacetyl-rifampicin in the estimation of rifampicin was observed by us when this procedure was followed, an experience shared by McConnell and others¹¹. We therefore modified this procedure by using chloroform for the initial extraction of rifampicin and its metabolites, and separating them from one another by TLC on silica gel. Rifampicin and desacetyl-rifampicin have absorption maxima at 335 and 475 nm; the other metabolites of rifampicin absorb strongly at 335 nm, but at 475 nm, the absorption is minimal¹⁰. We took advantage of these differences: the chloroform extract of urine was read at 475 nm, thus precluding, to a great extent, the contributions due to compounds other than rifampicin and desacetyl-rifampicin. The methanolic extract, after separation of the different compounds by TLC, was read at 335 nm to increase the sensitivity, as the molar extinction coefficients of both rifampicin and desacetyl-rifampicin were found to be higher at the shorter wave-length. Thus, the method described here is specific as well as sensitive, and the

recoveries of rifampicin and desacetyl-rifampicin were found to be quantitative. Care must be taken, however, to elute the compounds after separation from the chromatoplate as early as possible (within 30 min), as the colour of the spots change to violet on prolonged exposure to atmosphere.

Serum concentrations of rifampicin were estimated by the plate diffusion assay using *Staph. aureus*⁹. It has been demonstrated that the activity of desacetyl-rifampicin against this organism is only about 20-25 per cent of that of rifampicin⁹, and that the metabolite forms only a small proportion (< 15%) of the total circulating rifampicin in patients receiving this drug¹². Thus, the changes observed in serum concentrations would almost entirely reflect those of unchanged rifampicin.

Rifampicin is known to be rapidly absorbed from the gastro-intestinal tract, and peak concentrations in serum are attained within 2 h after drug administration¹³. Results reported in this communication show that the mean serum concentrations and half-life of rifampicin decreased significantly during daily administration up to the 7th day, and there was no further decrease when daily treatment was continued for 3 more days. These findings suggest that maximal induction of rifampicin metabolism, and presumably that of the hepatic microsomal enzyme system, in general, is probably attained with about 7 daily doses of this drug. These findings are similar to those of Acocella and others¹ who observed a decrease in serum concentrations and the half-life of rifampicin up to the 6th day during daily administration of the drug and not much change thereafter. When the investigations were repeated 4 and 8 days after daily treatment

had been discontinued, the serum concentrations and half-life showed a tendency to return to normal. However, the values at 8 days after cessation of treatment were still significantly lower than those on the first day of treatment. These findings suggest that it probably takes longer than a week after discontinuation of treatment for the induced enzymes to return to normal levels.

The urinary excretion of rifampicin and desacetyl rifampicin followed a pattern fairly similar to that of the serum half-life of the drug. There was a slight decrease in the excretion of both the compounds during daily treatment, and a tendency to return to initial levels after stopping treatment. The decrease in the excretion of rifampicin during treatment which has been recorded by several other workers¹⁴⁻¹⁶, is probably due to the enhanced metabolism of the drug. The decrease in the excretion of desacetyl rifampicin could either be due to an increase in the biliary excretion or even due to an increase of its metabolism caused by induction due to rifampicin. Acocella¹³ has shown that the rate of transfer of desacetyl rifampicin from blood to urine decreased and that from blood to bile increased after 7 days of daily treatment. He has also suggested the possibility of further biotransformation of desacetyl rifampicin and of an increase in this process during daily treatment. The decrease observed in the ratio of the urinary excretion of rifampicin to desacetyl rifampicin on induction is also in accord with the findings of earlier studies^{10,17,18}.

Controlled clinical trials of the treatment of pulmonary tuberculosis undertaken at our Centre^{19,20}, have demonstrated that regimens containing rifampicin (12

mg/kg body-weight) in addition to streptomycin, isoniazid and pyrazinamide are highly effective, suggesting that the decrease in the serum concentrations of rifampicin caused by daily administration of the drug does not affect the therapeutic efficacy of the regimens. It is possible, however, that self-induction of its metabolism exacts a therapeutic 'penalty', if rifampicin is used in lower dosages.

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