International Journal of Clinical Pharmacology, Therapy and Toxicology, Vol. 24 No. 7 – 1986 (pp. 344–348)

Hepatic mixed function oxidase induction during rifampicin/isoniazid therapy in Indian vegetarians

W. Perry and M. V. Jenkins

The Metabolic Unit, Royal National Orthopedic Hospital, Stanmore, Middlesex, England

Abstract. To determine the effect of rifampicin therapy on hepatic oxidase activity in animal protein deficient patients antipyrine and quinine $t_{1/2}$ and 6B-hydroxycortisol (6B-OHF) excretion was studied in 8 Indian vegetarians during treatment for tuberculosis. In 4 patients at the start of treatment rifampicin/streptomycin caused a steady decline in by time antipyrine $t_{1/2}$ which was complete in 3 weeks, in one patient introduction of isoniazid produced a temporary reversal. After 4 months rifampicin/isoniazid 6B-OHF excretion was increased 2 to 10 fold in all patients although one followed serially showed a marked fall when isoniazid was begun. Decline in antipyrine $t_{1/2}$ persisted in 4 patients at the end of 18 months therapy and in one of these concurrent quinine $t_{1/2}$ confirmed partial isoniazid reversal of this decline. Rifampicin-mediated mixed function oxidase induction appeared similar to that reported for non-vegetarians and largely persists with combination therapy throughout treatment. Isoniazid can act as a competitive inhibitor of hepatic oxidase activity in some patients.

Key words: rifampicin – enzyme induction – antipyrine half-life

Introduction

During the course of metabolic studies into the problem of late rickets and osteomalacia in Indian and European patients [Stamp et al. 1980] the possible role of rifampicin and isoniazid induction and inhibition of hepatic microsomal enzymes was examined but we were unable to show an effect on serum calcium or intestinal calcium absorption [Perry et al. 1982] although Brodie et al. [1982] reported a reduction in 25-hydroxycholecalciferol after 6-months combined treatment. In our view the deficiency of dietary vitamin D and avoidance of sunlight continued to be the main factor in causing osteomalacia in the Indian group. The absence of animal protein in the diet raised the question as to whether their susceptibility to rifampicin-mediated enzyme induction was similar to that reported in non-vegetarian volunteers and patients [Bennett et al. 1982, Brodie et al. 1982, Larousse et al. 1980]. Dietary factors have an important influence on the activity of enzymes [Brown et al. 1954] and Indian vegetarians living in London were shown to have slow microsomal oxidation rates as judged by prolonged antipyrine $t_{1/2}$ [Fraser et al. 1977]. Additionally there has been little study of the induction status of patients throughout long-term rifampicin therapy.

To determine the rate, degree and persistence of hepatic mixed function oxidase induction in Indian vegetarians antipyrine $t_{1/2}$ was measured serially at the beginning and end of treatment and 6B-OHF excretion after 4-months therapy. Quinine $t_{1/2}$ is a further useful test of drug oxidation rates [Padgham and Richens 1974] and a decline in $t_{1/2}$ was shown with rifampicin [Perry et al. 1978]. A similar response by antipyrine and quinine with corresponding changes in 6B-OHF excretion would be strong evidence that the observed effects of rifampicin and isoniazid were on oxidative enzyme activity rather than changes in volume distribution or clearance independent of enzyme activity.

Patients and methods

Eight adult Indians with pulmonary or bone tuberculosis who were strict vegetarians were studied on a metabolic ward (4 male and 4 female). All were non-smokers and non-drinkers with normal liver and renal function. Two were followed serially with antipyrine $t_{1/2}$'s for 40 days from the start of treatment using rifampicin 450 mg and streptomycin 0.75 g I.M. daily. At the end of 28 days isoniazid 300 mg daily was substituted for streptomycin

Reprint requests to Dr. W. Perry, 25, Station Road, Dersingham, Kings Lynn, Norfolk, England.

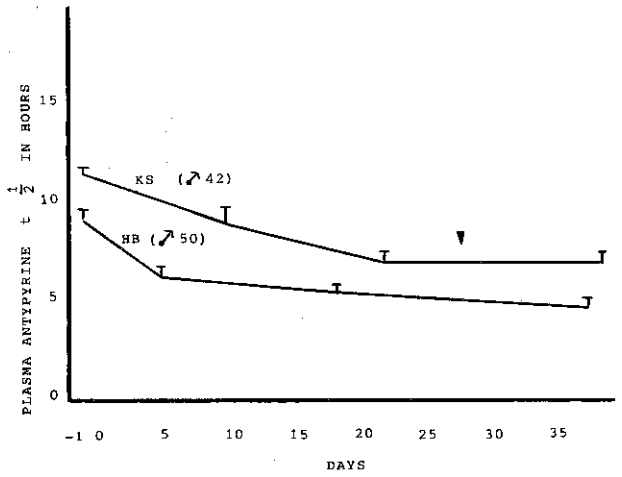

Fig. 1 Effect of rifampicin 450 mg daily on serial antipyrine $t_{1/2}$. Arrow denotes replacement of streptomycin by isoniazid. Vertical bars indicate s.e.m.

(Figure 1). Two patients were similarly studied but isoniazid replaced streptomycin at 14 days to test whether earlier introduction influenced the rifampicin induced decline (Figure 2). In the remaining 4 patients $t_{1/2}$ was tested at the end of 18 months rifampicin/isoniazid treatment at the above dosage. In two of them control values were obtained after 2 weeks on no drugs (Figure 3). The final two patients had repeat $t_{1/2}$'s on either rifampicin or isoniazid alone for 2 weeks and then on no drugs for 2 weeks, they also had quinine $t_{1/2}$ measured 2 days after each antipyrine $t_{1/2}$ (Figure 4). Informed consent was obtained for plasma $t_{1/2}$ studies. Twenty-four-hour urinary 6B-OHF was estimated before and after 4-months therapy. In one patient 6B-OHF was followed serially at the start of treatment and after introduction of isoniazid at 2 weeks (Figure 5).

Antipyrine was measured using the method of Brodie et al. [1949]: 18 mg/kg was given orally in water and $t_{1/2}$ obtained from plasma samples drawn at intervals over 12 hours and calculated by computer by the method of least squares. Quinine $t_{1/2}$ was measured by a method based on that of Brodie et al. [1947]. Quinine

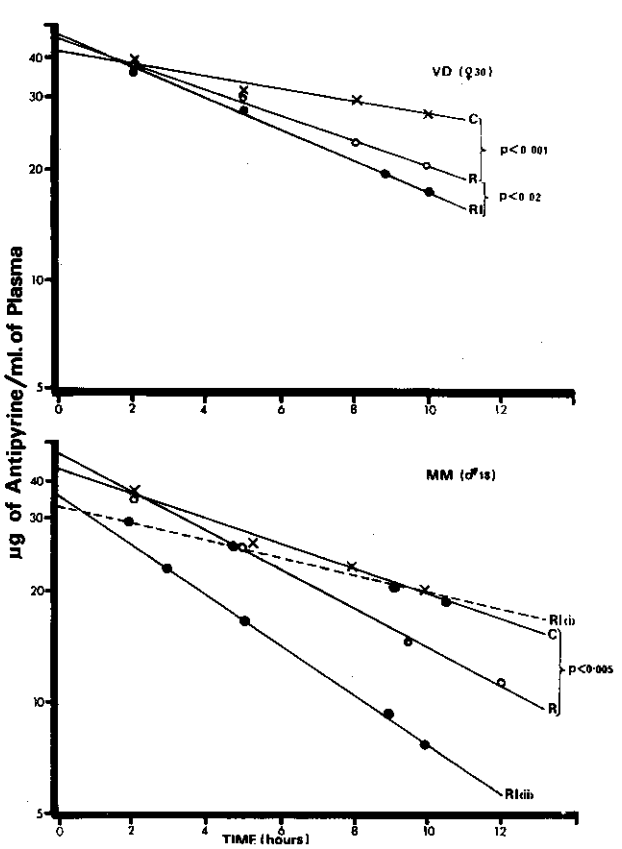

Fig. 2 Effect of rifampicin 450 mg daily on serial antipyrine $t_{1/2}$ at one week and following introduction of isoniazid at 2 weeks. Note the further significant decline at 3 weeks (RI and RI, ii) and the temporary reversal of $t_{1/2}$ in MM the day following isoniazid (RI, i). C = control $t_{1/2}$, R = rifampicin and streptomycin, RI = rifampicin and isoniazid.

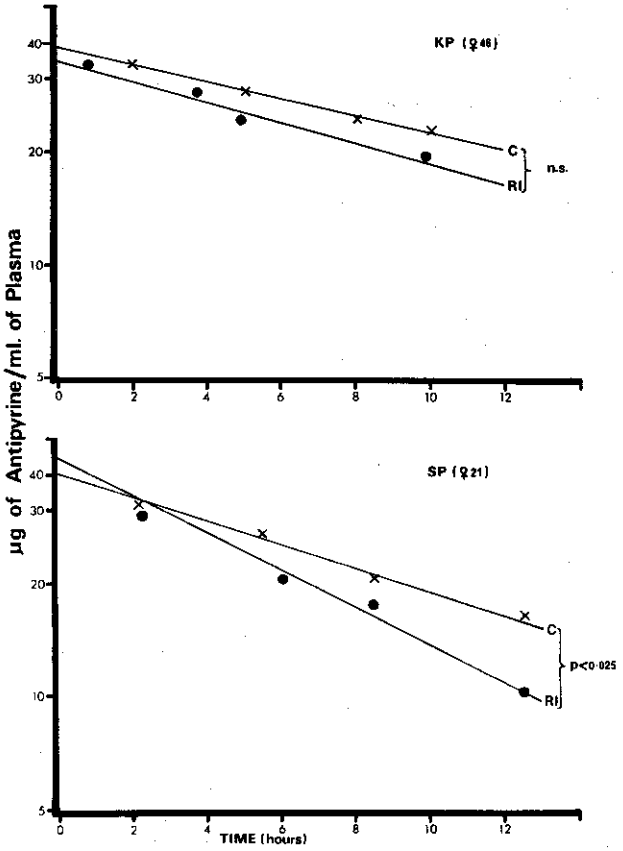

Fig. 3 Decline in antipyrine $t_{1/2}$ at the end of 18-months treatment. The control value was obtained after the drugs were stopped for 2 weeks.

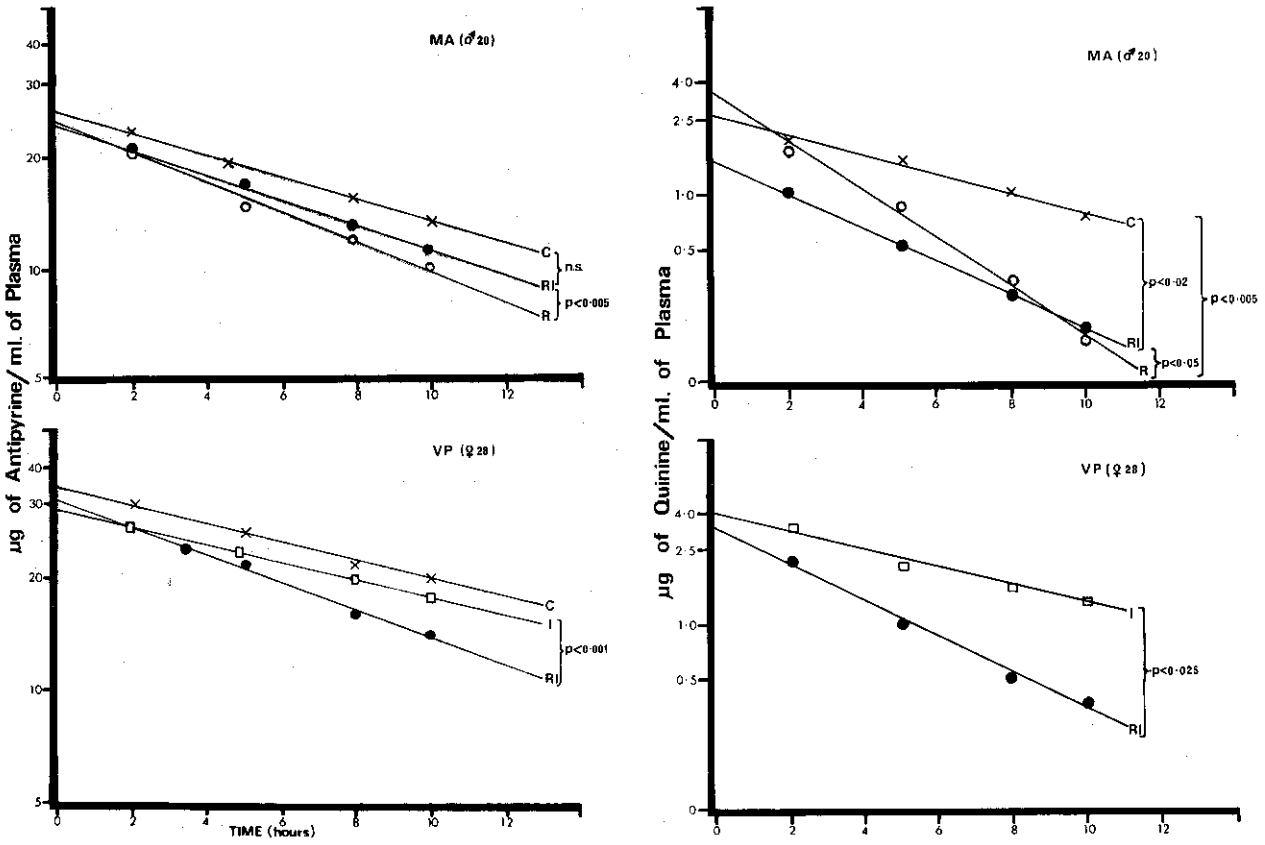

Fig. 4 Decline in antipyrine and quinine $t_{1/2}$ at the end of 18-months treatment. Note in MA only quinine $t_{1/2}$ showed a decline during combined therapy (RI) and in VP there was no difference between the control value (C) and isoniazid alone (I). R = rifampicin alone.

hydrochloride was given orally in a gelatin capsule containing 300 mg and blood was drawn at intervals over 8 hours. Twenty-four-hour urinary 6B-OHF was measured, after extraction and purification, by gas chromatography and mass spectrometry [Setchell et al. 1976]. Student's t-test was used for comparison of $t_{1/2}$'s and Sign test for 6B-OHF results.

Results

A steady decline in antipyrine $t_{1/2}$ induced by rifampicin occurred over a period of 3 weeks by which time it was complete. No effect by isoniazid was observed in these 2 patients when $t_{1/2}$ was repeated 10 days after its introduction (Figure 1). When isoniazid was started at 2 weeks further significant falls were seen but in one patient there was a pronounced increase the day after isoniazid was begun. This was a temporary phenomenon for $t_{1/2}$ one week later was further shortened (Figure 2, MM). Half-life continued to be decreased at the end of 18 months although in one patient this was not significant (Figure 3, KP). A partial reversal of the rifampicin induced decline was clearly shown in patient MA (Figure 4) in whom both antipyrine and quinine $t_{1/2}$ significantly shortened when isoniazid was discontinued. Isoniazid given alone in one patient showed no difference from the control value (Figure 4, VP), but the effect of rifampicin was clear

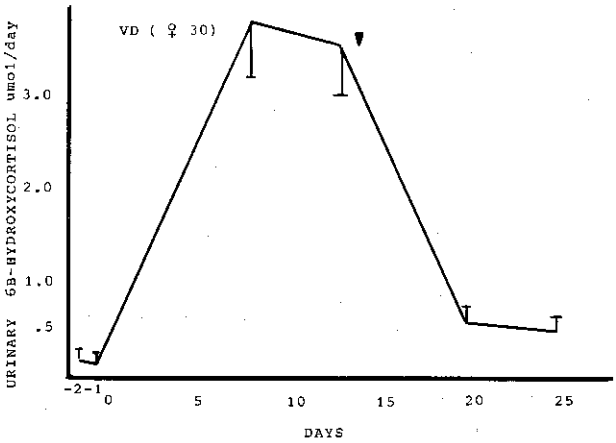

Fig. 5 Effect of rifampicin 450 mg daily with streptomycin on serial 6B-hydroxycortisol excretion and the inhibitory effect when isoniazid was introduced (arrow). Vertical bars indicate s.e.m.

Table 1

Patients	Control	Rifampicin 450 mg + Isoniazid 300 mg/day
VP	0.248	2.726
MS	0.240	0.457
MM	0.261	2.074
VD	0.232	0.626
CK	0.185	0.475
SK	0.190	0.610
HP	0.114	0.462
KP	0.243	0.534

Daily urinary 6B-OHF excretion (μmol) before and after 4 mos. antituberculous therapy. Note the exaggerated responses of VP and MM.

on antipyrine and quinine $t_{1/2}$. When control antipyrine $t_{1/2}$'s were compared with $t_{1/2}$ on rifampicin/isoniazid combination there was a highly significant difference. Mean ± SD = 13.36 ± 4.52 h cf. 7.35 ± 2.06 h, $p < 0.001$.

6B-OHF excretion was significantly increased at 4 months, $p < 0.01$ and 2 patients showed an exaggerated response (Table 1). One patient followed serially at the beginning of treatment showed a 15 fold increase which fell rapidly when isoniazid replaced streptomycin but was still twice the control value (Figure 5).

Discussion

From previous studies [Bennett et al. 1982, Brodie et al. 1982, Larousse et al. 1980] it has not been clear when maximum induction of antipyrine oxidation with rifampicin is reached and our observations indicate that at least 3 weeks is required using 450 mg daily. However, the rate and magnitude of the $t_{1/2}$ response is variable as is the case with similar indices such as urinary D-glucaric acid [Perry and Stamp 1984]. We believe this is genetically controlled. Streptomycin is not metabolized by the liver and is excreted unchanged by the kidney [Goodman et al. 1979], thus we do not think this has influenced our findings. Antipyrine and quinine oxidation were increased throughout therapy in contrast to urinary D-glucaric acid excretion where the increase is reversed in all patients by isoniazid [Perry et al. 1978, Perry and Stamp 1984]. Here the effect by isoniazid on $t_{1/2}$ was either temporary or partial.

The concurrent quinine findings, with the 6B-OHF results, indicate to us that the isoniazid effect is on direct inhibition of oxidative enzymes rather than other pharmacokinetic changes. We did not measure slow acetylator status of isoniazid but where we have observed a reversing effect on $t_{1/2}$ this seems to be the most likely explanation [Brodie et al. 1982]. Yet in contrast to their findings all our patients showed some decrease on combination therapy. It is of interest that since our first report on decline in quinine $t_{1/2}$ by rifampicin [Perry et al. 1978] clinical problems have arisen with the interaction of quinidine and rifampicin [Twum-Barima and Carruthers 1981].

The increase in 6B-OHF excretion demonstrates again the powerful effect of rifampicin on steroid metabolism [Ohnhaus and Parke 1979, Powell-Jackson et al. 1983] but isoniazid can partially oppose this induction (Figure 5) which supports our view that its action is on oxidative enzyme inhibition. This in turn may be related to slow acetylator status. Alternatively, as 2 patients had exaggerated responses (Table 1) the interesting possibility of individual genetic predisposition to enzyme induction is raised. Such a potentiality will be complex to predict for it may depend on the type of drug or drug combination, the diet and state of health and other environmental factors such as smoking, as to whether it is expressed. This may explain why clinical problems are relatively infrequent.

Vegetarian groups are of particular interest clinically because the absence of animal protein may lead to deficiency of certain elements and vitamins and this could be exacerbated by rifampicin-mediated enzyme induction. This possibility was considered in relation to the vitamin D status of Indian vegetarians but the evidence is conflicting [Brodie et al. 1982, Perry et al. 1982] since diet and sunlight deficiency already play such a large part in late rickets and osteomalacia in this population [Stamp et al. 1980]. European patients acting as non-deficient controls showed no evidence of bone disease during rifampicin therapy [Perry et al. 1982]. Here we have also found that such protein deficiency does not appear to grossly affect the capacity of hepatic microsomal enzymes to respond to drug-induced enzyme induction nor does it confer any protection against it.

REFERENCES

Bennett PN, John VA, Whitmarsh VB 1982 Effect of rifampicin on metoprolol and antipyrine kinetics. Br. J. Clin. Pharmacol. *13:* 387–391

Brodie BB, Udenfriend S, Baer JE 1947 The estimation of basic organic compounds in biological materials I. General Principles. J. Biol. Chem. *168:* 299–309

Brodie BB, Axelrod J, Soberman R, Levy BB 1949 The estimation of antipyrine in biological materials. J. Biol. Chem. *179:* 25–29

Brodie MJ, Boobis AR, Hillyard CJ, Abeyasekera G, Stevenson JC, MacIntyre I, Parke BK 1982 Effect of rifampicin and isoniazid on vitamin D metabolism. Clin. Pharmacol. Ther. *32:* 525–530

Brown RR, Miller JA, Miller EC 1954 The metabolism of methylated amino dyes. 4, Dietary factors enhancing demethylation in vitro. J. Biol. Chem. *209:* 211–222

Fraser HS, Mucklow JC, Bulpitt CJ, Khan C, Mould G, Dollery CT 1977 Environmental effects on antipyrine half-life in man. Clin. Pharmacol. Ther. *22:* 799–805

Goodman LS, Gilman A (Eds) 1979 In: The pharmacological basis of therapeutics, 4th ed., Collier-Macmillan, London, p 1245

Larousse C, Le Normand Y, Kergueris MF, Veyrac MJ, Chailleux E, Ordronneau J, Moigneteau Ch 1980 Tuberculosis therapy and enzyme induction in man. Int. J. Clin. Pharmacol. Ther. Toxicol. *18:* 163–168

Ohnhaus EE, Parke BK 1979 Measurement of urinary 6B-hydroxycortisol excretion as an in vivo parameter in the clinical assessment of the microsomal enzyme inducing capacity of antipyrine, phenobarbitone and rifampicin. Eur. J. Clin. Pharmacol. *15:* 139–145

Padgham C, Richens A 1974 Quinine metabolism – a useful index of hepatic drug-metabolising capacity in man? Br. J. Clin. Pharmacol. *1* (4): 352

Perry W, Jenkins MV, Erooga MA, Setchell KDR, Stamp TCB 1978 Induction and inhibition of hepatic microsomal enzymes by rifampicin and isoniazid. Clin. Sci. Mol. Med. *55:* 1–2P

Perry W, Erooga MA, Brown J, Stamp TCB 1982 Calcium metabolism during rifampicin and isoniazid therapy for tuberculosis. J. Roy. Soc. Med. *75:* 533–536

Perry W, Stamp TCB 1984 Urinary D-glucaric acid excretion during rifampicin/isoniazid and anticonvulsant enzyme induction. Clin. Pharmacol. Ther. *35:* 710–715

Powell-Jackson PR, Gray BJ, Heaton RW, Costello JF, Williams R, English J 1983 Adverse effect of rifampicin administration on steroid-dependent asthma. Am. Rev. Respir. Dis. *128:* 307–310

Setchell KDR, Almé B, Axelson M, Sjövall J 1976 The multicomponent analysis of conjugates of neutral steroids in urine by lipophilic ion exchange chromatography and computerised gas chromatography mass spectrometry. J. Steroid Biochem. *1:* 615–629

Stamp TCB, Walker PG, Perry W, Jenkins MV 1980 Nutritional osteomalacia and late rickets in Greater London 1974–1979, clinical and metabolic studies in 45 patients. Clin. Endocrinol. Metab. *9:* 81–105

Twum-Barima Y, Carruthers SG 1981 Quinidine-rifampicin interaction. N. Engl. J. Med. *304:* 1466–1469