Isoniazid Inhibition of Rifampicin-mediated Enzyme Induction

Wayne Perry[1] MB MRCP

يعالج هذا المقال مفعول الايسونيازيد في علاج السل ، وعلاقته ببعض الانزيمات التي تتفاعل مع الريفامبيسين الذي يستعمل في علاج السل أيضاً .

Summary

The effect of isoniazid on rifampicin-mediated enzyme induction was studied in 18 patients during treatment for tuberculosis using measurements of plasma antipyrine and quinine half-life and urinary excretion of D-glucaric acid and 6β-hydroxycortisol (6β-OHF). Antipyrine half-life decreased significantly in 11 patients whether rifampicin was combined with streptomycin or isoniazid. In 3 patients half-life increased when isoniazid was substituted for streptomycin. Quinine half-life decreased in 3 patients tested and in one this change was partially blocked by isoniazid. This effect was probably due to competition by isoniazid for enzyme-binding sites for antipyrine and quinine. Rifampicin combined only with streptomycin increased urinary D-glucaric acid and 6β-OHF two- to ten-fold, replacement of streptomycin by isoniazid restored D-glucaric acid to normal but not 6β-OHF in which only a partial decrease was suspected. It was concluded that isoniazid has a variable suppressant effect on rifampicin-mediated induction.

These findings emphasize the problems of interpreting indirect biochemical indices of enzyme induction during multiple therapy where drugs may have opposing effects. When the above indices are used preferably three should be studied before hepatic microsomal enzyme induction can be considered unlikely.

Introduction

Drug-mediated induction or inhibition of liver microsomal enzymes is well recognized to have important consequences for the metabolism of endogenous substrates and the activity of other drugs (Conney 1967, Vesell & Passananti 1973). The antibiotic

[1] Assistant Professor of Medicine, Faculty of Medicine, King Faisal University, PO Box 2114, Dammam, Saudi Arabia.

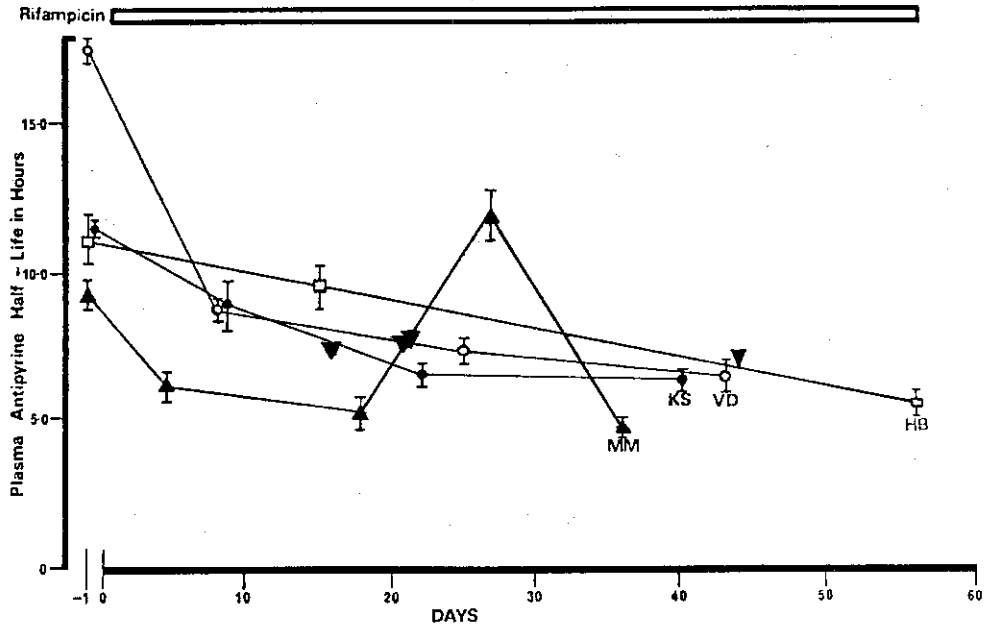

Figure 1. Variable decline in plasma antipyrine half-life during rifampicin therapy 450 mg daily and after substitution of isoniazid 300 mg daily for streptomycin (indicated by ▼).Note temporary increase of the half-life in MM. Bars = mean ± s.e.m.

rifampicin is a powerful inducer of human hepatic microsomal enzymes *in vitro* (Remmer *et al.* 1973). Conversely isoniazid was reported to inhibit some microsomal enzymes (Raisfeld *et al.* 1973, Brennan *et al.* 1970). Changes in plasma antipyrine and quinine half-life and increase in urinary D-glucaric acid and 6β-OHF have been used as indirect indices of hepatic enzyme induction or inhibition in man (Hunter *et al.* 1973, Padgham & Richens 1974, Breckenridge 1975). In a preliminary report (Perry *et al.* 1978) we confirmed the effect of rifampicin-mediated enzyme induction on decline in antipyrine half-life (Miguet *et al.* 1977) and increase in urinary D-glucaric acid and 6β-OHF excretion (Breimer *et al.* 1977, Yamada & Iwai 1976). We also showed a decline in quinine half-life. It was suggested then that isoniazid has an inhibitory effect on rifampicin induction.

Drug-mediated enzyme inhibition has been less well studied than induction. The possibility of both effects occurring during multiple therapy may cause difficulty in the interpretation of established indices. The present study investigates the response of these indices in the presence of two drugs which have opposing actions on hepatic enzyme induction.

Patients and Methods

Eighteen patients with pulmonary or bony tuberculosis were studied who were not receiving other drugs and who had normal renal function. Changes in plasma antipyrine and quinine half-life, urinary D-glucaric acid and 6β-OHF were determined before and during treatment with rifampicin 450 mg daily combined either with streptomycin or with isoniazid 300 mg daily. Streptomycin is excreted largely

unchanged by the kidney (Goodman & Gilman 1970) and is not known to affect hepatic microsomes. Plasma antipyrine half-life was studied in 11 patients, 4 of whom had serial estimations during which isoniazid was substituted for streptomycin (Figure 1). Antipyrine was measured using the method of Brodie *et al.* (1949): 18 mg/kg was given orally dissolved in water and half-life was obtained from 4 plasma samples taken at intervals over twelve hours and calculated by computer by the method of least squares. Quinine half-life was estimated in 3 patients by a method based on that of Brodie *et al.* (1947). Quinine hydrochloride was given orally in a gelatin capsule containing 300 mg and blood was taken at intervals over eight hours. Informed consent was obtained for plasma half-life studies.

Twenty-four-hour urinary D-glucaric acid was estimated using the enzymatic procedure of Simmons *et al.* (1947). Calcium hydrogen glucarate (Sigma Chemical Co.) was used for standard solutions and the sodium salt of phenolphthalein glucuronide as substrate. Daily urinary 6β-OHF was measured, after extraction and purification, by gas chromatography and mass spectrometry (Setchell *et al.* 1976).

The Student t-test was used for statistical analysis.

Results

Antipyrine half-life: These results are shown in Table 1. The range of decline in half-life during rifampicin and isoniazid therapy after at least one month's treatment was 13.7–62.6%. Serial determinations of antipyrine half-life in 4 patients showed variable rates of decline. In one patient, MM, a marked temporary increase was seen five days after the introduction of isoniazid (Figure 1). In the second patient a half-life of 5.93 ± 0.39 hr (mean $\pm$ s.e.m.) was reached after nine days of rifampicin and streptomycin; isoniazid was substituted for streptomycin and half-life eight days later was 7.62 ± 0.20 hr ($P<0.01$). In a third patient, MA, the increase in antipyrine half-life was mirrored by a corresponding effect on quinine half-life (Table 2).

Table 1

Decline in antipyrine half-life during rifampicin therapy for tuberculosis

	No. of patients	Mean duration of treatment (days)	Mean antipyrine half-life ± s.d. (hours)	Percentage decline in half-life	Paired Student t-test
Before treatment	8	–	13.36 ± 4.52	31	< 0.005
Rifampicin 450 mg + streptomycin 1g i.m. daily	8	10	8.95 ± 3.15		
Before treatment	9	–	11.57 ± 2.71	34.6	< 0.001
Rifampicin 450 mg + isoniazid 300 mg daily	9	240	7.35 ± 2.06		

Table 2

Plasma antipyrine and quinine half-life in a patient (MA) showing a blocking effect by isoniazid on rifampicin induction

Daily dosage (mg)	Antipyrine half-life (hr)		Quinine half-life (hr)	
Rifampicin 450 + isoniazid 300	● 9.10±0.19	$P<0.005$	■3·47±0·13	$P<0.05$
Rifampicin 450	7·55±0·14	$P<0.02$	2·67±0·19	$P<0.005$
No drugs	●10·91±0·76		■5·56±0·46	

Results show half-life (mean value ± s.e.m.) in hours during and after therapy and statistical analysis using the paired Student t-test. ●$0.1> P>0.05$, ■$P<0.02$.
The half-life was measured at the end of treatment, isoniazid was then stopped and half-life repeated after 11 days on rifampicin alone and then on no drugs.

Table 3

Plasma quinine half-life before and during rifampicin and isoniazid treatment

Patients	No drugs	Rifampicin 450 mg + isoniazid 300 mg daily	Percentage fall	*P*
VP	5·05±0·44	2·96±0·38 (500)	41·3	<0·02
MA	5·56±0·46	3·47±0·13 (12)	37·6	< 0·02
AM	6·07±0·67	4·89±0·53 (300)	19·4	n.s.

Results show half-life (mean value ± s.e.m.) in hours and the percentage decrease in mean half-life while on drugs. Number of days on drugs in parentheses. Statistical values using the paired Student t-test.

Table 4

Urinary D-glucaric acid excretion during rifampicin therapy for tuberculosis

	Urinary D-glucaric acid (μmol/day)		Mean duration of treatment (days)
Before treatment	23·80±20·4 (16)	(A)	
Rifampicin 450 mg + streptomycin 0·75–1·0 g i.m. daily	108·4 ±40·6 (10)	(B)	10
Rifampicin 450 mg + isoniazid 300 mg daily	29·40±19·60 (18)	(C)	120

Statistical analysis using unpaired Student t-test (values shown are mean ± s.d.) with number of patients in parentheses.
A v. C $P>0.20$; A v. B $P<0.001$; B v. C $P<0.0.001$.

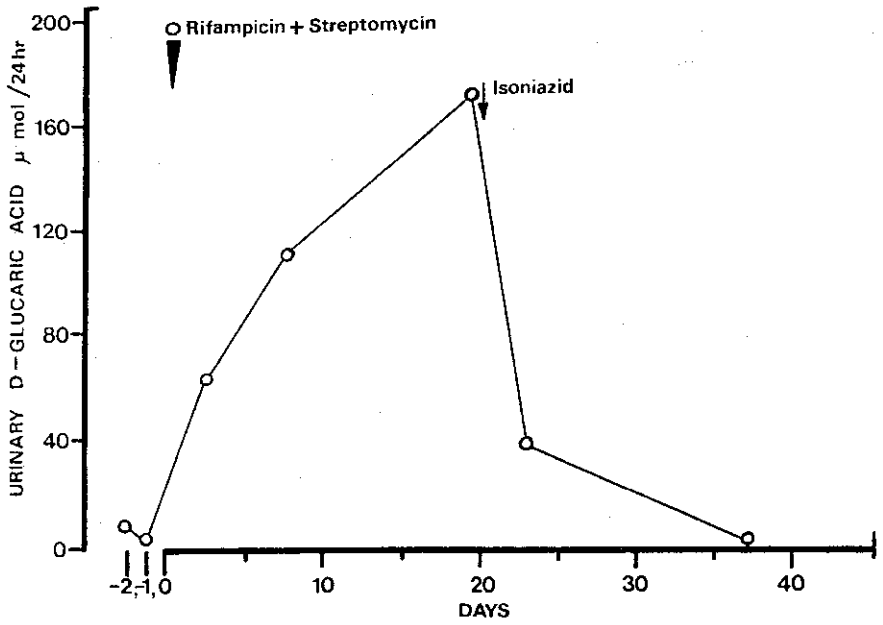

Figure 2. Increase in D-glucaric acid in a patient during rifampicin 450 mg daily and fall in excretion when isoniazid 300 mg daily was introduced (see text).

Quinine half-life: These results are shown in Table 3. All 3 patients showed a decline in quinine half-life during rifampicin and isoniazid. In 2 patients this was statistically significant.

Urinary D-*glucaric acid:* These results are summarized in Table 4. Group A were estimated prior to antituberculous treatment and Group B during rifampicin and streptomycin therapy. A significant increase in urinary D-glucaric acid excretion occurred in the latter group. In Group C patients taking rifampicin and isoniazid showed no significant difference in excretion from the control group. One patient was followed serially and a complete reversal of rifampicin-stimulated D-glucaric acid excretion was observed when isoniazid was substituted for streptomycin (Figure 2).

Urinary 6β-hydroxycortisol: These results are plotted in Figure 3: 8 patients were studied during rifampicin and isoniazid therapy and 3 of these during rifampicin and streptomycin. The range for urinary 6β-OHF excretion before treatment was 0.11–0.26 μmol/day and during therapy 0.46–3.81 μmol/day. In 3 patients the increase exceeded their control values by more than tenfold and in all patients excretion was at least doubled. In 3 patients tested during rifampicin and streptomycin therapy only one showed a clear reversal after isoniazid was substituted for streptomycin.

Discussion

The antipyrine results demonstrate that the decline in half-life due to rifampicin is maintained in the presence of streptomycin or isoniazid. However, in 3 patients out of 11 there appeared to be a significant reversal of this decline when isoniazid was substituted for streptomycin. In patient MM in Figure 1 the effect appeared to be

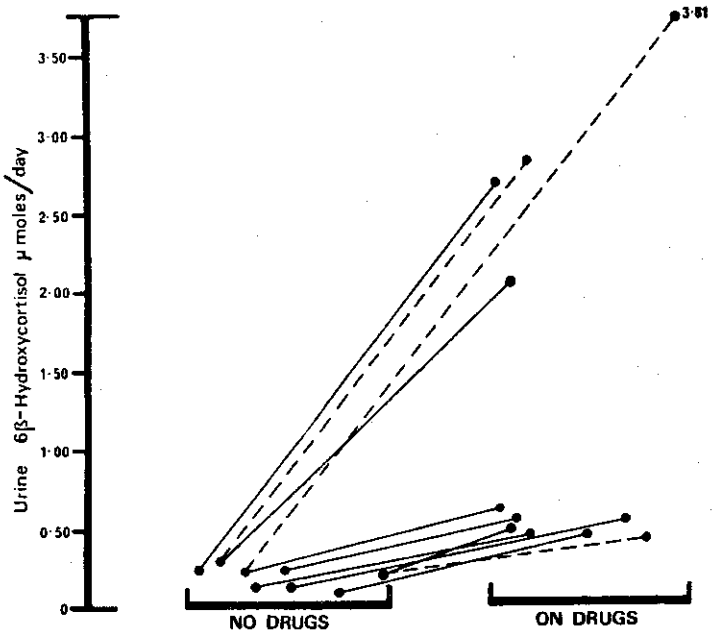

Figure 3. Increase in urinary 6β-OHF in 3 patients on rifampicin 450 mg and streptomycin 0.75 g i.m. daily for ten days (interrupted line) and in these 3 and in 5 other patients on rifampicin and isoniazid 300 mg daily for a mean of 120 days (continuous line). Note the fall in 6β-OHF after isoniazid was begun in the patient with the highest response (3.81 μmol/day).

short-lived. Patient MA in Table 2 confirmed that this was not a chance effect by showing that both antipyrine and quinine half-life were similarly altered in the same patient. Patients had similar plasma peak levels of antipyrine two hours after ingestion of the drug (unpublished observations) which was strong evidence against any subsequent alteration in absorption when test drugs were given or in the distribution of antipyrine in body water. The possibility of enzyme inhibition by isoniazid of the cytochrome p450 mixed function oxidase system responsible for antipyrine oxidation has to be considered. However, the rapid return of induction in the case of MM favours the possibility of competition, temporary or continuous, between isoniazid and antipyrine for enzyme binding sites thus altering the plasma kinetics of antipyrine. This was previously shown to occur between antipyrine and aminopyrine (Vesell *et al.* 1976). A decline in quinine half-life has not been reported with rifampicin. The results in these patients support its value as an index of enzyme induction (Padgham & Richens 1974).

The significant increase in urinary D-glucaric acid with rifampicin 450 mg daily and streptomycin demonstrates that the assay is quite sensitive; other workers have achieved a significant rise using a high dose of 1200 mg/day (Ohnhaus *et al.* 1979). When isoniazid was part of the anti-tuberculous regime urinary excretion of D-glucaric acid was normal in 18 patients. Edwards *et al.* (1974) reported increased levels in 3 patients they studied. In a further 5 patients followed serially all showed suppression of the rifampicin-induced rise in D-glucaric acid when isoniazid was substituted for streptomycin. The reversal of one of these patients with a high response to rifampicin is shown in Figure 2. The reason for this reversal is not clear although isoniazid inhibition of microsomal enzymes appears most likely. This brings into

question the value of using D-glucaric acid as a single test of drug-mediated enzyme induction, especially in the presence of multiple therapy.

The increase in urinary 6β-OHF excretion by rifampicin, similar to D-glucaric acid, is probably due to induction of the cortisol 6-hydroxylation pathway (Yamada & Iwai 1966). The possibility that isoniazid is partially inhibiting this effect is suggested from the data in Figure 3, where two groups appear to be separated. However, this may be due to the small number of patients tested although one patient had a pronounced fall in 6β-OHF after isoniazid was introduced.

The complexity of the interaction between rifampicin and isoniazid on endogenous substrate indices (D-glucaric acid and 6β-OHF) and the plasma kinetics of antipyrine and quinine is an interesting model for the effects of drugs which have opposing actions on hepatic enzyme activity. It may have important pharmacological implications for compound therapy.

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