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The Effect of Rifampicin on Norethisterone Pharmacokinetics

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Summary. The pharmacokinetics of norethisterone have been studied in 8 women during and one month after treatment with rifampicin (450–600 mg/day). Rifampicin caused a significant reduction in the A. U. C. of a single dose of 1 mg norethisterone from 37.8 ± 13.1 to 21.9 ± 5.9 ng/ml X h ($p < 0.01$). The plasma norethisterone half life (β-phase) was also reduced from 6.2 ± 1.7 to 3.2 ± 1.0 h ($p < 0.0025$). In one additional woman on long term oral contraceptive therapy the 12 hour plasma norethisterone concentration was reduced by rifampicin from 12.3 ng/ml to 2.3 ng/ml. Rifampicin caused a significant increase in antipyrine clearance, 6β-hydroxycortisol excretion and plasma gamma-glutamyltranspeptidase activity but there were no significant correlations between changes in these indices of liver microsomal enzyme induction. There was a significant correlation between the percentage increase in antipyrine clearance and the percentage decrease in norethisterone A. U. C. during rifampicin. The changes in norethisterone pharmacokinetics during rifampicin therapy are compatible with the known enzyme inducing effect of rifampicin.

Key words: norethisterone, rifampicin; enzyme induction, antipyrine, 6 beta-hydroxycortisol, gamma-glutamyltranspeptidase.

In 1971 Reimers and Jezek [1] reported that of 51 women with tuberculosis taking contraceptive steroids, 38 had increased intermenstrual or breakthrough bleeding, a sign of relative oestrogenic deficiency, when given rifampicin. Further, at least 14 pregnancies have now been reported in women taking rifampicin in conjunction with contraceptive steroids [2–9]. It has been shown [10] that rifampicin enhances the metabolic destruction of ethinyloestradiol in the livers of women receiving rifampicin as compared to controls. No information is available on the effect of the antibiotic rifampicin on the rate of elimination of the progestogenic component of oral contraceptives. We have thus studied the pharmacokinetics of norethisterone in a group of women being treated with rifampicin during and after stopping the rifampicin.

Materials and Methods

Patients

We have studied 9 female patients (aged 18–42) who were receiving treatment with rifampicin for tuberculosis. Clinical details are shown in Table 1. As shown all the patients were also receiving isoniazid and/or ethambutol. Patients K. P. and B. S. stopped rifampicin while continuing with the alternative therapy but the other women stopped all their antituberculous therapy at the same time. Five women were treated with rifampicin for one year and three (H. A., A. O'C., B. S.) for only 3 months. One woman was receiving long term contraceptive steroid therapy with Minovlar® (containing 1 mg norethisterone acetate and 50 µg ethinyloestradiol). All patients gave their written informed consent to the studies which were approved by the local ethical committee. No patient was taking any other drug during the period of the study.

Single Dose Studies

The eight women not on contraceptive steroid therapy each took a single tablet of Minovlar®, after

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Table 1. Patients studied

Patient	Age	Organ involved with TB	Daily Dose of Rifampicin (mg)	Other drugs & Dose/day
H. A.	18	Lung	450	Isoniazid 300 mg
G. B.	21	Lung	600	Isoniazid 300 mg Ethambutol 600 mg
I. C.	41	Lung	600	Isoniazid 300 mg
J. N.		Lung	600	Isoniazid 350 mg
A. O'C.	21	Lymph Nodes	450	Isoniazid 200 mg Ethambutol 600 mg
K. P.	42	Lymph Nodes	600	Isoniazid 300 mg
A. S.	23	Lung	450	Isoniazid 300 mg Ethambutol 600 mg
B. S.	26	Kidney	600	Pyrazinamide 2000 mg Isoniazid 300 mg
C. D.[a]	29	Lung	600	Isoniazid 300 mg

[a] On long term therapy with oral contraceptive steroids

an overnight fast, towards the end of rifampicin therapy and again one month after stopping rifampicin. Blood samples (10 ml) were taken via an indwelling catheter in an arm vein, prior to each dose of Minovlar®, and at 1, 2, 3, 4, 6, 8, 11, 14 and 24 h after dosing. The blood was taken into heparinized containers, centrifuged at 2000 rpm for 10 min and the supernatant plasma was removed and stored at −20° C prior to analysis. Plasma norethisterone concentrations were measured by a radioimmunoassay developed in this laboratory [11]. The plasma norethisterone concentration versus time curve was analysed according to a two compartment open model [12] and the plasma half life was calculated from the terminal apparently mono-exponential phase by least square regression (β-phase). The area under the plasma concentration versus time curve (A. U. C.) was calculated from 0 to 24 h by the trapezoidal rule [13]. The area from 24 h to infinity was always less than 5% of the area from zero to 24 h.

Multiple Dose Study

In the one patient (C. D.) who was taking long term oral contraceptive steroids, blood samples were taken 10–12 h after the daily dose of Minovlar® on alternate days for the last two weeks of the cycle (6 samples). The blood was centrifuged, the plasma stored at −20 °C and later analysed for norethisterone. This procedure was performed over two cycles towards the end of the rifampicin therapy and over two cycles after stopping rifampicin. She stopped all antituberculous therapy at the same time after one year's treatment.

Indices of Enzyme Induction

a. Change in Plasma Antipyrine Half Life. In seven patients a single dose of antipyrine (600 mg) was given by mouth during and one month after stopping rifampicin. Blood samples were taken at 3, 6, 9, 12 and 24 h on both occasions and the plasma was analysed for antipyrine by radioimmunoassay [14] with the following minor modifications. The extraction volume was reduced to 1.8 ml and after the addition of saturated ammonium sulphate, the amount of antipyrine bound to the antibody was measured directly by resuspending the ammonium sulphate precipitate in distilled water (400 µl) and counting the radioactivity in N. E. 260 (4.5 ml) (Nuclear Enterprises, Edinburgh, Scotland), in a liquid scintillation counter. The plasma half lives and plasma clearances of antipyrine were calculated as described previously [15].

b. Change in Urine 6β-hydroxycortisol Excretion. In the nine women, two, twenty-four hour urine collections were made both during and after rifampicin therapy. The volume of the urine was recorded and a 50 ml aliquot was stored at −20 °C. The urinary 6β-hydroxycortisol was measured by a radioimmunoassay method [16] which is both specific and sensitive. The urinary excretion of 17-hydroxy-corticosteroids was also measured in all samples [17].

c. Change in Plasma Gamma-Glutamyltranspeptidase. Gamma-glutamyltranspeptidase activity was measured by the method of Szasz [18] in 3 plasma samples taken during rifampicin as well as in 3 plasma samples taken one month after stopping rifampicin in nine women.

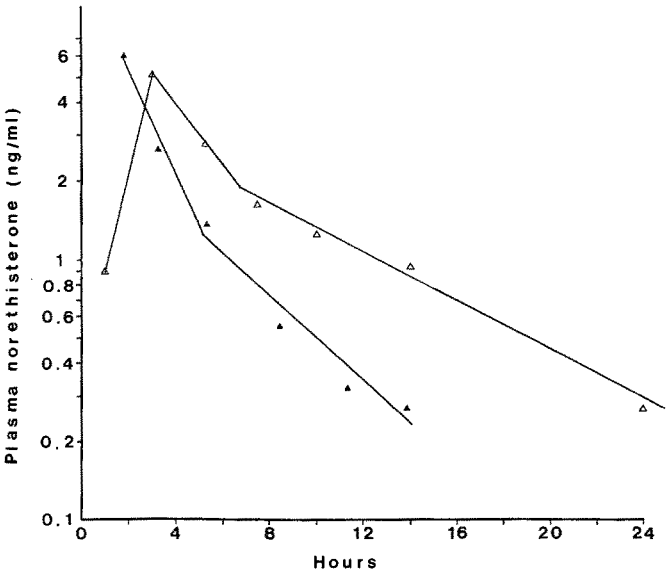

Fig. 1. Plasma norethisterone concentrations in patient A. O'C. after a single oral dose of Minovlar® during (▲–▲) and after (△–△) rifampicin therapy

Statistical Methods

The differences between the tests performed during and after rifampicin treatment were assessed by Students t-test and the correlations between the tests were measured using linear regression analysis.

Results

1. Single Dose Studies

The results of the norethisterone pharmacokinetics are shown in Table 2 with a typical example in Figure 1. The mean AUC was 21.9 ± 5.9 ng/ml × h (mean ± SD) during rifampicin and increased to 37.8 ± 13.1 ng/ml × h ($p < 0.01$) after rifampicin was stopped. The terminal plasma half-life increased significantly from 3.2 ± 1.0 h to 6.2 ± 1.7 h ($p < 0.0025$).

2. Multiple Dose Study

In patient C. D. the mean plasma norethisterone concentration determined 10–12 h after dosing rose from 2.3 ± 0.4 ng/ml during rifampicin to 12.3 ± 1.6 ng/ml (mean ± SD) after rifampicin was stopped ($p < 0.001$).

3. Indices of Enzyme Induction

a. Change in Plasma Antipyrine Kinetics. The plasma half-life of antipyrine increased on stopping rifampicin from 7.3 ± 2.1 h to 9.8 ± 1.7 h ($p < 0.05$) and the plasma clearance fell significantly from 72.4 ± 24.3 ml/h/kg to 42.6 ± 3.9 ml/h/kg ($p < 0.02$) (Table 3).

b. Change in Urine 6β-hydroxycortisol Excretion. There was a significant fall in the urinary excretion of 6β-hydroxycortisol when rifampicin was stopped, from 1177 ± 313 μg/day to 341 ± 65.0 μg/day. There was no significant change in the urinary excretion of 17 hydroxycorticosteroids (Table 3).

c. Change in γ-glutamyltranspeptidase Activity. There was a significant fall in the plasma gamma-glutamyltranspeptidase activity from 29.6 ± 20 I. U. during rifampicin to 18.0 ± 6.6 I. U. after rifampicin was stopped (Table 3). In only one patient (B. S.) did rifampicin cause an increase in gamma-glutamyl-

Table 2. Norethisterone pharmacokinetics during and after rifampicin

	A. U. C. ng/ml × h		Plasma half life h	
Patient	During	After	During	After
H. A.	22.3	32.8	3.3	5.2
G. B.	32.3	64.0	2.6	4.9
I. C.	19.7	25.9	4.0	5.5
J. N.	21.2	25.9	5.1	7.7
A. O'C.	22.5	35.4	3.5	6.0
K. P.	15.1	41.0	2.4	4.2
A. S.	14.9	48.4	2.8	6.3
B. S.	27.6	28.8	1.9	9.6
Mean	21.9	37.8	3.2	6.2
± SD	±5.9	±13.1	±1.0	±1.7
Significance		$p < 0.01$		$p < 0.0025$

Table 3. Plasma antipyrine half-lives and clearances, urinary excretion of 6β-hydroxycortisol and plasma gamma-glutamyltranspeptidase activities during and after rifampicin therapy

	(mean ± SD) During rifampicin	After rifampicin	Significance p
Plasma Antipyrine Half life h	7.3± 2.1	9.8± 1.7	<0.05
Plasma Antipyrine Clearance ml/h/kg	72.4± 24.3	42.6± 3.9	<0.02
6β-hydroxy-cortisol excretion μg/day	1177 ±313	341 ±65	<0.001
γ-glutamyl-transpeptidase activity I. U.	29.6± 20.0	18.0± 6.6	<0.05

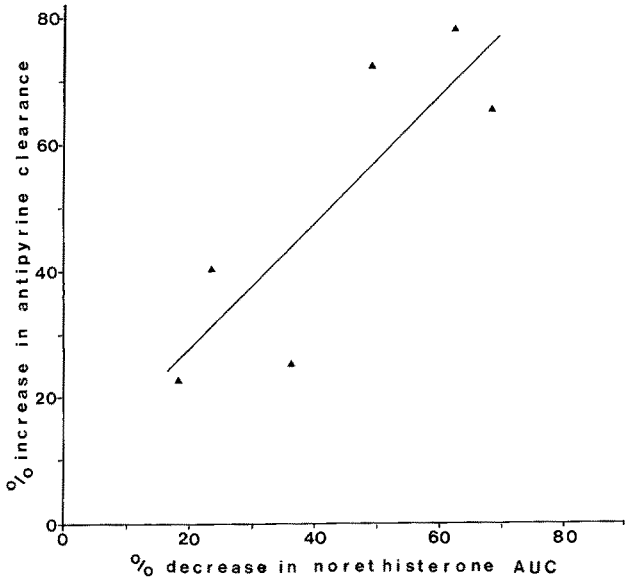

Fig. 2. Correlations between the percentage increase in antipyrine clearance and the percentage reduction in norethisterone A. U. C. during rifampicin therapy in six women ($r = 0.843$, $p < 0.01$, slope 0.994, intercept = 7.82)

transpeptidase above the upper limit of normal for this laboratory (53 I. U.).

Correlations

There were no significant correlations between the three tests used as indices of enzyme induction, plasma antipyrine clearance, 6β-hydroxycortisol excretion, and plasma gamma-glutamyltranspeptidase activity, whether expressed as absolute units or as percentage change. Figure 2 shows that there was a correlation between the percentage change in antipyrine clearance and the percentage change in the A. U. C. of norethisterone ($r = 0.843$, $p < 0.01$) although both tests were performed in only six subjects.

Discussion

We have shown that rifampicin administration will cause a significant reduction in the area under the plasma concentration time curve of norethisterone and its apparently terminal half life. Rifampicin is known to cause failure of oral contraceptive steroid therapy [1–9] and this has previously been attributed to changes in the kinetics of the oestrogen component. Rifampicin has been shown to enhance the metabolism of ethinyloestradiol in human liver microsomal preparations [10] and further work has shown that the terminal half life of ^{3}H-ethinyloestradiol is reduced following rifampicin administration [19]. Rifampicin is known to be an inducer of liver microsomal drug metabolising enzymes in man, based on changes in the elimination of hexobarbital, tolbutamide [20, 21], warfarin [22] and antipyrine [23, 24]. Our data is compatible with a similar basis for the interaction between rifampicin and norethisterone. The reduction in A. U. C. and plasma half-life of norethisterone is consistent with enzyme induction by rifampicin. A reduction in the A. U. C. of norethisterone might be due to a reduced absorption of norethisterone in the presence of rifampicin. The mechanism would be unlikely to result in a change in the terminal plasma half life; and we have evidence, in one patient given equal i. v. and oral doses of norethisterone whilst taking rifampicin, that the bioavailability of norethisterone is very similar to that found in normal volunteers not on rifampicin therapy [13]. A further possible method of interaction is by an alteration in the enterohepatic circulation of norethisterone. We have shown that both norethisterone and ethinyloestradiol undergo enterohepatic circulation in the rat and that antibiotics may reduce the enterohepatic circulation of these steroids [25]. Norethisterone however, unlike ethinyloestradiol, undergoes enterohepatic circulation only as the reduced metabolite and not as the unchanged drug [25].

Rifampicin caused significant increases in the urinary excretion of 6β-hydroxycortisol, plasma antipyrine clearance and plasma gamma-glutamyltranspeptidase activity, used as indirect indices of enzyme induction in man. Our data confirm the observations of Miguet et al. [23] who observed a reduction in the mean plasma half life of antipyrine from 11.7 to 6.9 h after 6 days of administration of rifampicin to 7 volunteers. Ohnhaus [24] has also noted an increase in antipyrine clearance following rifampicin as well as an increase in 6β-hydroxycortisol excretion [26]. In contrast, Breimer et al. [27] could find no change in plasma antipyrine half life or gamma-glutamyltranspeptidase activity after the administration of 1200 mg rifampicin daily for 8 days to 6 volunteers. In their patients the initial plasma antipyrine half life was short with a mean value of 6.9 h. Using the change in plasma antipyrine half-life as an index of the rate of drug oxidation, it has been suggested that patients who oxidize drugs slowly show a proportionately greater degree of enzyme induction [28].

We were unable to show any significant correlation between the individual indices of enzyme induction in man, in confirmation of earlier studies [29, 30]. There was however, a significant correlation between the increase in antipyrine clearance and the reduction in the norethisterone A. U. C. during rifampicin ($r = 0.843$, $p < 0.01$).

All our patients were taking other antituberculous drugs, most of which were stopped at the same time as rifampicin. Isoniazid, the most common additional drug, is reported to inhibit drug metabolism in

man [31] but any changes caused by the cessation of this drug would have been in the opposite direction to those seen with rifampicin.

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