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SERUM CONCENTRATIONS AND BIOAVAILABILITY OF RIFAMPICIN AND ISONIAZID IN COMBINATION

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1 The bioavailability of rifampicin and isoniazid from formulations containing these drugs in combination has been compared to that from formulations containing either drug alone.

2 No formulation-related differences in either rates or extent of bioavailability were found after administration of each formulation.

3 Mean peak serum concentrations of rifampicin (8.2-11.7 μg/ml) occurring 2 to 4 h after doses of 600 mg, and isoniazid (3.6-4.8 μg/ml) occurring 0.5 to 1 h after doses of 300 mg, were similar to those reported in the literature.

Introduction

Chemically equivalent dosage forms of a drug may not be therapeutically equivalent (Campagna, Cureton, Mirigian & Nelson, 1963; Levy, 1964; Clarke & Lasagna, 1965; Parfitt, 1968; Varley, 1968; Macdonald, Pisano, Burger, Dornbush & Pelak, 1969; Tyrer, Eadie, Sutherland & Hooper, 1970; Whittet, 1971; British Medical Journal, 1972; Chasseaud & Taylor, 1974) and therefore whenever significant changes in the dosage form or formulation of drugs are made, the bioavailability of the drug from the new product must be measured.

In the treatment of tuberculosis two or more antituberculous drugs are almost invariably used concurrently. Even when the two drugs are given separately, however, one may affect the absorption of the other, as shown by Boman, Lundgren & Stjernström, (1975). 'Rifampicin is a powerful, bactericidal antituberculosis drug, and in combination with isoniazid it forms a highly potent regimen' (British Thoracic and Tuberculosis Association, 1975). Furthermore 'Chemotherapy with rifampicin plus isoniazid for 9 months, supplemented initially by ethambutol, is more acceptable than standard chemotherapy for 18 months, is highly effective in sputum conversion, and has resulted in no relapses over a 9-month follow-up period' (British Thoracic and Tuberculosis Association, 1975). For these reasons, a dosage form combining rifampicin and isoniazid was considered desirable.

In a five-way crossover bioavailability study the effects of isoniazid and rifampicin on each other were evaluated when they were given together in the single dosage form (Rifinah®). The bioavailability of a new dosage form of isoniazid was also compared with that of the standard.

Methods

Ten healthy volunteer adults, seven males and three females were chosen. The subjects were aged between 18 and 50 years and weighed between 54 and 89 kg. Each subject was examined and blood and urine samples were taken for measurement of liver and kidney functions. The results of these observations showed that the subjects were in good health. The subjects consented in writing to participate after the nature and aim of the experiment had been suitably explained. It was also established that the subjects had no history of liver, kidney or gastrointestinal disease, or showed evidence of allergic diathesis. None of the female subjects was pregnant or considering pregnancy.

Five formulations were tested in these experiments. The isoniazid reference formulation (Formulation A, Rimifon®, Roche Products Ltd, lot no. 701081) contained isoniazid per tablet (100 mg) and each subject received a dose of three tablets, equivalent to isoniazid (300 mg). The rifampicin reference formulation (Formulation B,

Rifadin®, Lepetit Pharmaceuticals Ltd, lot no. 509/9/A) contained rifampicin per capsule (300 mg) and each subject received a dose of two capsules, equivalent to rifampicin (600 mg). The two combination formulations (Formulations C and D, Lepetit Pharmaceuticals Ltd, batch nos. 528 and 536, now marketed as Rifinah® 300 and Rifinah® 150, respectively contained isoniazid (150 mg) and rifampicin (300 mg) per tablet, and isoniazid (100 mg) and rifampicin (150 mg) per tablet respectively. Each subject received a dose of two tablets of Formulation C, equivalent to isoniazid (300 mg) and rifampicin (600 mg) and a dose of three tablets of Formulation D, equivalent to isoniazid (300 mg) and rifampicin (450 mg). The last formulation (Formulation E, Lepetit Pharmaceuticals Ltd, batch no. 500) contained isoniazid alone (300 mg per tablet), and each subject received a dose of one tablet, equivalent to isoniazid (300 mg).

All the subjects were required to fast from the midnight preceding drug administration, and to continue fasting for 3 h afterwards, during which time the subjects were ambulant. Administration was conducted in complete crossover fashion, and each subject received the appropriate formulation on each of 5 days of dosing, with a minimum of 1 week interval between administrations. The medication was taken with one glass of water, and blood samples (15 ml) were taken before dosing, and at 0.5, 1, 2, 4, 6, 8 and 12 h afterwards. After the samples had clotted, the separated serum was removed by centrifugation, and stored at -20°C until taken for analysis. Although only 1 week was allowed before administration of the next formulation, it was unlikely that there would be any influence on drug metabolism by the single doses of rifampicin, reported as an enzyme inducer in man (Jezequel, Orlandi & Tenconi, 1971; Nitti, Ninni, Meola, Iuliano & Curci, 1973) or isoniazid, reported as an enzyme inhibitor in man (Kutt, Brennan, Dehejia & Verebely, 1970).

Isoniazid concentrations in serum were determined by the fluorimetric method of Peters (1960). The recovery of isoniazid added to serum in the range 0.1-30.0 μg/ml was low (70%-75%) but consistent, and the fluorescence of extracts of serum from treated subjects was compared to a standard curve prepared by adding isoniazid to control human serum. Serum concentrations were therefore automatically corrected for losses in analysis. Rifampicin concentrations in serum were determined by a microbiological large plate assay technique using Oxoid antibiotic medium No. 1, seeded with *Sarcina lutea*. Diluted serum was assayed against a standard curve prepared by adding rifampicin to human serum.

Half-lives of drug elimination from serum were calculated by least squares regression analysis of log concentration against time, over the terminal linear section of the curve. Areas under the serum drug concentration-time relationships were calculated by the trapezoidal rule (Notari, 1971) and extended to infinite time by standard procedures (Wagner, 1967). The areas were corrected for variations in half-lives of elimination and in dose/bodyweight ratios using the expression

$$\frac{[\text{Area}]_0^{\infty}}{(\text{D/W})\,(T_{\frac{1}{2}})} = \frac{\text{F}}{0.693\,(\text{V/W})}$$

where D/W is the dose/bodyweight ratio, $T_{\frac{1}{2}}$ is the apparent half-life of elimination, V/W is the apparent volume of distribution per unit bodyweight, and F is the fraction of the dose absorbed (Wagner, 1967).

As the experimental design was unbalanced with respect to the drug content of the formulations, analyses of variance were performed by regression techniques (Draper & Smith, 1966). Formulation-related differences of area (scaled for a dose of 600 mg after administration of rifampicin), half-lives of drug elimination, peak serum concentrations (also scaled for a dose of 600 mg rifampicin), and times of occurrence of peak concentrations were isolated from subject and day of administration effects.

Results

After single oral doses of those formulations containing rifampicin, the peak of mean serum concentrations of drug generally occurred between 2 and 4 h after dosing (Figure 1). After administration of 600 mg in the reference Formulation B, a peak mean concentration of 11.1 μg/ml occurred 2 h after dosing (Table 1), and peak levels in the serum of individual subjects ranged between 5.2 μg/ml and 21 μg/ml. After administration of 600 mg in the isoniazid-rifampicin combination Formulation C, a peak mean serum concentration of 11.7 μg/ml occurred 2 h after dosing (Table 1), with a range of individual peak levels between 7.8 μg/ml to 17.0 μg/ml. After administration of 450 mg in the other combination Formulation D, a peak mean concentration of 8.2 μg/ml occurred 4 h after dosing (Table 1) with a range of individual peak levels between 4.7 μg/ml to 14.5 μg/ml.

The mean apparent half-lives of elimination of rifampicin from serum were 4.1 h after administration of the reference Formulation B, and 3.3 h and 2.8 h after administration of the two combination Formulations C and D respectively (Table 2).

Formulation-related differences in half-lives of elimination of rifampicin were not statistically

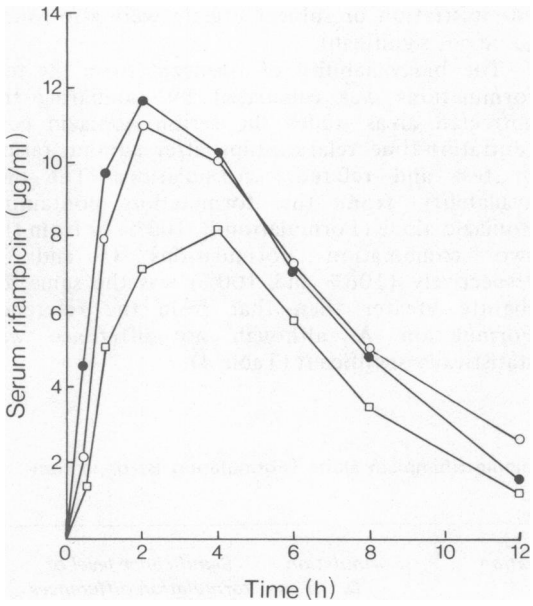

Figure 1 Mean serum concentrations of rifampicin after oral administration of Formulation B (rifampicin 600 mg (○), C (rifampicin 600 mg isoniazid 300 mg i.e. Rifinah® 300 x 2) (●), and D (rifampicin 450 mg, isoniazid 300 mg i.e. Rifinah® 150 x 3) (□) to human subjects.

significant and an analysis of variance showed that there were no formulation-related differences of areas, peak serum concentrations or times taken to reach peak levels (Table 2). Day of administration or subject effects were also found to be not significant (Table 3).

The bioavailability of rifampicin from the combination formulations was calculated by comparing the corrected areas under the concentration-time relationships after administration of the combination formulations with those after administration of the reference formulation. The bioavailability of rifampicin from the combination Formulations C and D (both 114%) was apparently greater than that from the reference Formulation A (Table 2), although these differences were not statistically significant (Table 2). The absence of formulation-related differences of peak serum concentrations and times of occurrence of peak concentrations implied that rates of absorption of rifampicin were not significantly different after administration of any formulation.

After single oral doses of those formulations containing isoniazid, peak serum concentrations of drug generally occurred between 0.5 and 1 h after dosing (Figure 2). After administration of 300 mg as the reference Formulation A, a peak mean concentration of 3.6 μg/ml occurred 1 h after dosing (Table 3) and peak levels in the serum of individual subjects ranged between 2.0 μg/ml and 5.8 μg/ml. After administration of 300 mg in the combination Formulations C and D, peak mean concentrations of 4.8 μg/ml and 3.8 μg/ml occurred 0.5 h after doses of each formulation (Table 3) and peak levels in the serum of individual subjects ranged between 2.6 μg/ml and 7.7 μg/ml (Formulation C) and between 1.4 μg/ml and 6.2 μg/ml (Formulation D). A peak mean concentration of 4.2 μg/ml occurred 1 h after administration of Formulation E, which contained 300 mg isoniazid alone (Table 3), and peak levels in the serum of individual subjects ranged between 2.8 μg/ml and 7.7 μg/ml.

The mean apparent half-lives of elimination of isoniazid from serum were 1.9, 1.8, 2.0 and 1.8 h after administration of Formulations A, C, D and E respectively (Table 4). These half-lives of elimination were not significantly different after administration of any formulation, but subject related differences were significant ($P < 0.05$). Half-lives in two of the male subjects were consistently longer than those in the other subjects

Table 1 Mean ± s.e. mean serum concentrations of rifampicin after single oral doses of formulations containing rifampicin alone (Formulation B) or rifampicin in combination with isoniazid (Formulations C and D).

	Serum concentration (μg/ml)		
Time (h)	*Formulation B*	*Formulation C*	*Formulation D*
0.5	2.1 ± 0.7	4.6 ± 1.0	1.4 ± 0.4
1	8.0 ± 1.3	9.8 ± 1.4	5.1 ± 0.8
2	11.1 ± 1.3	11.7 ± 1.0	7.2 ± 1.2
4	10.1 ± 1.2	10.3 ± 0.9	8.2 ± 0.8
6	7.3 ± 0.8	7.1 ± 0.8	5.8 ± 0.6
8	5.0 ± 0.6	4.9 ± 0.7	3.5 ± 0.4
12	2.5 ± 0.4	1.8 ± 0.4	1.2 ± 0.3

regardless of the formulation administered. These two individuals may have been members of a population group of 'slow inactivators' of isoniazid (Price Evans, 1968).

An analysis of variance of corrected areas, peak concentrations of isoniazid and times taken to reach peak levels showed no significant formulation-related differences for these parameters, although the analysis of variance model did not fit the data of the latter two parameters well. The absence of formulation-related differences in peak concentrations and time to reach peak concentrations implied that the rate of absorption of isoniazid was similar from any formulation. Day of administration or subject effects were also found to be not significant.

The bioavailability of isoniazid from the test formulations was calculated by comparing the corrected areas under the serum isoniazid concentration-time relationships after administration of test and reference formulations. The bioavailability from the formulation containing isoniazid alone (Formulation E, 100%) or from the two combination Formulations C and D respectively (106% and 100%) was the same, or slightly greater than that from the reference Formulation A, although no difference was statistically significant (Table 4).

Table 2 Bioavailability parameters of formulations containing rifampicin alone (Formulation B) or in combination with isoniazid (Formulations C and D).

Parameter	*Formulation B*	*Formulation C*	*Formulation D*	*Significance level of formulation differences*
Area to infinite time* ($\mu g\ ml^{-1}\ mg^{-1}\ kg$)	2.9	3.3	3.3	NS
Mean of individual peak concentrations ($\mu g/ml$)	11.5	12.1	11.6**	NS
Mean time of occurrence of peak concentrations (h)	2.5	1.8	3.3	NS
Apparent half-lives of elimination (h)	4.1	3.3	2.8	NS
Peak of mean serum concentrations ($\mu g/ml$)	11.1	11.7	11.0**	–
Time of peak mean serum concentrations (h)†	2.0	2.0	4.0	–

* Adjusted for variations in dose/bodyweight and half-lives
** Scaled for a dose of 600 mg
† Concentrations were not measured at 3 h
NS Not significant

Table 3 Mean ± s.e. mean serum concentrations of isoniazid after single oral doses of 300 mg in formulations containing isoniazid alone (Formulations A and E) or isoniazid in combination with rifampicin (Formulations C and D).

	Serum concentration ($\mu g/ml$)			
Time (h)	*Formulation A*	*Formulation C*	*Formulation D*	*Formulation E*
0.5	3.1 ± 0.6	4.8 ± 0.6	3.8 ± 0.6	3.5 ± 0.6
1	3.6 ± 0.4	3.6 ± 0.2	3.5 ± 0.3	4.2 ± 0.5
2	2.4 ± 0.3	2.5 ± 0.2	2.4 ± 0.3	2.4 ± 0.2
4	1.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.3	1.1 ± 0.2
6	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.5 ± 0.1
8	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
12	0.1 ± 0.06	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.05

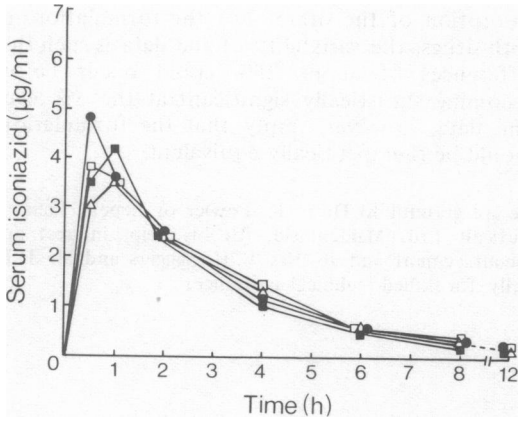

Figure 2 Mean serum concentrations of isoniazid after oral administration of Formulation A (isoniazid 300 mg) (△), C (isoniazid 300 mg, rifampicin 600 mg i.e. Rifinah® 300 x 2) (●), D (isoniazid 300 mg, rifampicin 450 mg i.e. Rifinah® 150 x 3) (□) and E (isoniazid 300 mg) (■).

Discussion

Reported concentrations of rifampicin in serum after oral doses vary widely, probably because of the experimental conditions employed. Binda, Domenichini, Gottardi, Orlando, Ortelli, Paccini & Fowst, (1971) cited concentrations of 5-8 μg/ml occurring 2 h after doses of 450 mg, and 7-10 μg/ml occurring 2 h after doses of 600 mg. Boman (1974) administered rifampicin in combination with isoniazid and found mean peak rifampicin concentrations of 7.8 μg/ml (range 2.2-14.5 μg/ml) after doses at 10 mg/kg, which are similar to those measured after administration of similar doses in the combination Formulations C and D. Virtanen & Tala (1974) found concentrations of up to 16 μg/ml (mean 7.8 μg/ml) occurring 1 h after oral doses of a single 450 mg tablet to patients free from tuberculosis, and up to 20 μg/ml (mean 9.0 μg/ml) occurring 1-2 h after administration of three 150 mg tablets. After administration of single 450 mg tablets to patients with pulmonary tuberculosis, mean peak concentrations of 7.9 μg/ml (range 2.5-16.0 μg/ml) were reached in patients on a rifampicin-free regimen, in contrast to peak concentrations of 5.9 μg/ml (range 3.4-9.6 μg/ml) in others after prolonged treatment with rifampicin. Acocella, Bonollo, Garimoldi, Mainardi, Tenconi & Nicolis, (1972) found reduced blood concentrations of rifampicin after prolonged treatment, as did Sunahara & Nakagawa (1972). Rifampicin has been reported to induce microsomal drug-metabolising enzyme systems (Jezequel *et al.*, 1971), but significant week effects in the analysis of variance which might have been expected had these doses of rifampicin caused induction, were not observed.

Serum concentrations of isoniazid after administration of the two combination Formula-

Table 4 Bioavailability parameters of formulations containing isoniazid alone (Formulations A and E) or in combination with rifampicin (Formulations C and D).

Parameter	*Formulation A*	*Formulation C*	*Formulation D*	*Formulation E*	*Significance of formulation differences*
Area to infinite time* ($\mu g\ ml^{-1}\ mg^{-1}\ kg$)	1.7	1.8	1.7	1.7	NS
Mean of individual peak concentrations (μg/ml)	4.2	5.0	4.3	4.6	NS
Mean time of occurrence of peak concentrations (h)	0.9	0.7	0.9	0.8	NS
Apparent half-lives of elimination (h)	1.9	1.8	2.0	1.8	NS
Peak of mean serum concentrations (μg/ml)	3.6	4.8	3.8	4.2	–
Time of peak mean serum concentrations (h)	1.0	0.5	0.5	1.0	–

* No significant differences were found for these parameters but the analysis of variance model did not fit the data well
NS Not significant

tions C and D were similar to those after administration of Formulations A and E, which contained isoniazid alone, and were also similar to those reported in the literature (Peters, 1969; Boman, 1974).

No formulation-related significant differences in extent of bioavailability of isoniazid or rifampicin were found between the combination and reference formulations. The rates of absorption of each drug from the formulations were similar and neither drug interfered with the absorption of the other. For the formulations of both drugs, the variability of the data is such that differences of about 25% could occur before becoming statistically significant at the 5% level. The data, however, imply that the formulations should be therapeutically equivalent.

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