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## Bioavailability of rifampicin following concomitant administration of ethambutol or isoniazid or pyrazinamide or a combination of the three drugs

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**Background & Objectives:** Poor bioavailability of rifampicin (R) in combination with other anti-tuberculosis drugs such as isoniazid (H), pyrazinamide (Z), and ethambutol (E) is a subject of much concern for the last few decades. This could be due to an interaction between R and other drugs. An investigation was therefore undertaken to examine the bioavailability of R in the presence of H, Z and E or a combination of the three drugs.

**Methods:** The study included eight healthy volunteers, each being investigated on four occasions at weekly intervals once with R alone and with three of the four combinations on the three remaining occasions. A partially balanced incomplete block design was employed and the allocation of R or the drug combinations was random. Plasma concentrations of R at intervals upto 12 h were determined by microbiological assay using *Staphylococcus aureus* as the test organism. The proportion (%) dose of R as R plus desacetyl R (DR) in urine excreted over the periods 0-8 and 8-12 h was also determined. Bioavailability was expressed as an index (BI) of area under time concentration curve (AUC) calculated from the plasma concentrations or proportion of dose of R excreted as R plus DR in urine with the combinations to that with R alone.

**Results:** The bioavailability indices based on AUC were 0.96 with RE, 0.76 with RH, 1.08 with RZ and 0.65 with REHZ. The indices based on urine estimations (0-8 h) were similar, the values being 0.94, 0.84, 0.94 and 0.75, respectively. A second investigation revealed that the decrease of bioavailability of R with H was not due to the excipients present in H tablets.

**Interpretation & conclusion:** Isoniazid alone or in combination with E and Z reduces the bioavailability of R. Urinary excretion data offer a simple and non invasive method for the assessment of bioavailability of R.

**Key words** Bioavailability - rifampicin - treatment - tuberculosis - urinary excretion

Current treatment of tuberculosis involves the use of multiple drug regimens containing rifampicin (R) in addition to ethambutol (E), isoniazid (H) and pyrazinamide (Z). Bioavailability of R is known to be affected by a number of factors such as the manufacturing process<sup>1</sup>, presence of food in the gastrointestinal tract<sup>2</sup>, acidity of the gastric juice<sup>3</sup> and excipients including those used with companion drugs such as *p*-amino salicylic acid<sup>4</sup>. Ethambutol has been

shown to have little or no effect on the gastrointestinal absorption of R<sup>5</sup> and concomitant administration of Z resulted in a lower bioavailability of R<sup>6</sup>. Published results with respect to H are equivocal. While Boman *et al*<sup>7</sup> and Acocella *et al*<sup>8</sup> did not observe any effect, Mouton *et al*<sup>9</sup> demonstrated a decrease in the plasma concentrations of R when administered together with H. In normal adults the peak plasma concentrations after

administration of 600 mg R, are in the range of 6-13 µg/ml, while on administration of R along with H and/or Z, the peak concentrations range from 3-6 µg/ml and AUC<sub>0-8</sub> values in the range of 30-50 µg/ml.h<sup>10-12</sup>. Shishoo *et al*<sup>13</sup> demonstrated decreased bioavailability of R in presence of H both by means of *in vitro* dissolution testing and *in vivo* bioavailability studies. Grosset *et al*<sup>14</sup> observed an antagonism between R and H in experimental murine tuberculosis and attributed this to a decreased bioavailability of R. To gain a better understanding of the interactions if any, an investigation was done to study the bioavailability of R when administered together with E, H, Z or a combination of the three drugs (EHZ). The conclusions are not only based on the traditional plasma levels (AUC), but also on the excretion of the proportion of the administered drug as R plus its primary metabolite, desacetyl rifampicin (DR) in urine. Since the results of this investigation revealed a significant decrease in the bioavailability of R when administered together with H alone or EHZ, another investigation based on urine excretion of R plus DR was undertaken to examine whether it was H *per se* or the excipients present in H tablets that was responsible for the decrease.

### Material & Methods

The investigations were undertaken in healthy male volunteers (college students and the centre staff) aged 18-56 yr with normal, hepatic and renal function. Rifampicin (Tata Pharma, India), ethambutol (Ciba-Geigy, India), isoniazid (Pfizer, India) and pyrazinamide (Lupin, India) were used in the first investigation. In the second investigation, due to the non-availability of the Tata Pharma Product, rifampicin (Lupin) was used and pure isoniazid powder was a Bayer product. Standard dosages of the drugs<sup>15</sup> were used in the investigations: 10 mg/kg body weight for R, 25 mg/kg for E, 12 mg/kg for H and 35 mg/kg for Z.

The investigations were started after getting approval from the institutional ethics committee and informed written consent from all the study subjects.

Plasma R concentrations were determined by the plate diffusion assay of Dickinson *et al*<sup>16</sup> employing a strain of *Staphylococcus aureus* [Sub Group I - NCTC 10702, a gift from Dr DA Mitchison, British Medical Research Council (BMRC), UK], resistant to streptomycin and other antibiotics. Rifampicin standards ranging from 0.04-1.28 µg/ml were set up in quadruplicate and the

concentrations of the drug in plasma (set up in quadruplicate in dilutions of 1 in 5, 1 in 10 and 1 in 20) were obtained from regression line of zone of inhibition on log concentrations of the standards.

Urinary excretion of R plus DR was estimated according to the procedure of Immanuel *et al*<sup>17</sup>. In brief, urine samples (3ml or larger according to volume excreted) and standard concentrations of R ranging from 10-50 µg/ml set up in normal urine were mixed with 1.5 ml of a citrate - phosphate buffer, pH 7.0 and extracted with 3 ml of chloroform. The extinction of the chloroform extract was recorded at 475 nm giving an estimate of the concentrations of R plus DR without interference from other metabolites of R.

### Conduct of the investigations:

Investigation I—A total of eight volunteers were investigated. Each volunteer was investigated on four occasions, once with R alone (control) and with 3 of the 4 drug combinations, RE, RH, RZ and REHZ on the other three occasions with an interval of at least one week between occasions. A partially balanced incomplete block design was employed and by using this design, information on R alone was available for all the 8 subjects and for 6 each with the four drug combinations. The sequence of administration of R and the combinations was random (Table I).

**Table I.** Sequence of administration of R alone and in combination with E, H and Z

Volunteer No.	Occasions			
	I	II	III	IV
1.	RE	REHZ	R	RH
2.	R	REHZ	RE	RZ
3.	R	RH	RZ	RE
4.	REHZ	R	RH	RZ
5.	RZ	RH	R	REHZ
6.	RZ	RE	REHZ	R
7.	RH	R	REHZ	RE
8.	RH	RZ	RE	R

R (n=8); RE, RH, RZ & REHZ (n=6)

R, rifampicin; E, ethambutol; H, isoniazid; Z, pyrazinamide

About eight ml of heparinised blood was collected at 1, 2, 3, 6, 9 and 12 h after administration of drug(s) and total urine excreted over the periods 0-8 and 8-12 h were collected following drug administration on an empty stomach. Uniform breakfast and lunch were provided at the end of 2 and 6 h respectively after drug administration. Plasma was separated and stored along with aliquots of urine at  $-20^{\circ}\text{C}$  until analysis. Estimations of plasma R concentrations and those of R plus DR in urine were undertaken within 48 h.

Investigation II—Six volunteers were investigated on two occasions. On the first occasion, 3 volunteers received R (10 mg/kg) plus pure H powder (12 mg/kg), while the other 3 received R plus H tablet in the same dosage. On the second occasion, a week later, the order of administration of H powder or the tablet was reversed, a cross-over design being employed. Urine excreted over the period (0-8 h) was collected and the proportion (%) of the dose of R excreted as R plus DR was calculated.

In both the investigations, all estimations were undertaken after randomising and coding the samples. In investigation-I comparison of the values between the control group (R only) and those who received RE, RH, or REHZ was restricted to the same subjects who received the particular combination and the control.

*Pharmacokinetics and statistical analysis:* Maximum concentration ( $C_{\max}$ ) and the time to attain  $C_{\max}$  ( $T_{\max}$ ) were determined by direct visual inspection of data. Linear trapezoidal rule was used to calculate area under time concentration curve ( $\text{AUC}_{0-12}$ )<sup>18</sup>. The elimination rate constant (Kel) was calculated from the terminal

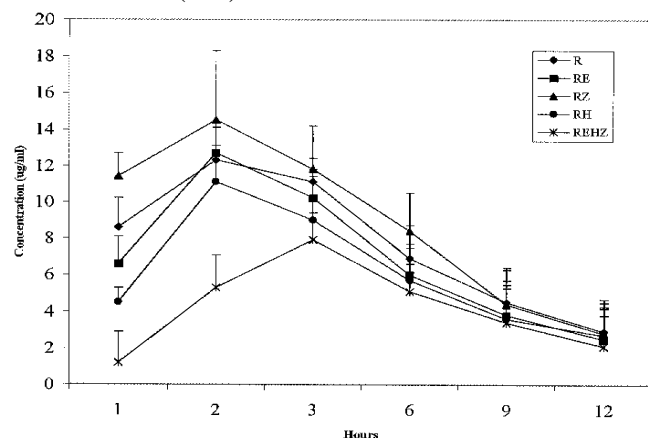


Fig. Plasma concentrations of rifampicin in different study groups.

log-linear decline of concentration. Terminal elimination half-life ( $t_{1/2}$ ) was calculated as  $0.693/\text{Kel}$ .

The bioavailability of R for each of the 4 drug combinations has been expressed as an index (BI) and is the ratio of AUC or the proportion of the dose excreted as R plus DR in urine with the four combinations (RE, RH, RZ and REHZ) to the respective control values obtained after the administration of R alone. Student's t-test (paired and unpaired) was used for testing the significance of the differences between the mean values.

## Results

*Investigation I:* The mean body weight of the 8 volunteers was  $66.1 \text{ kg} \pm \text{SD}$  (range 56.5-75.4 kg) and the mean dosages of R administered to the RE, RH, RZ and REHZ groups (and the respective controls) were 10.88, 11.43, 11.30 and 10.86 mg/kg, respectively.

The mean serial plasma R concentrations for all the 8 control subjects (R only) and for 6 each with RE, RH, RZ and REHZ are shown in the Fig. The mean peak concentrations and  $\text{AUC}_{0-12}$  calculated from those concentrations together with the proportion (%) of dose excreted as R plus DR in urine over the periods 0-8 and 0-12 h are presented in Table II.

Peak concentrations were attained before the third hour in all the subjects and the concentrations fell exponentially thereafter with mean half-lives ranging from 4.14 in the RZ group to 5.14 in the REHZ group, none of these differences being significant. Administration of E or Z did not appear to have any significant effect on the plasma concentrations or the urinary excretion of R. However, the  $C_{\max}$  and the AUC values were significantly lower in the RH (24%) and REHZ (39%) groups than in the respective controls who received R only ( $P < 0.01$ ). Corresponding to the above, the proportion (%) of dose excreted as R plus DR was lower in RH and REHZ groups than in the respective controls, the decrease with RH and REHZ being 18 and 25 per cent over the 0-8 h period and 15 and 21 per cent respectively over a 12h period, all the differences being significant ( $P < 0.01$ ) (Table II).

None of the differences in the mean values of  $C_{\max}$ ,  $\text{AUC}_{0-12}$  or the proportion of dose excreted as R plus

**Table II.** Pharmacokinetics of R given alone and in different combinations

Pharmacokinetic variable	Groups							
	R vs RE		R vs RH		R vs RZ		R vs REHZ	
	R	RE	R	RH	R	RZ	R	REHZ
<i>Plasma :</i>								
Peak concentration (µg/ml)	11.9 ±3.4	12.7 ±1.8	13.5 ±4.3	11.1* ±3.8	13.8 ±3.0	14.5 ±1.7	13.6 ±4.2	7.9* ±2.4
AUC <sub>0-12</sub> (µg/ml.h)	72.4 ±20.0	64.6 ±4.4	77.3 ±22.1	58.6* ±16.7	79.7 ±22.1	85.6 ±15.3	73.1 ±20.4	44.6* ±11.2
<i>Urine :</i>								
% dose excreted 0-8 h	12.8 ±2.3	11.8 ±1.1	14.1 ±1.8	11.9* ±2.0	13.1 ±2.2	13.7 ±2.1	12.6 ±2.0	9.5* ±2.1
% dose excreted 0-12 h	17.5 ±3.8	16.0 ±2.2	19.3 ±2.7	16.4* ±2.5	18.4 ±3.6	17.0 ±3.3	16.8 ±2.9	13.2* ±2.9

Values are mean±SD. n=8 for group R; n=6 for groups RE, RH, RZ and REHZ

\*P<0.01 compared to R

DR in urine was significant between the RE and the RZ groups or between the RE and the RH groups. Only the mean AUC<sub>0-12</sub> value of R was significantly higher in the RZ group than in the RH group (P=0.02). The mean values of all the variables in the RE and the RZ groups were, however, significantly higher than those in the REHZ group (P<0.03). Even though the mean values of all the variables were lower in the REHZ group than in the RH group too, non of the differences was significant.

**Table III.** Bioavailability indices for the various groups

R vs	Bioavailability index based on		
	Plasma levels	Excretion in urine	
		AUC <sub>0-12</sub>	0-8 h
RE	0.96±0.32	0.94±0.12	0.94±0.17
RZ	1.08±0.20	0.94±0.07	0.94±0.10
RH	0.76±0.11*	0.84±0.08	0.85±0.07*
REHZ	0.65±0.25*	0.75±0.09	0.65±0.25*

Values are mean ± SD. n=8 for group R; n=6 for groups RE, RH, RZ and REHZ; \* P<0.05 compared to RE and RZ

The BI (based on AUC and per cent dose excreted in urine) of RE and RZ groups were significantly higher than those obtained for the RH and REHZ groups (P<0.05). The mean values were consistently higher with RH than with REHZ, but none of these differences was significant (Table III).

*Investigation II:* The mean proportions of the dose of R excreted as R plus DR in urine excreted over the period 0-8 h were 9.3 per cent with R plus pure H powder and 10.6 per cent with R plus H tablets; the difference was not significant.

## Discussion

Good bioavailability leading to adequate plasma and tissue concentrations of R (and other drugs) is an absolute prerequisite for the success of treatment of tuberculosis. It has been postulated that peak plasma R concentrations should be of the order of 10-15 µg/ml with dosages of 10-12 mg/kg for good therapeutic response<sup>19</sup>. Together with Z, R is one of the key sterilizing drugs and its anti-TB potency is markedly dose dependent<sup>20</sup>. A United States Public Health System (USPHS) trial has shown that with lower dosages such as 9 mg/kg leading to lower plasma concentrations of R,

the speed at which sputum conversion occurs is also reduced<sup>21</sup>.

Evidence presented in this report suggests that H alone or in combination with E and Z reduces the bioavailability of R. Since the plasma elimination half-lives were similar with the different combinations employed, this could result only from a decreased gastrointestinal absorption of R caused by H. Impaired bioavailability of R in the presence of H as seen in the present study could be due to degradation of R in the presence of H as reported by Jindal *et al*<sup>22</sup> by *in vitro* dissolution testing. Similar results have been reported by Shishoo *et al*<sup>23</sup> who showed more degradation of R in combination with H (18-21%). They have further observed that after oral administration of R and H together, an appreciable amount of R is degraded in the acidic conditions of the stomach to 3-formyl rifampicin (3-FR), which is not absorbed and is inactive. 3-formyl rifampicin undergoes a reversible reaction to form a hydrazone with H. As a result, decomposition of R to 3-FR is pushed forward and an overall increase in the degradation of R is observed in the presence of H<sup>13</sup>. This observation is in agreement with that of Singh and co-workers<sup>24</sup>, who demonstrated degradation of R in the presence of H using a specific HPLC method showing a 3-FR hydrazone peak in the HPLC analysis which was confirmed with a synthetic 3-FR hydrazone.

The results of the second investigation show that it is H *per se* and not the excipient present in H tablet, that is responsible for the decrease in the absorption of R. These findings are in agreement with those reported earlier<sup>9,14</sup>. Further, our observation that Z does not affect the bioavailability of R contradicts the findings of Jain *et al*<sup>6</sup>. Whether the decreased bioavailability of R exerts any therapeutic penalty in the presence of three other powerful anti-tuberculosis drugs during the initial intensive phase of treatment is a matter of conjecture. Combining H with R during the continuation phase is unlikely to have any deleterious effect as there would have been a substantial reduction in the bacterial load by then.

We also examined the bioavailability of E, H and Z in a total of 17 healthy individuals (including the 8 volunteers in the first investigation of this study). Results (not reported) showed that concomitant administration

of the other drugs either alone or in combination did not in any way, affect the bioavailability of E, H or Z. This finding is in agreement with that reported by other workers<sup>25-27</sup>.

About 20 per cent of the administered dose of R is excreted as R plus DR over a 24 h period (TRC, unpublished findings). Of this, more than 70 per cent (*i.e.* about 14% of the dose) is excreted during the first 8 h period. Determination of plasma R concentrations is a complex process and the variation associated with these determinations is larger than that with the estimations of R plus DR in urine. Thus, on the basis of the results obtained in the eight volunteers following administration of R alone, the coefficient of variation associated with the AUC values (based on the determination of plasma concentrations) was 24.9 per cent as against coefficient of variations of 16.1 and 18.7 per cent with the estimation of R plus DR in urine collected over the periods 0-8 and 0-12 h respectively. Thus, the urine method reported in this paper, provides bioavailability indices similar to those based on plasma concentrations, which is in agreement with that reported by others<sup>28-30</sup>. The urine method, therefore, offers a simple and reliable non invasive procedure for investigations requiring an assessment of the bioavailability of R in double and triple drug formulations containing the drug.

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