

## GASTRO-INTESTINAL ABSORPTION OF ISONIAZID AND RIFAMPICIN IN PATIENTS WITH INTESTINAL TUBERCULOSIS

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**Summary.** The gastro-intestinal absorption of isoniazid and rifampicin was studied in 12 patients with intestinal tuberculosis (10 slow and 2 rapid acetylators of isoniazid) and compared with that in 18 patients with pulmonary tuberculosis (8 slow and 10 rapid acetylators). Exposure (area under the time-concentration curve), calculated on the basis of serum concentrations of the two drugs at 1, 2, 3, 6 and 8 hours, and the proportion of dose of isoniazid excreted in urine collected over the period 0-8½ hours as isonicotinyl compounds after drug administration were similar in the 2 groups suggesting no impairment of gastro-intestinal absorption of the 2 drugs in patients with intestinal tuberculosis. It was also observed that there was no delay in the absorption of the 2 drugs in patients with intestinal tuberculosis as compared to that in patients with pulmonary tuberculosis. The absorption of D-xylose, used to assess the absorptive capacity of the proximal small intestine, was also similar and normal in both groups of patients.

### Introduction

Response to treatment is dependent upon effective blood and tissue concentrations of the drugs administered, and these concentrations are, in turn, dependent on a number of factors such as absorption from the site of administration, distribution metabolism and excretion. Most anti-tuberculosis drugs are administered orally and are known to be really absorbed from the gastro-intestinal tract. Fully oral regimens containing drugs such as isoniazid, rifampicin, pyrazinamide and ethambutol have been shown to be highly effective in the treatment of pulmonary

tuberculosis and several extra-pulmonary forms of the disease such as tuberculous lymphadenitis and spinal tuberculosis. The efficacy of these oral regimens in patients with abdominal tuberculosis, including those with intestinal tuberculosis, is now being examined in a controlled clinical trial at the Tuberculosis Research Centre, Madras. Tuberculosis of the gastro-intestinal tract could lead to a destruction of the mucosal membrane resulting in a decreased absorption of drugs and nutrients. Further, bacterial overgrowth in the intestines has been shown to lead to the malabsorption of a number of substances (Theodossi and Gazzard, 1984). An investigation was, therefore, undertaken to study the absorption of isoniazid and rifampicin, two of the most commonly used anti-tuberculosis drugs, in patients with intestinal tuberculosis and compare the findings with those obtained in patients with pulmonary tuberculosis. In addition, the absorption of D-xylose, which has widely been used to assess the absorptive capacity of the proximal small intestine (Chanarin and Bennett, 1962; Sammons *et al.*, 1967), was also studied; this report presents the findings.

### Material and Methods

#### Patients

*Intestinal tuberculosis* : Twelve patients (7 male and 5 female), aged 13 to 45 years, with a mean body-weight of 36.7 kg (range : 23-52 kg) were admitted to the study. The diagnosis of intestinal tuberculosis was made on the basis of radiological, histopathological and bacteriological findings. Duodenum was predominantly involved in 1 patient, ileum in 1, ileocaecum in 2, and ileocaecum in combination with other viscera such as peritoneum, colon, liver or mesenteric

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lymph nodes in the remaining 8 patients. Further, 4 of the 12 patients had surgical resection of part of the affected portion of the intestines (right hemicolectomy in 2 patients and ileocaecal resection in 2 others).

*Pulmonary tuberculosis* : Eighteen sputum smear-positive patients (15 male and 3 female), aged 14 to 44 years with a mean body-weight of 41.5kg (range : 34-51 kg) and with no obvious clinical evidence of gastro-intestinal dysfunction were used as controls.

The renal and hepatic functions, as assessed by plasma creatinine concentrations, and by the activities of AST and ALT, respectively, were normal in all patients in both groups.

*Determination of the acetylator phenotype* : The acetylator phenotype was determined on the basis of the molar ratio of acetylisoniazid to isoniazid in urine. The ratio was determined in urine collected over the period 5-6 hours after a uniform oral dose of isoniazid 300 mg in patients with intestinal tuberculosis (Raghupati Sarma *et al.*, 1976), and over the period 24-25 hours following an oral dose of 30 mg/kg of a slow-release preparation of isoniazid ("matrix isoniazid") in patients with pulmonary tuberculosis (Kailasam *et al.*, 1975).

*Conduct of the investigation* : Isoniazid 300 mg and rifampicin 10 mg/kg were administered on an empty stomach to both groups of patients. Half-an-hour later, a uniform oral dose-of D-xylose 5 g was administered. Blood at 1, 2, 3, 6 and 8 hours, and urine excreted over the periods 0-½ hour and then at 2-hourly periods upto 8½ hours after administration of the anti-tuberculosis drugs were collected in plain bottles. (A small volume of the blood collected at 2 hours, *i.e.*, 1½ hours after xylose administration, was transferred to a bottle containing oxalate and fluoride for estimation of blood xylose concentrations). Serum was separated from the blood samples and stored at -20°C for a period not exceeding 3 days for the determination of the concentrations of isoniazid and rifampicin. For estimation of xylose, a protein-free filtrate (proteins having been precipitated from 1 ml of blood diluted with 7 ml water by the addition of 1 ml portions of 5 percent zinc sulphate and 5 percent barium hydroxide solutions) of the oxalated blood sample collected at 2 hours was stored at -20°C for a period not exceeding 3 days. The volume of the urine samples excreted was noted and aliquots

stored at -20°C for a period not exceeding 7 days before estimations of isoniazid and its metabolites were undertaken; xylose was estimated only in those samples collected after administration of D-xylose (*i.e.* from ½ hour to 8½ hours).

Serum concentrations of isoniazid were estimated by a standard spectrophotometric method involving condensation with vanillin (Rao *et al.*, 1971). Serum rifampicin concentrations were determined by the plate diffusion assay of Dickinson *et al.* (1974), employing a strain of *Staphylococcus aureus* (Sub group I, NCTC 10702), resistant to streptomycin and other antibiotics. Rifampicin standards ranging from 0.04 to 1.28 µg/ml were set up in quadruplicate, and the concentrations of the drug in the samples (set up in quadruplicate in dilutions of 1 in 5 and 1 in 10) were obtained from the regression line of the diameter of the zone of inhibition on log concentration of the standard. The proportion of the dose of isoniazid excreted as isonicotinylic compounds (*i.e.* isoniazid, acetylisoniazid, isonicotinic acid, isonicotinylic glycine) in urine excreted over the period 0-8½ hours was estimated after acid hydrolysis of the urine samples by which all isonicotinylic compounds were converted to isonicotinic acid (Ellard and Gammon, 1976). D-xylose in the protein-free filtrates and the urine collections was estimated by a standard procedure involving reaction with p-bromoaniline (Tietz, 1976), slightly modified to eliminate any possible interference due to rifampicin. (The protein-free filtrates and the suitably diluted urine specimens (1 in 10, 1 in 25 or 1 in 50) were extracted thrice with 3 ml portions of chloroform and the organic layer containing rifampicin was discarded). All estimations were undertaken after coding the specimens.

*Calculation of pharmacokinetic variables* : The peak concentration was the geometric mean of the highest concentration in individual patients. Exposure to isoniazid was calculated as the area under the time-concentration curve (AUC) from a plot of concentration versus time as linear co-ordinates. The serum half-lives of isoniazid and rifampicin were calculated from the terminal phase of the time-concentration curves.

## Results

As mentioned earlier, of the 12 patients with intestinal tuberculosis, 4 (3 slow acetylators) had

resection of a part of the diseased portion of their intestines, while the remaining 8 (7 slow acetylators) did not. The findings in the 2 groups were similar; thus, the mean values for peak concentration, exposure and the serum half-life of rifampicin were 9.5 and 10.2  $\mu\text{g/ml}$ , 65 and 60  $\mu\text{g/ml. hours}$ , and 4.4 and 3.7 hours, in patients who had resection and in those who did not, respectively. None of the differences was significant ( $P > 0.2$ ); the findings in the two groups have therefore been amalgamated. (The findings for isoniazid have not been compared due to differences in the proportions of rapid acetylators in the 2 groups).

Of the 12 patients with intestinal tuberculosis, 10 were slow acetylators and the remaining 2, rapid acetylators of isoniazid, of the 18 patients with pulmonary tuberculosis, 8 were slow and 10 were rapid acetylators. The mean dosage of isoniazid administered to the 10 slow acetylators with intestinal tuberculosis was 8.5 mg/kg while that administered to 8 slow acetylators with pulmonary tuberculosis was 7.1 mg/kg; the mean dosages of isoniazid in rapid acetylators in the 2 groups were 8.5 and 7.5 mg/kg, respectively. The mean dosages of rifampicin in the two groups of tuberculous patients were 9.9 and 10.1 mg/kg, respectively, and the mean dosage of xylose was 142 mg/kg in patients with intestinal tuberculosis and 122 mg/kg in those with pulmonary tuberculosis.

The distribution of patients by-the time after drug administration at which the highest serum concentrations of isoniazid and rifampicin were observed was similar in the two groups of patients. Thus, for isoniazid (amalgamating the findings in the slow and the rapid acetylators), among the 12 patients with intestinal tuberculosis, highest concentrations were observed in 8 at 1 hour, in 3 at 2 hours and in 1 at 3 hours, and in 15, 1 and 2, respectively among the 18 patients with pulmonary tuberculosis. For rifampicin, the corresponding numbers were 3, 6 and 3 in patients with intestinal tuberculosis, and 5, 8 and 5 in those with pulmonary tuberculosis.

The highest concentrations of isoniazid were attained by the 3rd hour in all the patients and the concentrations fell exponentially thereafter (Fig. 1). In slow acetylators, while the peak concentration for isoniazid was higher ( $P = 0.04$ ), the mean serum half-life was slightly lower ( $P = 0.05$ ) in patients with intestinal tuberculosis

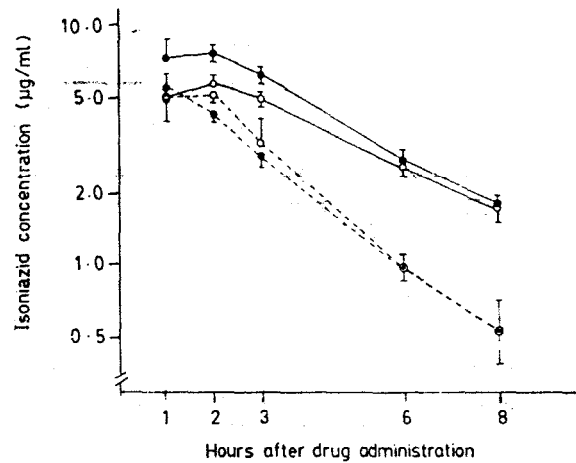


Fig. 1. Serial serum isoniazid concentrations ( $\pm$  standard error of the mean) in slow acetylators (solid line, —) and rapid acetylators (broken line, - - - -) among patients with intestinal tuberculosis (closed circles, ●) and those with pulmonary tuberculosis (open circles, ○).

than in those with pulmonary tuberculosis (Table 1); the difference between the mean values for exposure to isoniazid was not significant ( $P > 0.1$ ). None of the differences in the 3 parameters was significant ( $P > 0.2$ ) between the two groups of patients in the rapid acetylators.

The mean proportion of dose excreted as isonicotinylic compounds in urine collected over the 0-8½ hour period after administration of the anti-tuberculosis drugs (after amalgamating the findings in slow and rapid acetylators, which were similar) was 56 percent in patients with intestinal tuberculosis and 60 percent in those with pulmonary tuberculosis ( $P \geq 0.2$ ).

The highest concentrations of rifampicin were attained by the 3rd hour in both groups of patients and there was an exponential fall thereafter (Fig. 2). The peak concentration (Table 2) was about 30 percent higher in patients with intestinal tuberculosis than in those with pulmonary tuberculosis ( $P = 0.04$ ); the differences in the exposure to rifampicin and its serum half-life were, however, not significant ( $P > 0.2$ ).

The mean blood xylose concentrations at 1% hours and the mean proportions of the dose of xylose excreted over the periods 0-2 and 0-8 hours after administration of D-xylose in the 2 groups of patients are presented in Table 3. The mean

Table 1. Some pharmacokinetic variables of isoniazid in patients with intestinal tuberculosis and pulmonary tuberculosis

Pharmacokinetic variable	Slow acetylators		Rapid acetylators	
	Intestinal TB	Pulmonary TB	Intestinal TB	Pulmonary TB
Peak concentration ( $\mu\text{g/ml}$ )	8.8* (6.4-15.0)	6.7 (4.8-8.9)	5.2 (5.0-5.5)	6.1 (4.6-8.7)
Exposure ( $\mu\text{g/ml.hours}$ )	42 (33-62)	35 (21-51)	18 (16-21)	18 (11-27)
Serum half-life (hours)	2.8 (2.2-3.3)	3.3 (2.5-4.4)	1.8 (1.7-1.9)	1.9 (1.4-2.4)
No. of patients	10	8	2	10

\*Geometric mean with range in parentheses.

Table 2. Some pharmacokinetic variables of rifampicin in patients with intestinal tuberculosis and pulmonary tuberculosis

Pharmacokinetic variable	Intestinal TB	Pulmonary TB
Peak concentration ( $\mu\text{g/ml}$ )	10.0* (6.0-16.0)	7.7 (4.7-15.4)
Exposure ( $\mu\text{g/ml.hours}$ )	61 (33-157)	50 (28-107)
Serum half-life (hours)	3.9 (2.4-6.2)	(2.4-4.4)
No. of patients	12	18

\*Geometric mean with range in parentheses.

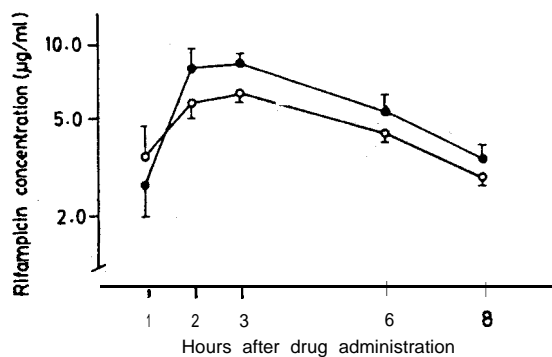


Fig. 2. Serial serum rifampicin concentrations in patients with intestinal tuberculosis (closed circles, ●) and those with pulmonary tuberculosis (open circles, ○).

blood concentration was about 23 percent higher in patients with intestinal tuberculosis than in those with pulmonary tuberculosis ( $P = 0.07$ ). On the contrary, the mean proportion of dose of xylose excreted in urine was lower in the former than in the latter, the difference being of the order of 7 percent over the 0-2 hour period ( $P > 0.2$ ), and about 13 percent over the 0-8 hour period ( $P = 0.04$ ).

#### Discussion

Absorption of drugs by transport across biological membranes in the stomach and the intestines is essentially by passive diffusion. The basic requirements of such a transport are lipid

Table 3. Blood xylose concentrations and proportions of dose of xylose excreted in urine in patients with intestinal tuberculosis and pulmonary tuberculosis

Body-fluid	Period of collection*	Intestinal TB	Pulmonary TB
<b>Blood</b>			
Concentration (mg/dl)	1½	17.5** (6.2-24.7)	14.2 (8.0-23.1)
<b>Urine</b>			
Proportion of 0-2 dose excreted (%)		15.0 (7.7-20.2)	16.2 (7.8-26.7)
	0-8	34.8 (26.0-44.4)	40.2 (29.5-54.3)
No. of patients		12	18

\*Hours after administration of D-xylose

\*\*Mean with range in parentheses

solubility and degree of ionisation at physiological pH of the drug, with the absorption being greater for compounds which are ionised less. Other factors that could influence the rate of absorption are molecular weight and configuration solubility of the drugs in body fluids and particle size. Most anti-tuberculosis drugs are compounds with a low molecular weight; they are lipid soluble and are weak electrolytes, and hence are readily absorbed from the gastro-intestinal tract. Results presented in this report demonstrate that the absorption of isoniazid and rifampicin is not affected in patients with intestinal tuberculosis. Indeed, the mean peak serum concentrations of isoniazid (in slow acetylators) and rifampicin were slightly higher in patients with intestinal tuberculosis than in those with pulmonary tuberculosis. Further, the similarity in the distribution of patients by the time-point of blood collection at which the highest serum concentrations of isoniazid and rifampicin were observed suggests that the absorption of both drugs in patients with intestinal tuberculosis is as good as that in those with pulmonary tuberculosis.

The jejunal portion of the intestines was not affected in any of the patients admitted to the study, and as such the absorption of D-xylose, which can detect only jejunal malabsorption, was not impaired in any of the patients. Investigations undertaken on an earlier occasion in healthy subjects (unpublished findings) had shown that the mean blood xylose concentration at 1½ hours and the mean proportion of the dose excreted over the period 0-2 hours after ingestion of D-xylose 5 g were 15.6 mg/dl and 17.0 percent, respectively, values similar to those observed in the present study in the two groups of tuberculous patients.

It has been demonstrated that surgical procedures such as partial gastric and duodenal resection (Robson and Sullivan, 1963), and jejunioleal by-pass (Bruce and Wise, 1976; Polk, Tenenbaum and Kline, 1978) did not significantly alter the absorption of isoniazid. Similarly, for rifampicin, sub-total or total gastrectomies, coeliac disease or small-bowel diverticulosis, did not affect the absorption of the drug as assessed by comparison of the exposure to the drug (AUC) between these patients and healthy subjects (Kenny and Strates, 1981). Further, the absorption of rifampicin was also shown to be normal in patients with Crohn's disease, a

chronic, granulomatous inflammation affecting the distal portion of the ileum (Kenny and Strates, 1981).

Tuberculosis rarely affects the stomach and the proximal loops of the small intestines. Thus, of the 106 patients with intestinal tuberculosis admitted to the controlled clinical trial of the treatment of abdominal tuberculosis at our Centre, none had tuberculosis of the stomach and the duodenum was affected in only 3 patients (unpublished findings). In the present investigation, only one of the 12 patients admitted had duodenal tuberculosis; further, in the 4 patients who had had resection of the affected portion of the intestines, only part of the distal portions of 'the small intestines were surgically removed. It is possible that isoniazid and rifampicin are readily absorbed from all sites of the gastro-intestinal tract, and the absorption of these drugs is not affected in patients with intestinal tuberculosis irrespective of the site of involvement.

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#### References

- Bruce R.M., Wise L. Tuberculosis after jejunioleal by-pass for obesity. *Ann Intern Med.* 1976, 87, 574-576.
- Chanarin I., Bennett M.C. Absorption of folic acid and D-xylose as tests of small intestinal function. *Br Med J.* 1962, 1, 985-998.
- Dickinson J.M., Aber V.R., Allen B.W., Eliard G.A., Mitchison D.A. Assay of rifampicin in serum. *J Clin Pathol.* 1974, 27, 457-462.
- Ellard G.A., Gammon P.T. Pharmacokinetics of isoniazid metabolism in man. *J Pharmacokinet Biopharm.* 1976 4, 83-113.
- Kailasam S., Immanuel C, Nair N.G.K., Radhakrishna S., Tripathy SP. Classification of subjects as slow or rapid inactivators of isoniazid : oral administration of a slow-release preparation of isoniazid and determination of the ratio of acetylisoniazid to isoniazid in urine. *Indian J Med Res.* 1975, 63, 323-328.
- Kenny M.T., Strates B. Metabolism and pharmacokinetics of the antibiotic rifampicin. *Drug Metab Rev.* 1981, 12, 159-218.

- Polk R.E., Tenenbaum M., Kline B. Isoniazid and ethambutol absorption with jejunoileal by-pass. *Ann Intern Med.* 1978, 89, 430-431.
- Raghupati Sarma G., Kannapiran M., Narayana A.S.L., Radhakrishna S., Tripathy S.P. Determination of acetylator phenotype based on the ratio of acetylisoniazid to isoniazid in urine following an oral dose of ordinary isoniazid. *Indian J Med Res.* 1976, 64, 1-8.
- Rao K.V.N., Kailasam S., Menon N.K., Radhakrishnan S. Inactivation of isoniazid by condensation in a syrup preparation. *Bull WHO.* 1971, 45, 625-632.
- Robson J.M., Sullivan F.M. Anti-tuberculosis Drugs. *Pharmacol Rev.* 1963, 15, 169-223.
- Sammons H.G., Morgan D.B., Frazer A.C., Montgomery R.O., Philip W.M., Philips M.J. Modification in the xylose absorption test as an index of intestinal function. *Gut.* 1967, 8, 348-353.
- Theodossi A., Gazzard B.G. Have chemical tests a role in diagnosing malabsorption? *Ann Clin Biochem.* 1984, 21, 153-165.
- Tietz N.W. Gastric, pancreatic and intestinal function. In : Tietz N.W., ed. *Fundamentals of clinical chemistry.* W.B Saunders Company, Philadelphia, London, Toronto, 1976, 1063-1099.
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