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ISONIAZID METABOLISM IN MAN AND ITS THERAPEUTIC IMPLICATIONS

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Isoniazid, ever since its introduction in 1952, continues to be the most widely used chemotherapeutic agent for the treatment of tuberculosis. It has all the essential properties of an ideal chemotherapeutic agent — it is highly effective, it is specific in its action against the tubercle bacilli, its toxicity is minimal and it is inexpensive. We, at the Tuberculosis Research Centre, Madras, have carried out extensive clinical, pharmacological and biochemical investigations with isoniazid and I may be pardoned if my presentation is confined mostly to the findings of our studies.

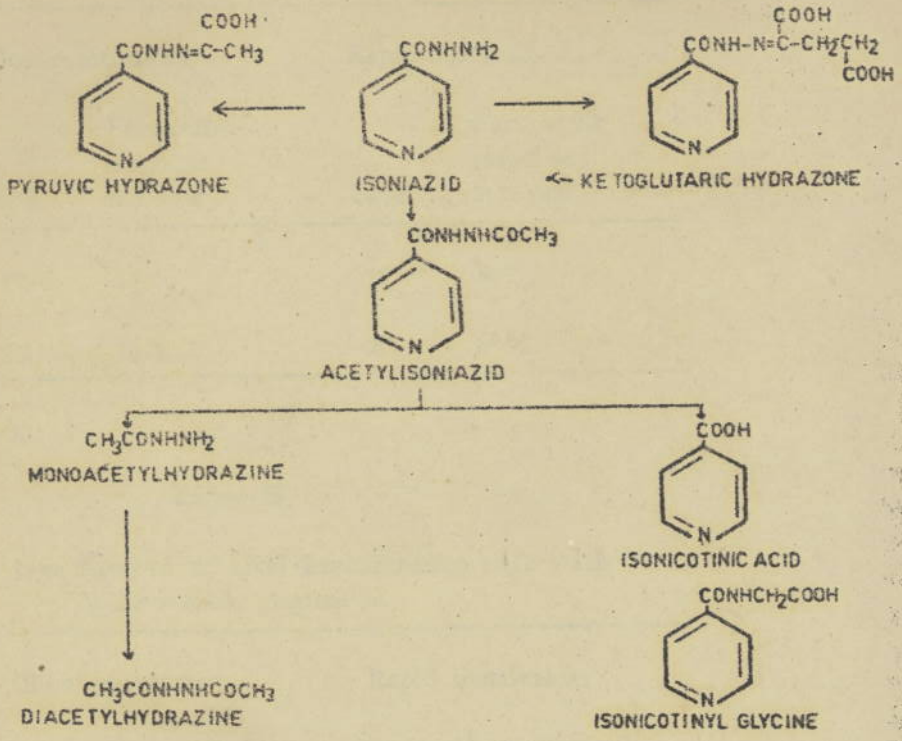
The main pathways of isoniazid metabolism are presented in Fig.I. Isoniazid is metabolised or inactivated by acetylation to acetylisoniazid and conjugated with pyruvic and ∝-ketoglutaric acids to form hydrazones. Acetylisoniazid is then hydrolysed to monoacetylhydrazine and isonicotinic acid. Monoacetylhydrazine is acetylated to diacetylhydrazine and isonicotinic acid conjugated with glycine to form isonicotinyl glycine. All the metabolites of isoniazid are devoid of antituberculous activity.

Numerous studies over the years have demonstrated that human subjects show a wide variation in their capacity to acetylate or inactivate isoniazid to acetylisoniazid, and a great majority of subjects can be clearly characterised as being either slow or rapid inactivators. The acetylation capacity which depends on the enzyme isoniazid acetyltransferase is genetically determined, the concentration of the enzyme being higher in rapid inactivators[1-4]. The frequency of the genes controlling the slow or rapid acetylation vary among different racial populations; thus, in people from the mongoloid race like the Eskimos, the Japanese and the Chinese, the proportion of rapid inactivators is 90% or more, while in the negroid, the Caucasian and the South Indian populations, this proportion is 40% or less.

The methods for the determination of the rate of isoniazid inactivation usually depend upon the estimation of either isoniazid in serum[5,6] or that of acetylisoniazid and isoniazid in urine[7-11]. Since sulphadimidine is acetylated by similar enzymatic processes and since its acetylation closely parallels that of isoniazid, quantitative methods using this sulpha drug have also been evolved[12,13]. We have recently developed a fairly simple qualitative test based on the free sulphadimidine content in urine for phenotyping subjects as slow and rapid inactivators of isoniazid[14].

Fig. I

Pathways of isoniazid metabolism



Several clinical trials at our Centre have shown that isoniazid acetylator status has little prognostic significance, if any, when isoniazid is given daily. The results of two such studies are presented in Table-I. Response to treatment is assessed bacteriologically and the patient is deemed to have a favourable response when all the sputum specimens collected during the last 3 months of chemotherapy (usually 7-9 specimens) are culture negative or even if only 1 isolated positive culture is obtained during this period. It can be seen that rapid inactivators respond just as well as the slow inactivators whether the companion drug is PAS or thioacetazone[15].

The next table (Table II) presents the prognostic significance of the rate of inactivation of isoniazid when chemotherapy was administered under supervision, twice-a-week. The dosages were 15 mg/kg body-weight for isoniazid, 1 g or 0.75 g for streptomycin, 200 mg/kg for PAS and 45 mg/kg for ethambutol. In slow inactivators, regardless of whether the companion drug is streptomycin[16,17] PAS[18] or ethambutol[19], the response is uniformly good. In rapid inactivators, when streptomycin is

Table I

Prognostic significance of the INH inactivation rate with daily regimens

Regimen	Slow inactivators		Rapid inactivators	
	Total	Favourable response at 1 year	Total	Favourable response at 1 year
PAS+INH	51	88%	32	84%
TB_1+INH	39	79%	26	85%

TB_1: Thioacetazone

Table II

Prognostic significance of INH inactivation rate with twice weekly regimens

Twice-weekly regimen	Slow inactivators		Rapid inactivators	
	Favourable response Total	at 1 year	Favourable response Total	at 1 year
Strep.+INH	136	94%	68	91%
PAS+INH	52	92%	38	82%
Emb.+INH	52	92%	48	83%

the companion drug, the response is as good as that in slow inactivators. However, when streptomycin is replaced with a weaker drug like PAS or ethambutol, the response at 1 year is less satisfactory in rapid inactivators, the difference being of the order of 10% (P=0.07).

The success of a twice-weekly supervised regimen of streptomycin plus high dosage isoniazid in both slow and rapid inactivators provided the stimulus for a series of controlled clinical trials with once-weekly regimens[6 16 17] sometimes preceded by an initial daily phase for 2 or 4 weeks. Table III summarises our cumulative experience regarding the

Table III

Prognostic significance of INH inactivation rate in studies of once weekly chemotherapy

Daily phase (weeks)	Once weekly phase	Total patients		Favourable response at 1 year		
		Slow	Rapid	Slow	Rapid	Diff.
-	SH	38	39	82%	60%	22%
-	SHZ	67	34	87%	53%	34%
SH (4)	SH	109	67	94%	73%	21%
SPH (4)	SPH	98	72	95%	76%	19%
SEH (2)	EH	57	51	91%	57%	34%

S: Streptomycin H: Isoniazid P: Sodium PAS
E: Ethambutol Z: Pyrazinamide

prognostic significance of isoniazid inactivation rate in these trials. It is abundantly clear that slow inactivators respond substantially better than rapid inactivators with every one of the once-weekly regimens, the difference being of the order of 20-35% ($P < 0.0001$).

To elucidate the reasons for the inferior response of rapid inactivators, we undertook studies of serial serum isoniazid concentrations in slow and rapid inactivators following a dose of isoniazid 15 mg/kg bodyweight[6]. Figure II depicts the time-concentration curves. The peak concentration attained in slow inactivators is slightly higher than that in rapid inactivators; however, the fall-off is rapid in the rapid inactivators, while it is more gradual in the slow inactivators. Coverage, defined as the number of hours for which a bacteriostatic concentration of isoniazid 0.2 µg/ml is maintained, is appreciably greater in slow inactivators. Similarly exposure, defined as the area under the time-concentration curve, is also substantially greater in slow inactivators.

Table IV presents the peak concentration, coverage and exposure to isoniazid attained with a dose of isoniazid 15 mg/kg. A mean peak concentration of isoniazid 13.8 µg/ml was attained in the slow inactivators while it was 10.8 µg/ml in rapid inactivators. Coverage, however, is only 14 hours in rapid inactivators, while it is 30 hours in the slows. Exposure is 36 µg/ml hours in the rapids, while it is 85 µg/ml hours in the slows. The differences between the two groups in coverage and ex-

Fig. II

Serial plasma isoniazid concentrations with isoniazid 15 mg/kg in slow and rapid inactivators of isoniazid

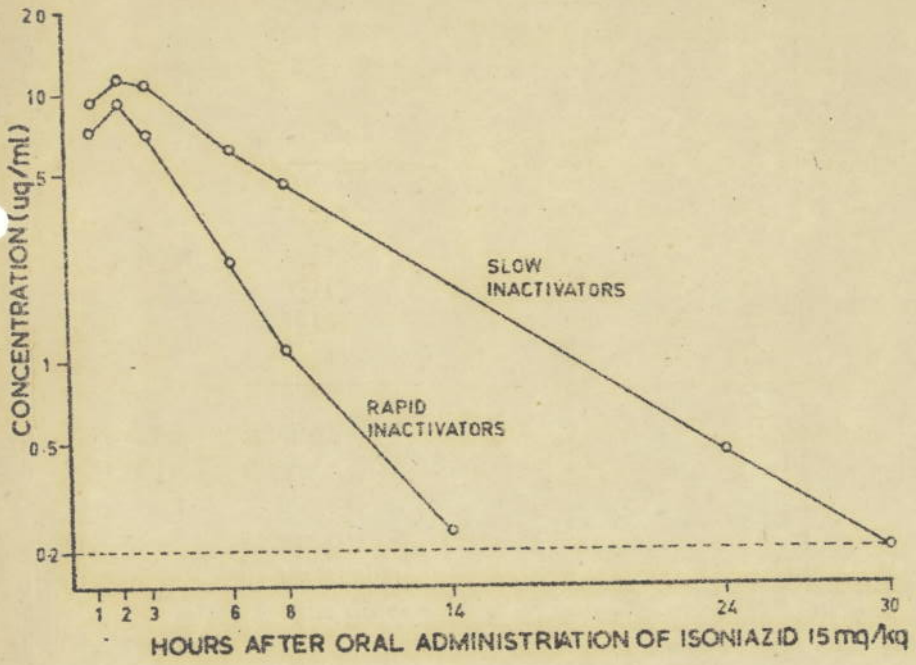


Table IV

Peak, coverage and exposure following a dose of isoniazid 15 mg/kg body-weight

INH inact. rate	Peak (µg/ml)	Coverage (hrs.)	Exposure (µg/ml.hrs.)
Slow	13.8	30	85
Rapid	10.8	14	36

posure are substantial and it may therefore be concluded that the failure of once-weekly regimens in rapid inactivators is predominantly due to inadequate coverage and exposure. It is, therefore, reasonable to expect that

if the coverage and exposure to isoniazid could be doubled in rapid inactivators by some means, it is possible that the efficacy is likely to be raised to the level of that in slow inactivators.

Unfortunately, isoniazid cannot be administered in much higher doses for fear of acute toxicity like convulsions. Further, the concomitant administration of a third drug, PAS 6 g, both in the daily and in the once-weekly phases did not redress the isoniazid inadequacy[6]. It was therefore, decided to investigate whether a slow-release preparation of isoniazid, matrix isoniazid (Smith and Nephew HS82), producing a lower peak concentration per unit dose and hence capable of being administered in much larger doses, could be used instead of ordinary isoniazid for obtaining adequate coverage and exposure. In this preparation, isoniazid is held within a matrix of hydroxymethyl cellulose; about 20% of the isoniazid is present in the free form and the rest as the matrix component with a half-life of 4 1/2 hours. Preliminary investigations with this compound had shown that isoniazid released from this preparations is totally absorbed, that the peak concentrations attained with this compound were about one third of that obtained with an equivalent dose of ordinary isoniazid, and that the levels were sustained confirming the slow-release nature of the product[20-22].

Based on these findings, pharmacological studies with two doses of matrix isoniazid, 30 mg/kg and 40 mg/kg, in both slow and rapid inactivators were undertaken. The peaks, coverages and exposures are presented in Table V; those obtained with ordinary isoniazid 15 mg/kg are also presented for purposes of comparison. In rapid inactivators, doubling the dose from 15 mg/kg (ordinary isoniazid) to 30 mg/kg (matrix isoniazid) did not result in a doubling of the exposure. However, when the dose was increased to 40 µg/kg, the exposure was 89 µg/ml. hours. The disproportionately large increase in exposure, in comparison with that obtained

Table V

Peak, coverage and exposure with different doses of isoniazid

Preparation and dose	Peak (µg/ml)		Coverage (hrs.)		Exposure (µg/ml.hrs.)	
	Slow	Rapid	Slow	Rapid	Slow	Rapid
Ord. INH 15 mg/kg	13.8	10.8	30	14	85	36
Matrix INH 30 mg/kg	11.4	7.0	38	22	147	51
Matrix INH 40 mg/kg	16.3	9.9	41	27	198	89

with the 30 mg/kg dose, is possibly due to a greater degree of saturation of the enzyme, isoniazid acetyltransferase. Exposure obtained in rapid inactivators with matrix isoniazid 40 mg/kg is fairly similar to the 85 µg/ml. hours obtained in slow inactivators with ordinary isoniazid 15 mg/kg. Again, coverage with the 40 mg/kg dose in rapid inactivators was 27 hours which is fairly similar to the 30 hours obtained in slow inactivators with ordinary isoniazid 15 mg/kg. It may, therefore, be expected that matrix isoniazid 40 mg/kg would be therapeutically effective in rapid inactivators. In slow inactivators, no additional benefit would be expected from the increase in coverage and exposure obtained with matrix isoniazid in high dosage, although chronic toxicity could well become a problem[28].

The findings of our clinical trials with matrix isoniazid[23] are presented in Table VI. The relatively poor response obtained in rapid inactivators with matrix isoniazid 30 mg/kg is readily understood since the exposure to isoniazid was not substantially more than that with ordinary isoniazid 15 mg/kg. However, the response to treatment with the 40 mg/kg dose was not very much better than that obtained with the 30 mg/kg dose despite the fact that an adequate coverage and an excellent isoniazid exposure were obtained. It was felt that this surprising finding may be due to some metabolite of isoniazid, present in high concentrations in rapid inactivators after a matrix isoniazid dose, antagonising the antituberculous-activity of isoniazid.

In consequence, various metabolites of isoniazid were tested for their antagonism to the activity of isoniazid *in vitro* against *M. tuberculosis* H_{37} R_V in 7H9 liquid medium without Tween. The test was read at both 7 and 14 days. Acetylisoniazid, isonicotinic acid and diacetylhydrazine had

Table VI

Exposure to INH and therapeutic efficacy in once weekly chemotherapy with ordinary and matrix isoniazid

INH inact. rate	Preparation and dose	Exposure (µg/ml.hrs.)	No. of patients	Favourable response at 1 year
Slow	Ord. INH 15 mg/kg	85	219	95%
Rapid	Ord. INH 15 mg/kg	36	138	75%
Rapid	Matrix INH 30 mg/kg	51	71	80%
Rapid	Matrix INH 40 mg/kg	89	59	83%

Table VII

Antagonism of monoacetylhydrazine to INH activity

Concentration of MAH (μg/ml)	MIC of INH 7 days	14 days
Nil	0.1	0.2
0.16	0.1	0.4
0.8	0.1	0.8
4.0	0.4	>1.6

no effect. Monoacetylhydrazine, however, was found to definitely antagonise the action of isoniazid[24]. The findings are presented in Table VII. It can be seen that a minimal inhibitory concentration of isoniazid of 0.1 μg/ml was increased 4-fold in the presence of monoacetylhydrazine 4 μg/ml when the test was read at 7 days. When the test was read at 14 days the minimal inhibitory concentration of 0.2 μg/ml was increased to greater than 1.6 μg/ml.

We then undertook a pilot study to determine the serial plasma monoacetylhydrazine concentrations in 3 slow inactivators receiving ordinary isoniazid 15 mg/kg and 2 rapid inactivators receiving matrix isoniazid 40 mg/kg. Results (not presented here) showed that peak monoacetylhydrazine concentrations were about 2 1/2 times higher, on average, in rapid inactivators receiving matrix isoniazid 40 mg/kg than in slow inactivators receiving ordinary isoniazid 15 mg/kg. Although it is not possible to draw firm conclusions on the basis of an investigation based on such small numbers of patients, these findings, coupled with the outcome of the *in vitro* experiments, offer a possible explanation for the inferior response in rapid inactivators receiving matrix isoniazid 40 mg/kg once a week.

With the introduction of rifampicin, a powerful antituberculosis drug, various intermittent regimens containing this drug have been evolved for the treatment of tuberculosis. In a recently concluded clinical trial at Singapore[25], all patients were treated with streptomycin, isoniazid and rifampicin for 2 weeks, followed by intermittent chemotherapy with isoniazid 15 mg/kg once-weekly and rifampicin once or twice a week. The patients were divided into 4 groups; the first group received rifampicin 900 mg twice weekly, the second 600 mg twice weekly, the third 900 mg once weekly, and the last 600 mg once weekly. The results at the end of 1 year are presented in Table VIII. In slow inactivators, regardless of

Table VIII

Singapore study of intermittent chemotherapy with isoniazid and rifampicin

INH inact. rate	Weekly dose of rifampicin (mg)	No. of patients	Favourable response at 1 year
Slow	600-1800	117	100%
Rapid	900 x 2	78	100%
	600 x 2	77	99%
	900 x 1	70	96%
	600 x 1	83	89%

the size of the weekly dose of rifampicin there is 100% bacteriologically favourable response at 1 year. In rapid inactivators, however, reduction of the weekly dose of rifampicin from 1800 to 600 mg resulted in a declining trend in response to treatment suggesting that low dose rifampicin is not fully capable of redressing the isoniazid inadequacy in rapid inactivators.

Mainly because of the need to reduce the duration of treatment and partly because of the lack of success of once weekly regimens, emphasis has now shifted to treatment of tuberculosis with short-course regimens. In a recently concluded clinical trial at our Centre (Table IX), we investigated two rifampicin containing regimens, one for 5 months and the other for 7 months and a non-rifampicin regimen for 7 months. The patients in all 3 groups received streptomycin plus isoniazid plus pyrazinamide daily

Table IX

Madras study of short-course chemotherapy

Daily phase (months)	Twice weekly phase (months)	Total patients	Favourable response at 5/7 mts.	Relapse
RSHZ (2)	SHZ (3)	129	100%	5%
RSHZ (2)	SHZ (5)	132	100%	0%
SHZ (2)	SHZ (5)	129	100%	4%

for 2 months followed by the same 3 drugs twice weekly for 3 or 5 months. Patients in the two rifampicin groups received rifampicin additionally in the daily phase. Results were highly satisfactory in that virtually 100% of the patients had attained bacteriological quiescence at the end of chemotherapy in all the 3 groups and relapse rates in patients followed up for 11-13 months after chemotherapy did not exceed 5% in any of the groups. Isoniazid inactivation rate was of no prognostic significance.

In all our studies, regularity of drug intake was very high due to the intensive efforts on part of our clinical staff. This degree of regularity cannot be attained under conditions existing in the National Tuberculosis Programme. In the presence of irregularity, it is conceivable that the isoniazid inactivation rate may acquire some importance in daily regimens also, and may have an appreciable effect in twice weekly regimens. One solution appears to be to reduce the duration of treatment with effective short-course regimens and this is bound to have a salutary effect on patient-holding and his regularity of drug-intake.

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