Effect of Prednisolone and Rifampin on Isoniazid Metabolism in Slow and Rapid Inactivators of Isoniazid

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The effect of prednisolone and rifampin, alone and in combination, on the biodisposition of isoniazid in slow and rapid inactivators of isoniazid was investigated. In one investigation, we made serial determinations of plasma isoniazid concentrations up to 8 h and of isoniazid and acetylisoniazid in excreted urine up to 8.5 h in patients receiving isoniazid alone on one occasion and isoniazid plus prednisolone or isoniazid plus rifampin on another. Prednisolone caused a significant decrease in the plasma isoniazid concentrations in both slow and rapid inactivators. It also enhanced the renal clearance of isoniazid in both slow and rapid inactivators and increased the rate of acetylation of isoniazid in slow inactivators only. Rifampin had no effect on the biodisposition of isoniazid in either slow or rapid inactivators. In a second investigation, one group of slow and rapid inactivators received isoniazid and rifampin, and a different group received prednisolone, in addition. Plasma isoniazid concentrations in slow inactivators receiving prednisolone were significantly lower than in those who received isoniazid and rifampin only. In rapid inactivators, plasma isoniazid concentrations were similar in the two groups of patients, suggesting that concomitant administration of rifampin had considerably modified the prednisolone effect on the biodisposition of isoniazid in these patients.

Corticosteroids are known to affect the biodisposition of a number of drugs (17). They have been shown to inhibit the metabolism of pethidine, promaxine (5) and cyclophosphamide (11) , and to affect the renal clearance of salicylates (14). Similarly, rifampin, a known inducer of the hepatic microsomal enzyme system, has been shown to affect the metabolism of cardiac glycosides (16), corticosteroids (3, 6), and dapsone (10). In a recent controlled clinical trial in. the treatment of pulmonary tuberculosis with short-course regimens at our Centre, rifampin was used in combination with isoniazid, streptomycin, and pyrazinamide. To investigate the role of corticosteroids in the treatment of tuberculosis, we used prednisolone as an adjuvant to chemotherapy in half the patients admitted to the study. As part of our program to study multiple-drug pharmacokinetic interactions in the chemotherapy of tuberculosis, we investigated the effect of prednisolone and rifampin, alone and in combination, on serial plasma isoniazid concentrations and excretion of isoniazid and acetylisoniazid in urine; this paper presents the results of our investigations.

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MATERIALS AND METHODS

Subjects. The subjects were ambulatory South Indian patients under treatment at this Centre, with a mean body weight of 39 kg (95% range: 27 to 51 kg). They had been previously classified as slow or rapid inactivators of isoniazid on the basis of standard procedures described earlier (13, 18).

Investigation I. Isoniazid (10 mg/kg) was administered to 13 slow and 13 rapid inactivators on the day they were admitted to the clinical study, and serial plasma isoniazid concentrations were determined up to 8 h. At 5 or 6 days, isoniazid (10 mg/kg) plus 20 mg of prednisolone was administered to the same patients, and serial plasma isoniazid concentrations were determined at the same times. On both occasions, urine excreted over the period 0 to 0.5 h and then hourly up to 8.5 h was collected, and concentrations of isoniazid and acetylisoniazid were determined.

The effect of rifampin on the biodisposition of isoniazid in 14 slow and 12 rapid inactivators was investigated in a similar manner. The same patient was tested twice, after administration of isoniazid (10 mg/kg) on the day of admission, and 5 or 6 days later, after a dose of isoniazid (10 mg/kg) plus rifampin (12 mg/kg). On both occasions, we determined plasma isoniazid concentrations serially up to 8 h and isoniazid and acetylisoniazid concentrations in urine excreted over the period 0 to 0.5 h and then hourly up to 8.5 h.

Investigation II. At 5 or 6 days after the admission of the patients to the clinical study, serial plasma isoniazid concentrations were determined at intervals

up to 8 h (i) in 25 slow and 27 rapid inactivators after a dose of isoniazid (10 mg/kg) plus rifampin (12 mg/kg) and (ii) in a different group of 25 slow and 24 rapid inactivators after a dose of isoniazid (10 mg/kg) plus rifampin (12 mg/kg) plus prednisolone (20 mg).

Conduct of the investigations. Isoniazid in investigation I and isoniazid and rifampin in investigation II were withheld for 48 h before the start of the test (there was no interruption of prednisolone). On the day of the test, a specimen of the patient's urine was verified to be negative for acetylisoniazid by the qualitative test of Eidus and Hamilton (7), and the drug(s) was administered under supervision. Blood was collected in heparinized bottles; plasma was separated immediately and stored at –20°C for not more than 48 h. The volume of the total urine excreted over the different periods (from patients in investigation I) was noted, and samples were stored at –20°C for not more than 3 days. After randomization of the specimens, plasma and urine isoniazid concentrations were determined by the method of Rao et al. (19), and acetylisoniazid was measured by the method of Raghupati Sarma et al. (18).

Calculation of pharmacokinetic parameters. Assuming that elimination of isoniazid follows first-order kinetics, the rate constant for elimination of isoniazid and the half-life of isoniazid were calculated on the basis of the concentrations at 2, 3, 6, and 8 h in slow inactivators, and at 2, 3, and 6 h only in rapid inactivators as some of them had indeterminate concentrations (<0.2 μg/ml) at 8 h. The geometric mean of plasma isoniazid concentrations at the various times was calculated for each patient, and the geometric mean of these mean values was then computed for each group. Exposure to isoniazid was calculated as the area under the time-concentration curve from a plot of concentration versus time as linear coordinates. The apparent distribution volume of isoniazid was calculated by dividing the dose administered (in milligrams) by the product of exposure to isoniazid and the rate constant for elimination of isoniazid (4). The rate constant for acetylation of isoniazid was calculated by multiplying the first-order rate constant for elimination of isoniazid with the proportion of dose excreted as acetylisoniazid in urine collected over the period 0 to 8.5 h. The renal clearance of isoniazid was calculated from the concentration of isoniazid in urine collected during a l-h period and the plasma isoniazid concentration at the midpoint of that period. The renal clearance was calculated at 1, 2, 3, and 6 h only, since at 8 h the plasma isoniazid concentrations were less than 0.2 μg/ml in some of the rapid inactivators.

RESULTS

Investigation I. The plasma isoniazid concentrations in slow and rapid inactivators receiving isoniazid and isoniazid plus prednisolone are shown in Fig. 1, and those in patients receiving isoniazid and isoniazid plus rifampin are shown in Fig. 2. The geometric mean of plasma isoniazid concentration, exposure to isoniazid, and the half-life of isoniazid were computed for both slow and rapid inactivators and are shown in Table 1.
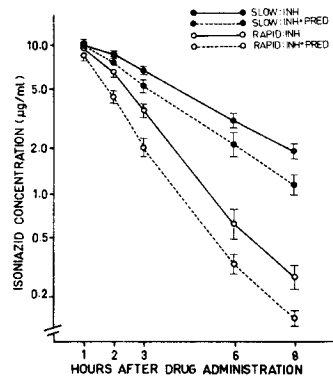

FIG. 1. *Serial plasma isoniazid concentrations (mean ± standard error of the mean) in slow and rapid inactivators receiving isoniazid (INH) only or isoniazid plus prednisolone (PRED).*
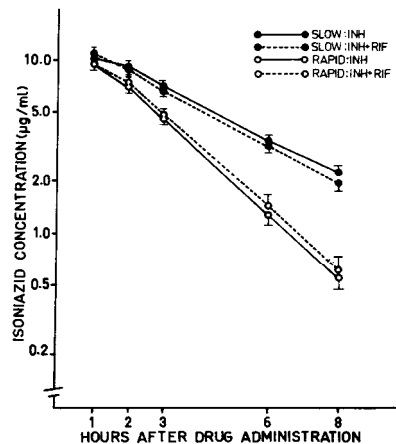

FIG. 2. *Serial plasma isoniazid concentrations (mean ± standard error of the mean) in slow and rapid inactivators receiving isoniazid (INH) only or isoniazid plus rifampin (RIF).*

Administration of prednisolone caused a 25% decrease in plasma isoniazid concentrations in slow inactivators and a 40% decrease in rapid inactivators ($P < 0.001$). The difference in the magnitude of decrease of isoniazid concentrations between slow and rapid inactivators was not statistically significant ($P > 0.2$). Corresponding to the decreased isoniazid levels, exposure to isoniazid in both slow and rapid inactivators and half-life of isoniazid in slow inactivators were significantly smaller in patients who received prednisolone ($P < 0.001$). The half-life of isoniazid was 1.2 h in rapid inactivators receiving isoniazid only and 1.1 h in the same patients receiving prednisolone also; the difference was, however, not statistically significant ($P > 0.2$).

Administration of rifampin had no effect on

TABLE 1. *Effect of prednisolone and rifampin on certain pharmacokinetic parameters of isoniazid inpatients receiving isoniazid[a]*

INH inactivation status	Drugs administered	No. of patients	Mean INH concn[b] (µg/ml)	Exposure to INH[b] (µg/ml x h)	Half-life of INH[b] (h)
Slow	INH	13	5.10 (3.81-6.83)[c]	45 (34-60)	2.80 (2.10-3.74)
	INH + Pred		3.92 (2.41-6.38)	35 (23-53)	2.24 (1.68-2.99)
	INH	14	5.53 (4.31-7.11)	50 (37-67)	3.03 (2.34-3.92)
	INH + Rif		5.29 (4.23-6.61)	47 (36-60)	2.82 (2.11-3.77)
Rapid	INH	13	2.06 (1.13-3.76)	23 (14-37)	1.20 (0.83-1.74)
	INH + Pred		1.28 (0.82-1.99)	16 (11-24)	1.10 (0.91-1.34)
	INH	12	2.95 (2.16-4.03)	28 (21-36)	1.65 (1.21-2.25)
	INH + Rif		3.12 (2.05-4.75)	29 (21-42)	1.76 (1.19-2.61)

[a] Abbreviations: INH, isoniazid (10 mg/kg); Pred, prednisolone (20 mg); Rif, rifampin (12 mg/kg).
[b] Based on the geometric mean of estimates for individual patients.
[c] Figures in parentheses are the 95% confidence limits.

the mean concentration, exposure, or half-life of isoniazid in either slow or rapid inactivators ($P > 0.2$).

Investigation II. The results of investigation II are shown in Fig. 3 and Table 2. Administration of prednisolone to patients receiving isoniazid and rifampin significantly decreased the geometric mean concentration, exposure, and half-life of isoniazid in slow inactivators; the magnitude of decrease was of the order of 15% ($P < 0.001$). In rapid inactivators, however, there was no significant effect.

Apparent distribution volume of isoniazid. The apparent distribution volume of isoniazid in patients receiving isoniazid only was 32 liters in slow inactivators and 33 liters in rapid inactivators. Since it is not expected that the effect of prednisolone or rifampin on the apparent distribution volume of isoniazid would be different in slow and rapid inactivators, findings in both groups of patients (from investigations I and II) were amalgamated. The mean values (not tabulated here) were 32 liters in patients receiving isoniazid only and 36 liters in those receiving prednisolone or rifampin or both, in addition to isoniazid. An analysis of variance indicated that none of the differences was statistically significant ($P > 0.2$).

Rate constant for acetylation of isoniazid. The rate constant for acetylation of isoniazid was calculated using the data from investigation I. In slow inactivators, the rate constant for acetylation of isoniazid was 0.034 per h with isoniazid only and 0.059 per h in the same patients receiving isoniazid plus prednisolone, a significant difference ($P < 0.001$). In rapid inactivators, the rate constant for acetylation of isoniazid was 0.186 per h with isoniazid only and 0.183 per h with isoniazid plus prednisolone ($P > 0.2$).
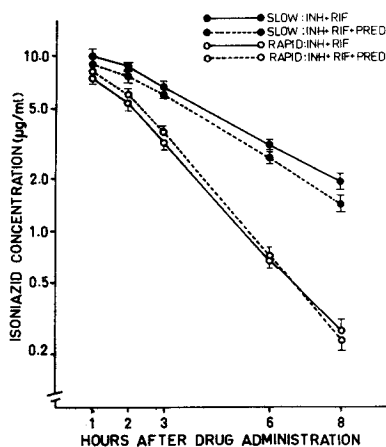

FIG. 3. *Serial plasma isoniazid concentrations (mean ± standard error of the mean) in slow and rapid inactivators receiving isoniazid (INH) plus rifampin (RIF) or isoniazid plus rifampin plus prednisolone (PRED).*

Results (not shown) indicated that rifampin had no effect on the rate constant for acetylation of isoniazid in either slow or rapid inactivators.

Renal clearance of isoniazid. The effect of prednisolone on the renal clearance of isoniazid (based on data from investigation I) is shown in Table 3. These findings show that the renal clearance of isoniazid increased with time and the mean value obtained at 6 h is approximately double that obtained at 1 h in both slow and rapid inactivators. It is also observed that the renal clearance of isoniazid is broadly similar in slow and rapid inactivators. Administration of prednisolone caused a significant increase in the renal clearance of isoniazid in both slow inactivators ($P < 0.001$) and rapid inactivators ($P = 0.01$).

TABLE 2. *Effect of prednisolone on certain pharmacokinetic parameters of isoniazid in patients receiving isoniazid and rifampin[a]*

INH inactivation status	Drugs administered	No. of patients	Mean INH concn [b] (µg/ml)	Exposure to INH[b] (µg/ml x h)	Half-life of INH[b] (h)
Slow	INH + Rif	25	5.08 (4.71-5.48)[c]	44 (42-48)	2.78 (2.62-2.95)
	INH + Rif + Pred	25	4.26 (3.94-4.61)	37 (34-40)	2.44 (2.29-2.60)
Rapid	INH + Rif	27	1.87 (1.61-2.18)	19 (16-21)	1.37 (1.29-1.46)
	INH + Rif + Pred	24	1.98 (1.69-2.32)	21 (18-24)	1.32 (1.22-1.43)

[a] Abbreviations: INH, isoniazid (10 mg/kg); Rif, rifampin (12 mg/kg); Pred, prednisolone (20 mg).
[b] Based on the geometric mean of estimates for individual patients.
[c] Figures in parentheses are the 95% confidence limits.

TABLE 3. *Effect of prednisolone on the renal clearance of isoniazid*

INH inactivation status	No. of patients	Drugs administered	Mean renal clearance (ml/min) at the following times (h):				GM [a] renal clearance (ml/min)
			1	2	3	6	
Slow	13	Isoniaaid	27	35	41	52	37.7
		Isoniaaid + prednisolone	33	50	59	66	50.4
Rapid	13	Isoniazid	27	33	53	56	40.5
		Isoniazid + prednisolone	31	44	67	76	51.4

[a] GM, Geometric mean of the renal clearance at 1, 2, 3, and 6 h.

Results (not tabulated here) showed that rifampin had no effect on the renal clearance of isoniazid. Thus, the mean clearance in slow inactivators was 33.2 ml/min with isoniazid only and 33.5 ml/mm with isoniazid plus rifampin; the corresponding values in rapid inactivators were 37.2 and 35.3 ml/mm, respectively.

DISCUSSION

Results presented in this paper clearly demonstrate that prednisolone caused an appreciable decrease in plasma isoniazid concentrations, leading to lower exposure (area under the time-concentration curve) and half-life of isoniazid. This decrease could have been caused by an enhanced acetylation or renal clearance of isoniazid or even by an increase in the total body water.

The mean body weight of the patients admitted to this study was 39 kg, and the total body water would therefore be about 28 liters (approximately 70% of body weight). It has been shown (9, 12) that the apparent distribution volume of isoniazid is similar to that of total body water, and the value calculated from the findings reported in this paper is approximately 32 liters.

Administration of prednisolone did not cause any appreciable increase in the apparent distribution volume of isoniazid in either slow or rapid inactivators. This suggests that the decrease in plasma isoniazid levels in both groups of patients is not due to an increase in the total body water caused by fluid retention due to prednisolone.

The rate constant for acetylation of isoniazid was five times greater in rapid inactivators than in slow inactivators, demonstrating that rapid inactivators acetylate isoniazid five times more rapidly then slow inactivators, an observation in agreement with that reported by Ellard and Gammon (8). In slow inactivators, prednisolone caused a significant increase in the rate constant for acetylation of isoniazid, possibly by enhancing the activity of isoniazid acetyltransferase. In rapid inactivators, however, prednisolone had no effect on the rate of acetylation of isoniazid. This indicates that the effect of prednisolone in increasing the rate of acetylation of isoniazid is negatively correlated with the concentration of isoniazid acetyltransferase and, therefore, is not felt in rapid inactivators who have about five times as many enzyme molecules as slow inactivators.

The renal clearance of isoniazid (including acid-labile hydrazones) at 6 h was approximately double the value at 1 h in both slow and rapid inactivators. A possible explanation for this increase with time might be that the renal clearance of acid-labile pyruvic and a-ketoglutaric hydrazones of isoniazid is considerably higher than that of isoniazid, and it has been observed that the ratio of these hydrazones to unchanged isoniazid increases with time (G. A. Ellard, per-

sonal communication). The mean renal clearance of isoniazid (38 and 40 ml/min in slow and rapid inactivators) was similar to the values of 41 ml/min reported by Jenne et al. (12) and 46 ml/min reported by Ellard and Gammon (8) in combined groups of slow and rapid inactivators.

Administration of prednisolone caused a significant increase in the renal clearance of isoniazid in both slow and rapid inactivators. It is therefore likely that the decrease in plasma isoniazid concentrations in these patients is due to an increase in the renal clearance of isoniazid.

It may therefore be concluded that prednisolone causes an appreciable decrease of plasma isoniazid concentrations, by enhancing both the rate of acetylation and the renal clearance of isoniazid in slow inactivators, and by increasing the renal clearance only in rapid inactivators.

Administration of rifampin alone did not affect the biodisposition of isoniazid in either slow or rapid inactivators, a finding in agreement with those of Acocella et al. (l), Boman (2), and Modai et al. (15).

Exposure to isoniazid in rapid inactivators receiving isoniazid plus rifampin in investigation II (19 µg/ml . h) was significantly lower than in patients receiving the same drugs in investigation I (29 µg/ml - h). This is probably due to genuine differences between the two groups of patients, for there was corroborative evidence on another occasion that the former metabolized isoniazid more rapidly than did the latter.

Results from investigation II show that rifampin had largely counteracted the prednisolone effect of lowering plasma isoniazid concentrations in rapid inactivators. In slow inactivators, however, it appears that the effect of prednisolone is maintained even in the presence of rifampin. Rifampin has been shown to increase the catabolism of corticosteroids in humans by induction of steroid-metabolizing enzymes (3,6). It is therefore likely that rifampin has caused a more rapid metabolism of the administered prednisolone leading to an abolition of the prednisolone effect of lowering plasma isoniazid concentrations in rapid inactivators. In slow inactivators, who have much less isoniazid acetyltransferase than rapid inactivators, it is probable that even small amounts of prednisolone could significantly enhance the activity of the acetylating enzyme, resulting in decreased plasma isoniazid levels.

The short-course regimens used in the clinical trial were highly effective, and there was little scope for additional therapeutic benefit from the use of prednisolone (20). On the contrary, the decrease in plasma isoniazid levels caused by prednisolone would suggest that steroids may lower the efficacy of treatment regimens containing isoniazid. The response to treatment was, however, excellent regardless of whether the patients received prednisolone or not, suggesting that such a decrease in plasma isoniazid levels was not of therapeutic significance. It is, however, possible that prednisolone may exact a therapeutic penalty if isoniazid is employed in lower dosages.

ACKNOWLEDGEMENTS

LITERATURE CITED

1. **Acoceh, G., L. Bonollo, M. Garimoidi, M. Mainardi, L. T. Tenconi, and F. B. Nicolis.** 1972. Kinetics of rifampicin and isoniazid, administered alone and in combination to normal subjects and patients with liver disease. Gut **13:** 47-53.

2. **Bornan, G.** 1974. Serum concentration and half-life of rifampicin after simultaneous oral administration of aminosalicylic acid or isoniazid. Eur. J. Clin. Pharmacol. **7:** 217-225.

3. **Buffington, G. A., J. H. Dominguez, W. F. Piering, L. A. Herbert, H. M. Kauffman, and J. Lemam.** 1976. Interaction of rifampin and glucocorticoids: adverse effect on renal allograft function. J. Am. Med. Assoc. **236:** 1958-1960.

4. **Butler, T.** 1971. The distribution of drugs, p. 44-62. In B. N. La Du, H. G. Mandel, and E. L. Way (ed.), Fundamentals of drug metabolism and disposition. The Williams & Wilkins Co., Baltimore.

5. **Crawford, J. S., and S. Rudofsky.** 1966. Some alterations in the pattern of drug metabolism associated with pregnancy, oral contraceptives, and the newly born. Br. J. Anaesth. **38:** 446-454.

6. **Edwards, O. M., R. J. Courtneay-Evans, J. H. Galley, J. Hunter, and A. D. Tait.** 1974. Changes in cortisol metabolism following rifampicin therapy. Lancet ii: 549-551.

7. **Eidus, L., and E. J. Hamilton.** 1964. A new method for the determination of N-acetylisoniazid in urine of ambulatory patients. Am. Rev. Respir. Dis. **89:** 587-588.

8. **Ellard, G. A., and P. T. Gammon.** 1976. Pharmacokinetics of isoniazid metabolism in man. J. Pharmacokinet. Biopharm. **4:** 83-113.

9. **Ellard, G. A., P. T. Gammon, and H. Tiitinen.** 1973. Determination of the acetylator phenotype from the ratio of the urinary excretion of acetylisoniazid to acidlabile isoniazid. A study in Finnish Lapland. Tubercle **54:** 201-210.

10. **Gelher, R. H., H. C. Gooi, and R. J. W. Rees.** 1975. The effect of rifampicin on dapsone metabolism. Proc. West. Pharmacol. Soc. **18:** 330-334.

11. **Hayakawa, T., N. Kanai, R. Yamada, R. Kuroda, H. Higashi, H. Mogami, and D. Jinnai.** 1969. Effect of steroid hormone on activation of endoxan (cyclophosphamide). Biochem. Pharmacol. **18:** 129-135.

12. **Jenne, J. W., F. M. MacDonald, and E. Mendoza.** 1961, A study of renal clearances, metabolic inactivation rates, and serum fall-off interaction of isoniazid and para-aminosalicylic-acid in man. Am. Rev. Respir. Dis. **84:** 371-378.

13. **Kailasam, S., C. Immanuel, N. G. K. Nair, S. Radhakrishna, and S. P. Tripathy.** 1975. 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. **63:** 323-328.

14. **Klinenberg, J. R, and F. Miller.** 1965. Effect of corti-
costeroids on blood salicylate concentrations. J. Am.
Med. Assoc. **194:** 601-604.
15. **Modai, J., J. P. Couland, J. M. Vivien, G. Berthelot.,
and E. Bergogne-Berezin.** 1976. Influence de la rif-
ampicine sur le métaboliame de l'isoniazide. Nouv.
Presse Med. **7:** 1263-1267.
16. **Peters, U., T. U. Hausamden, and F. Grosse-Brock-
hoff.** 1974. Einflusa van Tuberculosta auf die Pharma-
cokinetic des Digitoxins. Dtsch. Mad. Wochenschr. **99:**
2381-2386.
17. **Prescott, L. F.** 1969. Pharmacokinetic drug interactions.
Lancet ii: 1239-1243.
18. **Raghupati Sarma, G.. C. Immanuel, S. Kailasam, M.
Hannapiran, N. G. H. Nair, and S. Radhakrishna.**
1974. A modified method for the estimation of acetyl-
isoniazid in urine. Indian J. Med. Res. **62:** 945-962.
19. **Rao. K. V. N., S. Kailasam, N. K. Menon, and S.
Radhakrishna.** 1971. Inactivation of isoniazid by con-
densation in a syrup preparation. Bull. W.H.O. **45:** 623-
632.
20. **Tripathy, S. P.** 1979. Madras study of short-course
chemotherapy in pulmonary tuberculosis. Bull. Int. Un-
ion Tuberc. **54:** 28-30.