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Elevation of Plasma Levels of Lysosomal Enzymes during Treatment with Rifampicin and Isoniazid

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Chemotherapy of tuberculosis with rifampicin and isoniazid can produce several hepatic complications. Isoniazid alone is an established cause of hepatitis (1), and the simultaneous administration of rifampicin has been reported to increase the frequency of liver injury (2). Rifampicin is an inducer of microsomal liver enzymes (3), and it has been suggested this drug may enhance the biotransformation of isoniazid to toxic metabolites (4). The inductive effect of rifampicin on oxidative metabolism in microsomes has clinical implications for other drug interactions (5) and may accelerate the metabolism of endogenous substrates such as oestradiol (6) and cortisol (7). Conversely, isoniazid has been shown to have an inhibitory effect on the normal hydroxylation of phenytoin (8).

Plasma γ-glutamyl transpeptidase is a microsomal enzyme (9) which has been of value in measuring the effect of enzyme-inducing drugs on liver cell microsomes (10), and of particular help in identifying high-alcohol consumers (11). β-Glucuronidase and β-*N*-acetylglucosaminidase are representative lysosomal enzymes (12,13) which may be accurately measured in plasma. When tissue levels of lysosomal enzymes are raised, this has been interpreted on the basis of lysosomal damage and release (14). The cytoplasmic enzyme alanine transaminase is a widely accepted index of more generalised hepatocellular damage (15). To assess the relative contribution of these drug effects during the treatment of tuberculosis, the plasma activities of these enzymes were therefore measured.

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PATIENTS AND METHODS

Twenty-seven patients (17 European and 10 Indian) were used as controls. Twenty-four had previously undergone treatment for tuberculosis and three were pretreatment. The treatment group comprised 24 patients (10 European and 14 Indian) who were receiving standard dosage regimes of rifampicin and isoniazid, with or without ethambutol, for periods of up to 18 months. Both groups were drawn from the Out-Patient Department of the Chest Clinic, St. Mary's Hospital, Luton. Heparinised blood was centrifuged immediately after collection, and plasma samples kept at 4°C for estimation the following day.

Methods

γ-Glutamyl transpeptidase was measured by the method of Rosalki *et al.* (16) using 6.25 mmole/liter L-γ-glutamyl-4-nitroanilide as substrate and 50 mmole/liter glycylglycine as the glutamyl group acceptor in tris(hydroxymethyl aminomethane) buffer, pH 9.0. After incubation for 30 min at 37°C the reaction was terminated with acetic acid, and the extinction was determined at 410 nm.

β-Glucuronidase was estimated using the fluorimetric method of Woollen and Walker (17). The substrate was 0.057 mmole/liter methylumbelliferyl β-glucuronide, and this was dissolved in acetate buffer, pH 3.6. The reaction was terminated after 120 min at 37°C by adding glycine buffer, pH 10.6, and the free methylumbelliferone was estimated fluorimetrically.

β-*N*-Acetylglucosaminidase was determined by the method of Woollen and Walker (18). The assay mixture comprised nitrate buffer containing 0.026 mmole/liter 4-methylumbelliferyl β-*N*-acetylglucosaminide. After incubation for 30 min at 37°C the reaction was terminated and the free methylumbelliferone was measured as above. Alanine transaminase was measured using a standard laboratory method (19).

RESULTS

Plasma alanine transaminase levels were within the normal range for all subjects. Plasma γ-glutamyl transpeptidase values showed no significant difference between the control (Mean and SD = 27.1 ± 23.7 IU/liter, n = 27) and treatment group (Mean and SD = 49.5 ± 50.2 IU/liter, $n = 24$) (t = 1.99, $0.1 > p > 0.05$). Seven patients out of 24 in the treatment group and 2 patients out of 27 in the control group had abnormally raised values (males, > 60 IU/liter; females, >50 IU/liter).

The control group had normal levels for plasma β-glucuronidase and β-*N*-acetylglucosaminidase (Table 1), in agreement with previous data (20). Compared to controls the treatment group showed significantly elevated levels of both lysosomal enzymes, and this effect was

TABLE I

EFFECT OF ANTI-TUBERCULOUS TREATMENT ON TWO LYSOSOMAL ENZYMES[a]

Population	β-Glucuronidase			β-N-Acetylglucosaminidase		
	Controls	Treated	p	Controls	Treated	p
Males and females	0.450 ± 0.230 (27)	1.573 ± 0.853 (24)	<0.001	0.651 ± 0.148 (27)	1.046 ± 0.237 (24)	<0.001
Males and females (deleting subjects with raised γGT)	0.420 ± 0.199 (25)	1.454 ± 0.639 (17)	<0.001	0.640 ± 0.141 (25)	0.984 ± 0.185 (17)	<0.001
Males	0.543 ± 0.256 (11)	1.616 ± 1.053 (13)	<0.005	0.661 ± 0.177 (11)	1.057 ± 0.308 (13)	<0.001
Females	0.386 ± 0.200 (16)	1.523 ± 0.580 (11)	<0.001	0.645 ± 0.131 (16)	1.032 ± 0.134 (11)	<0.001

[a] The values shown express enzyme activity (micromoles per liter per minute) as the mean ± SD and the number of observations is given in parentheses.

the same in males and females (Table 1). To show that this effect was not dependent on the presence of a raised plasma γ-glutamyl transpeptidase, subjects with abnormal values for this enzyme were then deleted from the control and treatment groups; nevertheless, both lysosomal enzymes remained significantly elevated compared to controls (Table 1). Comparison within the treatment group between rifampicin and isoniazid, and rifampicin, isoniazid, and ethambutol showed no significant difference (Table 2); ethambutol therefore did not appear to be contributing to this lysosomal effect. Both lysosomal enzymes remained elevated during all periods of treatment between 1 and 18 months, and this effect was clearly independent of the duration of treatment (Fig. 1) and was confirmed by regression analysis. Although the scatter of β-glucuronidase levels was much greater than those of β-*N*-acetylglucosaminidase, there was a highly significant correlation between the levels of both enzymes ($r = 0.661, p < 0.001$).

DISCUSSION

These results clearly show marked elevation of plasma levels of the lysosomal enzymes β-glucuronidase and β-*N*-acetylglucosaminidase during therapy with rifampicin and isoniazid. The increased plasma levels of these enzymes are mainly due probably to an hepatocellular response, since the liver is a large organ which receives high concentrations of orally administered drugs via the hepatic portal system. Although the levels of both lysosomal enzymes are as high as those reported in infective hepatitis (20), the possibility that this effect represented generalised liver damage seemed unlikely, as all patients had normal alanine transaminase levels. However, lysosomal damage and release of enzymes in the absence of other evidence of liver injury cannot be excluded. The much greater scatter of β-glucuronidase levels (Fig. 1) compared to β-*N*-acetylglucosaminidase may be explained by the distribution of the macromolecular β-glucuronidase complex between microsomes and lysosomes shown in animal studies (21), whereas β-*N*-acetylglucosaminidase is a mainly lysosomal enzyme (13).

TABLE 2
COMPARISON OF THE TWO ANTI-TUBERCULOUS TREATMENT GROUPS[a]

Enzyme	Rifampicin + isoniazid	Rifampicin + isoniazid + ethambutol	*p*
β-Glucuronidase	1.496 ± 0.712 (14)	1.680 ± 1.050 (10)	NS
β-*N*-Acetylglucosaminidase	1.073 ± 0.182 (14)	1.008 ± 1.050 (10)	NS

[a] The values shown express enzyme activity (micromoles per liter per minute) as the mean ± SD, and the number of observations is given in parentheses.

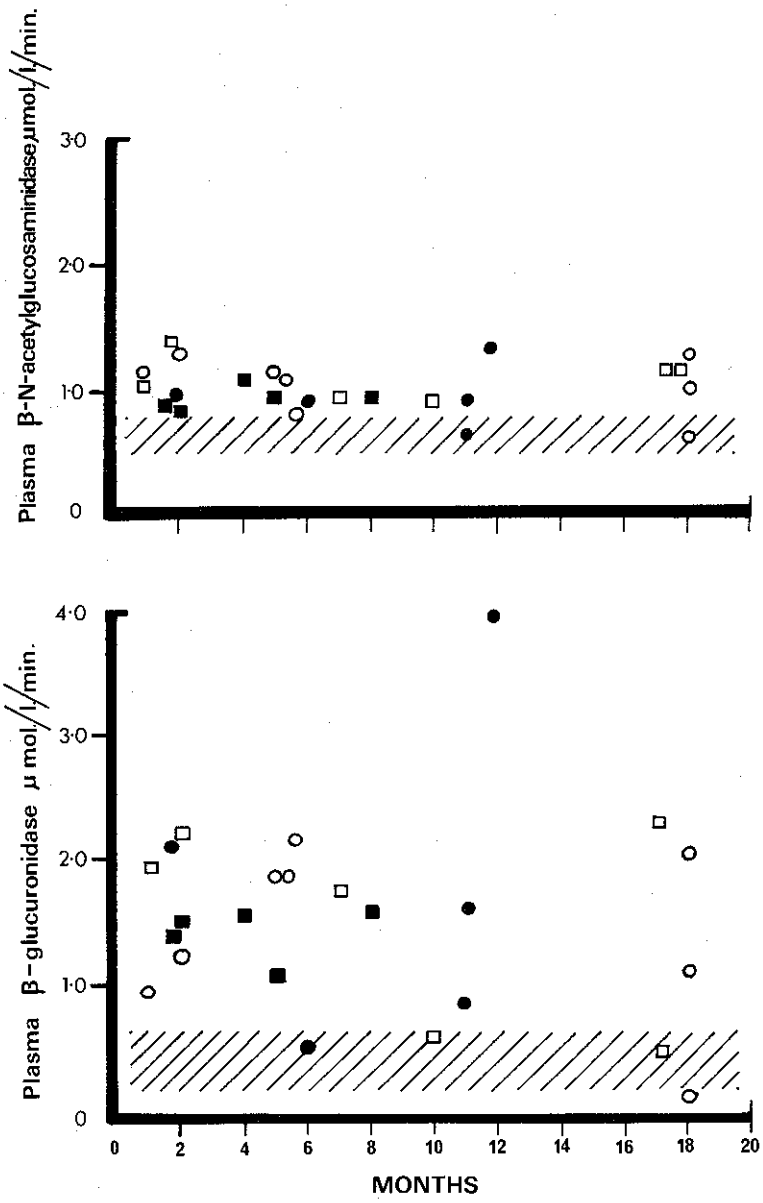

FIG. 1. Plasma activities of β-glucuronidase and β-*N*-acetylglucosaminidase in relation to duration of treatment. Males and females are shown as circles and squares, respectively. Open symbols represent rifampicin and isoniazid, and shaded symbols represent rifampicin, isoniazid, and ethambutol therapy. The hatched areas denote the mean and SD of the control groups. For β-glucuronidase: The slope ± SE of the slope = −0.0149 ± 0.0294 (n = 24). For β-*N*-acetylglucosaminidase: The slope ± SE of the slope = 0.0008 ± 0.0082 (n = 24).

Infection of laboratory animals with BCG, an attenuated strain of tubercle bacillus, causes a general elevation of the level of some lysosomal enzymes, with no further increase after the 14th day from inoculation (22). It seems unlikely that this could be the explanation here; all patients were attending the Out-Patient Department clinically well after at least 1 month of treatment and both enzymes remained raised in patients at the end of treatment. In this study 17 patients out of 24 in the treatment group, covering periods of therapy from 1 to 18 months, had normal plasma levels of γ-glutamyl transpeptidase. This finding confirms a recent study by Breimer *et al.* (5) using rifampicin alone but over a short period of 2 weeks. There were seven patients in our treatment group with raised plasma γ-glutamyl transpeptidase, of whom five were known to be high-alcohol consumers, and thus we believe this is the more likely reason for their abnormal values. An additive effect of alcohol and drugs may be a further reason, but on the evidence we have presented any effect by rifampicin and isoniazid alone on plasma γ-glutamyl transpeptidase activity is not a significant one.

We have not been able to study the single effect of rifampicin or isoniazid between 1 and 18 months to conclude whether it is one drug or the combination which is responsible for this lysosomal effect. Since lysosomal enzymes, like other proteins, are synthesised in the endoplasmic reticulum, the raised activity we have shown in the plasma may be due to the inductive property of rifampicin at this level. A more speculative possibility is suggested by the capacity of lysosomes to accumulate certain drugs (23) which might lead to an effect on lysosomal enzyme synthesis. We believe that more attention needs to be paid to the possible role of enzyme-inducing drugs in the lysosomal system.

SUMMARY

The activities of the enzymes γ-glutamyl transpeptidase, β-glucuronidase, β-*N*-acetylglucosaminidase, and alanine transaminase were measured in the plasma of 24 patients receiving treatment for tuberculosis with rifampicin and isoniazid. Except for seven patients, five of whom were known to be high alcohol consumers, γ-glutamyl transpeptidase levels were normal and did not reflect the known inducing effects of rifampicin on liver cell microsomal enzymes. The predominantly lysosomal enzymes β-glucuronidase and β-*N*-acetylglucosaminidase were significantly raised compared to controls ($p < 0.001$), and there was a significant correlation between the two enzymes ($r = 0.661$, $p < 0.001$). This elevation occurred within 4 weeks and was then independent of the duration of treatment. Alanine transaminase levels were normal, excluding any more general hepatocellular damage. These findings suggest an increase in lysosomal enzyme synthesis and release during rifampicin and isoniazid therapy.

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