URINARY D-GLUCARIC ACID EXCRETION DURING RIFAMPICIN/ISONIAZID AND ANTICONVULSANT ENZYME INDUCTION

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Reprinted from
CLINICAL PHARMACOLOGY AND THERAPEUTICS
St. Louis

Vol. 35, No. 5, pp. 710-715, May, 1984

Urinary D-glucaric acid excretion during rifampicin/isoniazid and anticonvulsant enzyme induction

As measured by urinary D-glucaric acid excretion, an index of hepatic enzyme induction, glutethimide was the most powerful of six such inducers tested. In patients with tuberculosis, rifampicin, 450 mg daily, induced excretion rates of the lower dose range of anticonvulsants in epileptics. The effect was detectable in the first few days but the degree and rate of rise to maximum excretion were variable. This may be due either to diposition of rifampicin or to genetic susceptibility to enzyme induction. Plasma β-glucuronidase, an essential enzyme of the glucuronic acid pathway, could be induced independently of an increase in D-glucaric acid excretion. Plasma γ-glutamyltranspeptidase-levels, an index of hepatic microsomal enzyme induction, were elevated in only 20 of 83 subjects receiving rifampicin and isoniazid, and in all of them urinary D-glucaric acid excretion was normal. Neither of these indices, therefore, showed hepatic enzyme induction during combined therapy when other pathways such as oxidative metabolism continued to be induced. Different active sites of rifampicin and isoniazid on glucuronic acid and other biochemical pathways emphasize the complexity of final metabolic effects in patients on long-term therapy.

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Urinary D-glucaric acid excretion has been used as a screening test for drug-mediated hepatic enzyme induction.[2, 10] To determine the effect of rifampicin in patients with tuberculosis, we compared its excretion to that of anticonvulsants and other known inducing drugs. Isoniazid, however, inhibits rifampicin-induced excretion of D-glucaric acid,[5, 6] and further evidence of the rate of rise and its inhibition when followed serially is presented here. We have shown a significant increase in plasma β-glucuronidase caused by rifampicin,[7] and if this is of microsomal origin it may correspond with the increase in D-glucaric acid excretion and plasma β-glucuronidase observed during anticonvulsant therapy.[11] We therefore studied the rise in D-glucaric acid excretion and β-glucuronidase in one patient receiving sodium phenytoin in comparison with a second patient who received rifampicin followed by isoniazid.

Plasma γ-glutamyltranspeptidase (γGT) is a rapid and useful indicator of drug-mediated hepatic microsomal enzyme induction,[8] but in patients with tuberculosis, who commonly have alcohol problems, it was these factors rather than antituberculous therapy that caused a rise in γGT in a minority of patients.[7] In this study we measured urinary D-glucaric acid excretion of patients with raised γGT levels to confirm that induction of that part of the glucuronic acid pathway affected by hepatic enzyme inducers was absent during combined rifampicin/isoni-

Received for publication July 21, 1983; accepted Nov. 1, 1983.

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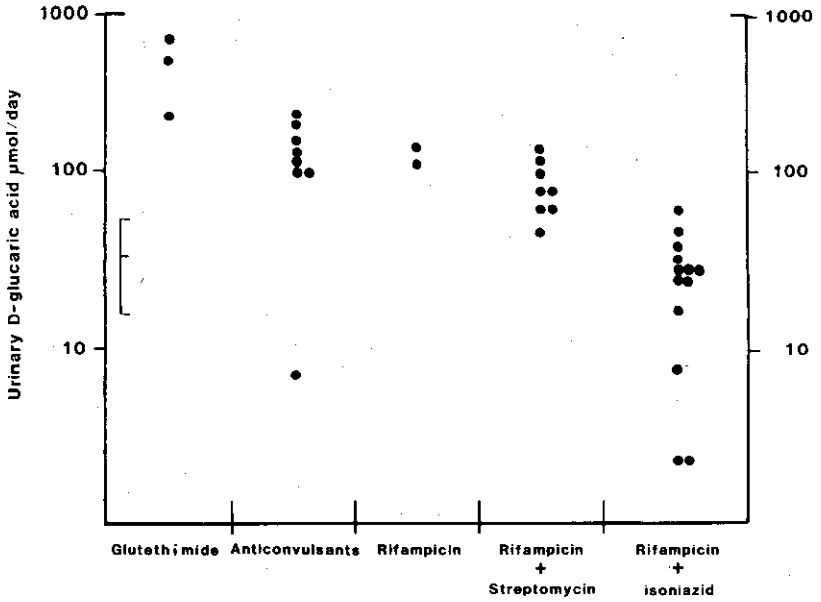

Fig. 1. Urinary D-glucaric acid excretion with different enzyme-inducing drugs. Vertical bars denote $\bar{X} \pm SD$ of controls. Note that all groups have clearly elevated levels of excretion [except for the reversal to normal induced by isoniazid in the rifampicin isoniazid group ($\bar{X} \pm SD = 28.80 \pm 20.81$; $n = 13$)] compared to rifampicin and rifampicin/streptomycin groups ($\bar{X} \pm SD = 108 \pm 40.55$; $n = 10$; $P < 0.001$).

azid treatment. This has some importance because induction of other pathways such as oxidative metabolism, as indicated by antipyrine and quinine t½s or 6β-hydroxycortisol excretion, persists in patients on combined therapy.[3, 5, 6, 14]

Methods

The enzymatic procedure of Simmons et al.[9] was used to measure urinary D-glucaric acid. The normal range in our laboratory was ($\bar{X} \pm SD$) 41.5 ± 25.6 µmol/day ($n = 29$). Five groups of patients on different enzyme-inducing drugs were compared (Fig. 1). In the glutethimide group (three patients) dose levels in descending order were 1 gm daily, glutethimide addiction, and 500 mg daily. In the anticonvulsant group (eight patients) daily dose levels in descending order were 300 mg phenytoin and 45 mg phenobarbital; 300 mg phenytoin with 750 mg primidone and 300 mg carbamazepine; 200 mg phenytoin and 100 mg phenobarbital; 180 mg phenobarbital and 1.5 gm primidone; 180 mg phenobarbital and 1 gm primidone; 200 mg phenytoin; 45 mg phenobarbital daily; and 100 mg butobarbital. Rifampicin, 450 mg daily, alone was given to two patients for 10 days (Fig. 1) and was combined with streptomycin, 0.75 gm im daily, in eight patients for a mean duration of 10 days and with isoniazid in 13 patients for a mean duration of 120 days. All patients receiving rifampicin had pulmonary or skeletal tuberculosis. In three of these patients initial rifampicin D-glucaric acid excretion was measured serially and after the introduction of isoniazid (Fig. 2). In two patients either rifampicin or isoniazid was stopped at the end of 18 mo and D-glucaric acid excretion was followed on the single drug (Fig. 3).

To compare the activities of rifampicin and sodium phenytoin on the glucuronic acid pathway, D-glucaric acid and plasma β-glucuronidase were measured serially (Figs. 4 and 5). β-Glucuronidase was determined according to the method of Wollen and Walker.[12] Plasma γGT levels were screened in 83 patients with tuberculosis (52 Indian and 31 European) 14 to 76 yr old ($\bar{X} = 39.7$ yr). There were 45 women and

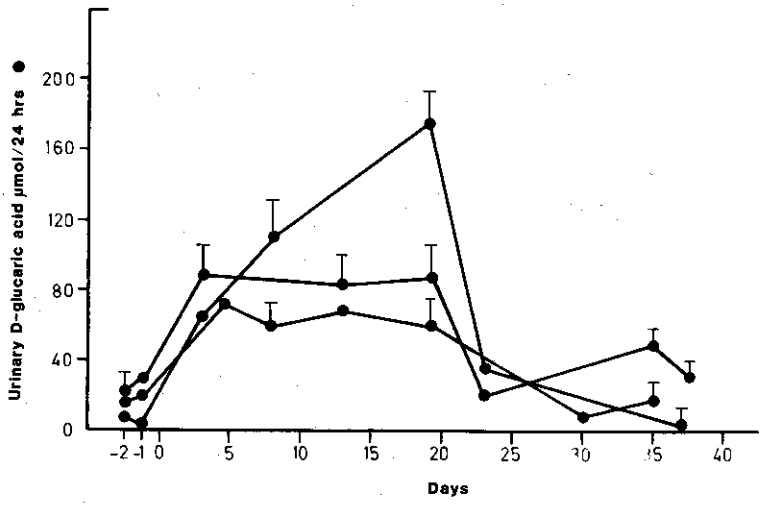

Fig. 2. Serial rise in D-glucaric acid in three patients on rifampicin, 450 mg, and streptomycin, 0.75 gm im daily, and decline to pretreatment levels when isoniazid, 300 mg daily, is added. Bars denote $\bar{X} \pm SD$.

Table I. *Comparison of γGT and D-glucaric acid during antituberculous treatment*

Sex	*Race*	*Serum alanine transaminase* (IU/l)*	*Plasma γGT (IU/l)*	*Urinary D-glucaric acid (µmol/day)*
M†	E	—	201	13.0
M	I	8	65	48.5
F	I	9	87	94.0
M	E	—	90	—
F†	E	17	309	15.0
M†	I	7	77	76.0
M	E	30	73	—
F†	E	—	91	35.0
M†	E	10	161	2.0
M†	E	7	95	5.0
M†	E	6	322	9.0
F	I	6	125	48.0
F	I	13	164	23.0
F	I	—	224	17.5
F	I	4	80	12.0
F†	E	21	95	16.0
M†	E	3	91	9.0
M	E	15	121	—
M†	E	10	78	2.0
F	I	6	93	20.9

Elevated plasma levels of γGT activity (>60 IU/l) in 20 of 83 patients receiving rifampicin and isoniazid. Note normal urinary D-glucaric acid excretion. E = European; I = Indian.
*Normal range 0 to 12 IU/l.
†History of high alcohol intake.

38 men receiving rifampicin, 450 or 600 mg daily, and isoniazid, 300 mg daily. The γGT level was measured after 6 mo therapy by the method of Rosalki et al.[8] Those with abnormally high levels (>60 IU/l) were further evaluated for cytoplasmic damage by measurement of serum alanine transaminase levels as determined by a standard laboratory method[13] and for separate evidence of enzyme induction by measurement of D-glucaric acid levels (Table

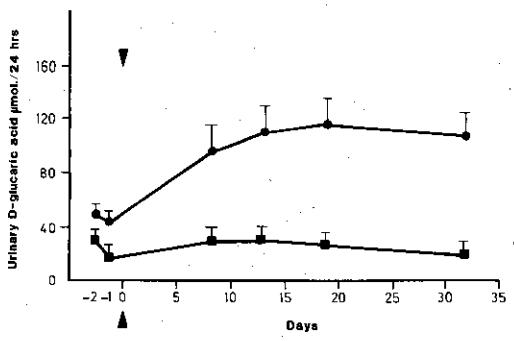

Fig. 3. Serial rise in D-glucaric acid excretion on rifampicin, 450 mg daily (●), after isoniazid, 300 mg daily, was stopped at the end of 18 mo of treatment (▼). Excretion was unchanged when isoniazid was continued (■) after rifampicin was stopped. Bars denote $\bar{X} \pm SD$.

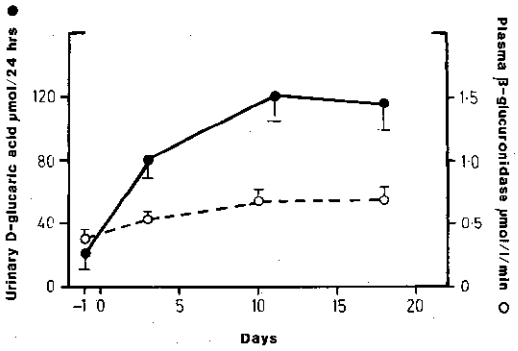

Fig. 4. Serial rise in D-glucaric acid excretion and plasma β-glucuronidase on sodium phenytoin, 200 mg daily. Bars denote $\bar{X} \pm SD$.

I). They were also interviewed for high alcohol intake, defined as equal to or more than 4 oz whiskey or 4 pt beer a day.

Results

Of nine drugs tested, glutethimide induced the highest level of urinary D-glucaric acid excretion (708 μmol/day; Fig. 1). Combined anticonvulsant therapy induced more excretion than did a single anticonvulsant. Butobarbital had no effect in one patient, presumably because of its short t½. Rifampicin alone or with streptomycin induced levels of the range of the lower doses of anticonvulsants. The highest excretion was 188 μmol/day. Streptomycin appeared to have no effect. As already reported,[5, 6] D-glucaric acid excretion returned to pretreatment levels after isoniazid was substituted for streptomycin.

Serial rises in D-glucaric acid excretion by rifampicin were variable and the suppressive effect of isoniazid was confirmed (Fig. 2). This was also seen in a patient at the end of 18 mo treatment when isoniazid was stopped and rifampicin continued alone (Fig. 3), whereas isoniazid alone after rifampicin was stopped had no effect (Fig. 3). There was a similar serial rise in D-glucaric acid excretion accompanied by a small rise in plasma β-glucuronidase (Fig. 4) after phenytoin, 200 mg daily. The rise in β-glucuronidase with rifampicin was much clearer (Fig. 5), however, and reversal of the D-glucaric acid rise by isoniazid had no effect on the steady high level of β-glucuronidase.

Levels of γGT were elevated in 20 of 83 patients receiving rifampicin and isoniazid. Ten of these patients had histories of high alcohol intake. Urinary D-glucaric acid excretion was normal in all patients (Table I).

Discussion

Glutethimide was reported to have caused osteomalacia in one patient, probably by a mechanism of hepatic enzyme induction and vicarious hydroxylation of vitamin D.[1] We confirmed its powerful inducing effect through the use of D-glucaric acid as the test compared to six other inductive drugs. In our hands the enzyme assay was sensitive and the activity of rifampicin, 450 mg daily, was detected. Others have shown an increase in D-glucaric acid with 1200 mg daily.[4] Since β-glucuronidase levels remain elevated both in the short term (Fig. 5) and throughout treatment,[7] isoniazid suppression of the D-glucaric acid rise might develop at the level of uronolactonase or glucuronolactone dehydrogenase in the pathway of D-glucaric acid metabolism. Such a theory depends on whether or not the measured β-glucuronidase level is of hepatic microsomal origin, but it might equally be derived from lysosomes.[7]

Differences in rates of rise and maximum excretion of D-glucaric acid during rifampicin induction are probably partly results of variations in rifampicin concentrations at the cellular enzyme level, although some subjects may have greater genetic susceptibility to enzyme induc-

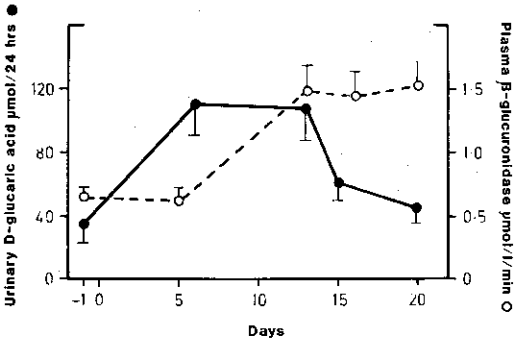

Fig. 5. Serial rise in D-glucaric acid excretion and plasma β-glucuronidase during rifampicin, 450 mg daily, and streptomycin, 0.75 gm im daily. Note the persistent high plasma level of β-glucuronidase after D-glucaric acid returned to pretreatment levels after addition of isoniazid, 300 mg daily. Bars denote $\bar{X} \pm SD$.

tion. In our high responder (Fig. 2), who began with an extremely low level of D-glucaric acid excretion, other indices such as antipyrine t½ and 6β-hydroxycortisol excretion were correspondingly affected more than in other patients tested.* Nonetheless, we have not found evidence to suggest a separate genetic group, which would be of great interest. Many of our patients receiving rifampicin were Indian vegetarians (including the above patient) and the absence of animal protein and other dietary factors may be as important as genetic determination in response to enzyme-inducing drugs.

Plasma γGT levels are not significantly elevated in most patients receiving rifampicin and isoniazid,[7] and of those patients with elevated levels, half in our group had high alcohol intake (Table I). Elevated levels in Indian women and others in whom high alcohol intake was unlikely were explained by observations of elevated γGT before treatment caused by tuberculosis itself.† When this enzyme is raised, therefore, it suffers from lack of specificity between the hepatic effects of infection, alcohol, and drug enzyme induction. Since D-glucaric acid excretion was normal in these patients, it might have been assumed that hepatic enzyme induction was absent, but oxidative enzymes as judged by antipyrine and quinine t½s and 6β-hydroxycortisol excretion continue to be induced.[3, 5, 6, 14] Consequently, no prediction of oxidative metabolism should be made on the basis of changes in glucaric acid metabolism or γGT levels. This emphasizes that, while these indirect indices have general screening value, they are often unequally affected and are unlikely to predict the fate of other endogenous substrates or drugs. A direct investigation of the suspected substrate or drug is still necessary.

*Perry W: Unpublished observation, 1980.

†Perry W: Unpublished observation, 1978.

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