ETHOSUXIMIDE : LIVER ENZYME INDUCTION AND D-GLUCARIC ACID EXCRETION

J.C. GILBERT & A.K. SCOTT

Department of Pharmacology, University Medical Buildings, Aberdeen

D.B. GALLOWAY & J.C. PETRIE

Department of Therapeutics and Clinical Pharmacology,
University Medical Buildings, Aberdeen

- 1 A study has been carried out to determine if ethosuximide induces liver enzymes.
- 2 Ethosuximide did not affect the urinary excretion of D-glucaric acid by healthy adult subjects nor was the mean daily D-glucaric acid excretion of three epileptic children on long term ethosuximide therapy different from that of three matched controls.
- 3 Ethosuximide (10 mg/kg or 50 mg/kg daily) did not influence D-glucaric acid excretion or liver microsomal protein and cytochrome P450 contents of guinea pigs but at a dose of 100 mg/kg daily in rats it increased liver microsomal protein and cytochrome P450 without altering D-glucaric acid excretion.
- 4 These results suggest that at anticonvulsant doses ethosuximide is unlikely to induce liver enzymes. The precise relationship between D-glucaric acid excretion and liver enzyme induction remains in doubt.

Introduction

A number of anticonvulsant drugs are known to cause induction of enzymes and proliferation of the endoplasmic reticulum of the liver cell. It has been suggested (for instance see Hunter, Maxwell, Carrella, Stewart & Williams, 1971a) that as enzyme induction occurs in patients treated with combinations of anticonvulsant drugs, there is a parallel increase in the production of D-glucaric (saccharic) acid by the liver. This acid is excreted unchanged and it has been claimed that its concentration in the urine is directly related to the degree of liver enzyme induction.

In some of the studies purporting to support this hypothesis, D-glucaric acid excretion has been related to total anticonvulsant intake, and the multiple anticonvulsant therapy has included not only phenytoin and phenobarbitone, drugs clearly shown to induce enzymes, but also ethosuximide, the drug of choice in the treatment of petit mal epilepsy. To the best of our knowledge no data are available to support the assumption that ethosuximide has effects on the liver cell similar to those of phenytoin and phenobarbitone.

In the present study, therefore, we have determined the urinary excretion of D-glucaric acid by adult human volunteers before and during ethosuximide administration, and we have compared the excretion of D-glucaric acid by

normal children with the excretion by epileptic children on long term ethosuximide administration. In addition, we have attempted to determine if ethosuximide induces liver enzymes upon administration to guinea-pigs and to rats. The parameters used as a measure of enzyme induction were the liver microsomal protein content, and the liver microsomal cytochrome P450 content.

Methods

Human experiments

(a) Four healthy adult males received 250 mg ethosuximide thrice daily (total daily dose 12 mg/kg approximately) in capsule form, and the urinary excretions of D-glucaric acid and creatinine were determined over a 13 day period—4 days prior to receiving drug and 9 days during drug treatment. In all cases complete 24 h urine collections were obtained.

(b) D-glucaric acid and creatinine excretions were determined in three children, living at home, who suffered from petit mal epilepsy and who were on long term ethosuximide (mean 23 mg/kg daily) in capsule form, and also in control children matched for age and sex.

Urinary D-glucaric acid concentrations were calculated by measuring the degree of inhibition of rat liver β -glucuronidase (Marsh, 1963) as suggested by Hunter *et al.* (1971a) using glucaro-1, 4-lactone (Sigma Chemical Co.) standards. Urinary creatinine was determined by the method of Folin (1904) as modified by Briggs & Cooper (1961).

Animal experiments

Male albino guinea-pigs or Sprague-Dawley rats were injected with ethosuximide or saline twice daily. After sacrifice the livers were removed, blotted free of superficial blood, placed on ice, weighed and homogenized in 1.15% KCl solution to give a 10% (w/v) homogenate. Microsomal fractions were prepared by centrifuging the homogenate at 100,000 *g* for 60 min after removing the nuclei, cell debris and mitochondria (600 *g* for 10 min, then 18,000 *g* for 30 minutes).

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) and cytochrome P450 by the method of Omura & Sato (1964).

Results

Table 1 shows that ethosuximide (approximately 12 mg/kg daily) had no effect upon the excretion of creatinine or D-glucaric acid in four adult volunteers over a 9 day period. The daily excretions of creatinine by the subjects showed only small variations throughout the period of the test (13 days) and the excretion of D-glucaric acid in 24 h urine samples was not significantly altered by drug administration. The D-glucaric acid excretion results are considerably lower than those obtained by Latham & Richens (1973) but they are similar to those of Marsh (1963) and of Hunter *et al.* (1971a). The mean daily excretion of D-glucaric acid over a 3 day period by three children receiving ethosuximide (mean dose 23 mg/kg daily; no other therapy) for the treatment of petit mal epilepsy was 1.8 ± 0.2 μ mol/g creatinine and this was not significantly different from the mean excretion of 4.0 ± 2.0 μ mol/g creatinine by three matched controls over the same period.

Table 2 shows that with regard to the experiments with rodents, ethosuximide

Table 1 The urinary excretion of D-glucaric acid and creatinine by adults

	No ethosuximide				Ethosuximide (approximately 12 mg/kg)								
	Day				Day								
Creatinine (g/24 h)	1	2	3	4	1	2	3	4	5	6	7	8	9
	1.55	1.80	1.85	1.65	1.80	1.75	1.75	1.80	1.70	1.55	1.55	1.70	1.80
D-glucaric acid* (μ mol/g creatinine)	2.7 (0.7)	1.4 (0.4)	2.3 (0.8)	3.8 (0.4)	3.0 (0.7)	3.7 (1.1)	5.5 (0.4)	3.3 (0.9)	2.9 (0.8)	3.8 (1.5)	4.0 (1.4)	2.9 (0.5)	1.8 (0.9)

Each result is the mean from four adult volunteers. S.e. means for the D-glucaric acid excretions are given in parenthesis while those of the creatinine excretions were small and have been omitted.

* As glucaro-1, 4-lactone.

Table 2 The effects of ethosuximide (10 mg/kg daily) on the protein and cytochrome P450 contents of microsomal fractions prepared from guinea-pigs

	5 day experiments		10 day experiments	
	C	D	C	D
Microsomal protein (mg/g liver)	14.8 (1.2)	16.0 (1.7)	14.1 (1.0)	14.7 (1.2)
Microsomal cytochrome P450 (nmol/mg microsomal protein)	0.65 (0.10)	0.61 (0.12)	0.65 (0.16)	0.70 (0.10)

Each result is the mean of three experiments. The numbers in parenthesis are the s.e. means. The results for drug-treated (D) and control animals (C) are not significantly different.

(10 mg/kg daily) did not significantly affect either the protein content or the cytochrome P450 content of microsomal fractions prepared from the livers of guinea-pigs which received the drug for either 5 or 10 days.

This dose of 10 mg ethosuximide/kg daily, whilst being an acceptable therapeutic dose, might be considered too low to exert a pronounced effect upon the enzymes of the endoplasmic reticulum of the liver cell. In another series of experiments, therefore, guinea-pigs were injected with a larger dose of ethosuximide (50 mg/kg daily) or with saline intraperitoneally. The excretion of D-glucaric acid was not altered by drug administration and although the protein and cytochrome P450 contents of microsomal fractions prepared from the livers of animals which had received the drug for 10 days were more variable than when the lower dose of drug was administered they were not significantly different from the results obtained for the control series (Table 3).

Finally, the parameters of liver enzyme induction and also the excretion of creatinine and D-glucaric acid, were determined in rats which had been treated with either ethosuximide (100 mg/kg) or saline daily by intraperitoneal injection. This dose of ethosuximide proved fatal to guinea-pigs in a similar series of experiments. Ethosuximide increased both the microsomal protein content (23.4 ± 1.8 mg/g liver as compared with 12.6 ± 1.3 mg/g liver, $P < 0.025$, $n = 4$) and cytochrome P450 content (0.70 ± 0.01 nmol/mg

microsomal protein as compared with 0.47 ± 0.06 nmol/mg microsomal protein, $P < 0.01$, $n = 4$). The drug did not significantly influence D-glucaric acid excretion by the animals before sacrifice ($2.5 \mu\text{mol}$ D-glucaric acid/g creatinine throughout).

Discussion

The results indicate that at anticonvulsant doses it is unlikely that ethosuximide induces liver enzymes. The dose of 10 mg/kg daily increased neither the excretion of D-glucaric acid by adult human subjects nor the protein or cytochrome P450 contents of microsomal fractions prepared from the livers of guinea-pigs which had received the drug. It could be argued that any enzyme induction might not be maximal during the period of the experiment and consequently that it would have been valuable to extend the period of ethosuximide administration to the human volunteers to at least 14 days. This point may be valid but there was no evidence of increased D-glucaric acid excretion by the human volunteers even by the ninth day. Furthermore, the excretion of D-glucaric acid by the small group of epileptic children was not different from the excretion by matched controls. The results obtained using higher doses of ethosuximide in guinea-pigs lead to similar conclusions. The dose of 50 mg ethosuximide/kg daily caused a greater degree of variability, but again the generally accepted indices

Table 3 Effects of ethosuximide (50 mg/kg daily) on liver microsomal protein and cytochrome P450 contents and D-glucaric acid excretion by guinea-pigs

	5 day experiments		10 day experiments	
	C	D	C	D
Microsomal protein (mg/g liver)	13.5 (0.1)	15.3 (1.8)	17.3 (0.6)	20.3 (2.5)
Microsomal cytochrome P450 (nmol/mg microsomal protein)	0.48 (0.08)	0.53 (0.10)	0.51 (0.15)	0.58 (0.12)

	No ethosuximide		Ethosuximide					
	Day		Day					
	1	2	1	2	3	4	5	6
D-glucaric acid* ($\mu\text{mol/g}$ creatinine)	3.0 (2.5)	1.6 (1.1)	2.1 (0.4)	5.2 (1.0)	3.8 (2.6)	4.3 (1.7)	1.0 (1.4)	3.5 (1.0)

Each result is the mean from three experiments. S.e. mean is given in parenthesis. The results for drug-treated (D) and control (C) animals are not significantly different.

* As glucaro-1, 4-lactone.

of enzyme induction (liver microsomal protein and cytochrome P450 concentrations) were not significantly altered, nor was the excretion of D-glucaric acid. In contrast, the studies of liver microsomal fractions prepared from rats which had received 100 mg ethosuximide/kg daily for 10 days suggested that significant protein synthesis had been induced. However, this effect was not reflected by parallel increases in D-glucaric acid excretion. A similar observation was made by Aarts (1971) and it does throw some doubt upon the value of measurements of D-glucaric acid excretion as a true measure of the degree of induction of liver enzyme by drugs. Hunter, Maxwell & Williams (1971b) have pointed out that the rat synthesizes ascorbic acid from glucuronic acid so that urinary D-glucaric acid (which is synthesized from glucuronic acid in man and guinea-pig) would not be expected to increase in that species when enzyme induction occurs. Whilst this argument may be correct it is surprising if any increased metabolic disposal of glucuronic acid in the rat due to enzyme induction is reflected solely by an increase in ascorbic acid production where the D-glucaric acid pathway remains available.

It is now a frequent practice (Hunter *et al.*, 1971b; Hunter, Maxwell, Stewart & Williams, 1973; Latham & Richens, 1973) to determine the concentration of D-glucaric acid in the urine of patients and to employ the level of D-glucaric acid expressed either per 24 h or per g creatinine, as a measure of the extent of induction of liver enzymes by a drug. Whilst this practice may turn out to be soundly based, and although there is

evidence that it is valid in the cases of specific drugs, a general relationship between D-glucaric acid excretion and liver enzyme induction is not clearly established.

It should also be mentioned that the technique of assaying D-glucaric acid involves determining the degree of inhibition of liver homogenate β -glucuronidase activity by urine samples heated at different pH values. It is assumed that the main inhibitory compound in urine which has been acidified, compared to that which has been made basic, is glucaro-1, 4-lactone formed from D-glucaric acid. Like other workers, we have used this technique without ascertaining that the assumption is correct, although the work of Marsh (1963) gives it adequate support, and results have been expressed as 'D-glucaric acid' rather than 'apparent D-glucaric acid' or 'acid potentiated inhibitor'. In addition, we have performed experiments to ensure that any ethosuximide excreted unchanged in the urine does not itself interfere with the assay technique.

It can be concluded on the basis of the results of this study that, in contrast to phenobarbitone and phenytoin, the anticonvulsant ethosuximide probably does not induce liver enzymes at therapeutic doses. However the drug may induce enzymes if it is administered to some species in high doses.

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