MICROSOMAL ENZYME INDUCTION IN CHILDREN: THE INFLUENCE OF CARBAMAZEPINE TREATMENT ON ANTIPYRINE KINETICS, 6 β -HYDROXYCORTISOL EXCRETION AND PLASMA γ -GLUTAMYLTRANSPEPTIDASE ACTIVITY

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1 The influence of carbamazepine (CBZ) therapy on saliva antipyrine kinetics, urinary 6 β -hydroxycortisol excretion and plasma γ -glutamyltranspeptidase activity was determined in nine children aged 6–14 years.

2 During 5 weeks of CBZ therapy the mean ($\pm$ s.d.) antipyrine clearance increased from 65 ± 12 ml $\text{kg}^{-1} \text{h}^{-1}$ to 143 ± 34 ml $\text{kg}^{-1} \text{h}^{-1}$ ($P < 0.001$) and the mean half-life declined from 6.24 ± 1.23 h to 2.78 ± 0.59 h ($P < 0.001$). The apparent volume of distribution of antipyrine did not change during CBZ treatment.

3 Urinary 6 β -hydroxycortisol excretion increased markedly during the study period from 5.10 ± 1.77 $\mu\text{g day}^{-1} \text{kg}^{-1}$ to 17.85 ± 6.75 $\mu\text{g day}^{-1} \text{kg}^{-1}$ ($P < 0.01$).

4 Plasma γ -glutamyltranspeptidase activity increased in eight out of nine children during CBZ therapy but the change was not statistically significant.

5 CBZ appears to have a marked enzyme inducing effect in young epileptics as indicated by antipyrine kinetics and 6 β -hydroxycortisol excretion.

Introduction

Many drugs known to induce drug metabolism in adults are used in paediatric practice but relatively little is known of the effect of these drugs in children. It has recently been shown that carbamazepine (CBZ) induces its own metabolism in children and that steady-state levels are approximately half those predicted from single dose kinetics (Bertilsson *et al.*, 1980; Moreland *et al.*, 1982).

A number of indices have been proposed, and used to assess the extent of enzyme induction in adults (Park & Breckenridge, 1981) but their usefulness in children is unknown. One of the more widely used measures of induction is the change in the rate of

elimination of antipyrine. The systemic clearance of this drug is significantly increased by a number of enzyme inducing agents in adults (Vesell, 1979) and it has recently been reported that antipyrine elimination increased in three children given long-term CBZ therapy (Lindgren *et al.*, 1980).

Enzyme induction has also been assessed by measuring changes in the disposition of endogenous compounds. Plasma γ -glutamyltranspeptidase (γ GT) activity is elevated in groups of young epileptics receiving anticonvulsant drugs (Bartels, *et al.*, 1975; de Wolff *et al.*, 1980) but the influence of CBZ monotherapy on γ -glutamyltranspeptidase activity in individual children is unknown.

Urinary 6 β -hydroxycortisol has also been proposed as an index of enzyme induction. It may now be determined conveniently and specifically by radioimmunoassay (Park, 1978) and has been shown to be a useful non-invasive index of enzyme induction

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in man (Ohnhaus & Park, 1979).

In the present study, the influence of CBZ therapy on the elimination of antipyrine and 6 β -hydroxycortisol excretion and on plasma γ -glutamyltranspeptidase activity in children has been examined.

Methods

Nine children aged 6–14 years, who were to receive CBZ therapy as part of the normal therapeutic management of their newly diagnosed epilepsy, took part in the study, which was approved by the local hospital ethical committee.

A single oral dose of antipyrine, 15 mg kg⁻¹, dissolved in orange juice was administered to each child at 08.00 h on the first day of the study and again after 5 weeks of twice-daily CBZ therapy, 400 mg day⁻¹ (mean $\pm$ s.d., 14.1 $\pm$ 2.7 mg kg⁻¹ day⁻¹). Mixed saliva was collected after stimulation with citric acid and flavouring (Bacon *et al.*, 1978) at hourly intervals throughout the remainder of the day after antipyrine administration and at 07.00 and 08.00 h the next day. Saliva was stored at -20°C until assayed for antipyrine by gas chromatography (Huffman *et al.*, 1974). Throughout the study period the children received no other drug therapy.

Saliva antipyrine half-life, apparent volume of distribution and clearance were calculated from the slope and zero-time intercept of the logarithm of the concentration versus time curve.

Twenty-four hour urines and single blood samples were collected at the beginning and end of the 5-week period. Urine was assayed for 6 β -hydroxycortisol by radioimmunoassay (Park, 1978) and plasma γ -glutamyltranspeptidase activity by routine biochemical analysis. Urinary 17-hydroxycorticosteroids could not be measured spectrophotometrically (Sanghvi *et al.*, 1973) because of interference from a carbamazepine metabolite.

Statistical comparison of the results was carried out using Student's paired *t*-test. Absolute values of antipyrine clearance, antipyrine half-life, 6 β -hydroxycortisol excretion and γ -glutamyltranspeptidase activity were compared against each other, both before and after induction by means of linear regression using the method of least squares of errors. The percentage change in the value of each parameter during the study period was calculated relative to the initial value to determine whether the capacity for induction is correlated with indices of mixed function oxygenase activity in the non-induced state. The changes were compared against each other and against the initial values by linear regression using the same method. In calculating the change in antipyrine half-life, reciprocal values were used (Sultatos *et al.*, 1980).

Results

Saliva antipyrine clearance, apparent volume of distribution and half-life at the beginning and end of 5 weeks carbamazepine therapy in nine children are listed in Table 1. Each of the saliva antipyrine concentration-time curves was determined from at least ten samples with correlation coefficients ranging from 0.76 to 0.99 ($P < 0.05$).

Antipyrine clearance increased in the children during CBZ treatment by 60% to 219%. The mean ($\pm$ s.d.) clearance increased significantly from 65 $\pm$ 12 ml kg⁻¹ h⁻¹ to 143 $\pm$ 34 ml kg⁻¹ h⁻¹ ($P < 0.001$). Antipyrine half-life fell by 56% to 317% over the 5 weeks of CBZ treatment from a mean value of 6.24 $\pm$ 1.23 h to 2.78 $\pm$ 0.59 h ($P < 0.001$). Children with initially longer half-lives tended to have a greater percentage change in half-life ($r = 0.75$, $P < 0.02$), but there was no significant correlation between initial antipyrine clearance and percentage change in clearance ($r = 0.29$). The apparent volume of distribution of antipyrine did not change significantly during the study period.

The 24 h urine collections in patients 1, 3 and 9 were incomplete and so 6 β -hydroxycortisol output was determined in only six children. In these patients 6 β -hydroxycortisol excretion increased during CBZ therapy by amounts ranging from 86% to 642% (Table 1). The mean excretion of 6 β -hydroxycortisol increased from 5.10 $\pm$ 1.77 μ g day⁻¹ kg⁻¹ to 17.85 $\pm$ 6.75 μ g day⁻¹ kg⁻¹ ($P < 0.01$).

The mean plasma γ -glutamyltranspeptidase activity did not change significantly during the 5 weeks of CBZ therapy.

The absolute value of saliva antipyrine clearance was significantly correlated to the urinary 6 β -hydroxycortisol output in the children both before and after induction ($n = 12$, $r = 0.80$, $P < 0.01$), but there was no significant correlation between the percentage changes in antipyrine clearance, 6 β -hydroxycortisol excretion and γ -glutamyltranspeptidase.

Discussion

Three proposed indices of hepatic microsomal enzyme induction have been used to assess the enzyme inducing capacity of carbamazepine therapy in children. Clearance of the model drug, antipyrine, showed that after 5 weeks of CBZ treatment, metabolic activity had increased markedly. The mean increase in saliva antipyrine clearance, 122%, was more than twice that previously observed in a group of children given a similar weight-related dose of CBZ (Lindgren *et al.*, 1980). In that study, the children were older (9–16 years, mean 12.7 years) compared with 6–14 years, mean 9.33 years) and only

Table 1 Antipyrine pharmacokinetics, γ -glutamyltranspeptidase activity and 6 β -hydroxycortisol excretion in children before and after 5 weeks of carbamazepine therapy. Mean $\pm$ s.d.

Patient	Sex	Age (years)	Weight (kg)	Clearance		Antipyrine Half-life		Volume of distribution		6 β -hydroxycortisol		γ -GT	
				Before	After	Before	After	Before	After	Before	After	Before	After
				(ml kg ⁻¹ h ⁻¹)	(%)	(h)	(h)	(ml mg ⁻¹)	(%)	(μ g day ⁻¹ kg)	(i.u.)	(%)	(%)
1	F	14.33	41.5	52	140	7.03	2.67	534	539	—	—	15	13
2	F	10.25	31.3	71	155	5.67	2.73	578	610	4.47	25.30	14	16
3	F	10.00	31.8	51	109	6.30	3.83	465	605	—	—	12	13
4	F	6.66	22.8	64	204	7.10	2.35	665	640	2.94	21.80	9	11
5	F	11.50	26.4	60	112	7.76	1.86	669	350	3.26	6.06	12	16
6	F	9.75	31.4	53	117	5.54	3.29	420	554	6.59	17.64	10	16
7	F	8.00	18.5	72	115	3.57	2.29	369	382	6.43	21.08	10	12
8	M	6.22	27.2	86	187	6.35	2.85	787	770	6.91	15.22	7	8
9	F	7.33	22.9	78	149	6.87	3.18	770	684	—	—	13	14
Mean		9.33	28.2	65	143	6.24	2.78	584	570	5.10	17.85	11	13
s.d.		2.59	6.8	12	34	1.23	0.59	150	25	1.77	6.75	3	3
P					<0.001		<0.001		NS		<0.01		NS

three out of thirteen CBZ-treated patients acted as their own controls; the remainder being compared with a separate group of CBZ-free children. These differences may account for the discrepancy.

In the present study urinary 6 β -hydroxycortisol showed a large, extremely variable increase in excretion rate after 5 weeks of CBZ treatment; the mean increase was 285% but individual changes ranged from 86% to 642%. The absolute values of antipyrine clearance and 6 β -hydroxycortisol output at the start and end of the study showed a significant correlation. This is in agreement with the findings of Ohnhaus & Park (1979) in adults, and indicates that cortisol and antipyrine are metabolised by similar enzyme systems.

In adults, daily fluctuation in 6 β -hydroxycortisol output may be reduced by expressing output as a proportion of total excretion of 17-hydroxycorticosteroids (Ohnhaus & Park, 1979). This procedure also allows single urine samples to be used instead of complete 24 h collections and would offer a great advantage in paediatric studies. Unfortunately, the presence of an acridine-aldehyde metabolite of CBZ in urine leads to false high values when 17-hydroxycorticosteroids are determined using the Porter-Silber reaction (Arisue *et al.*, 1976; Salti & Zeid, 1979) and so 6 β -hydroxycortisol output cannot be expressed in this way when CBZ is the inducer or the induced drug.

The third measure of drug metabolising ability which was examined in the present study, γ -glutamyltranspeptidase activity, showed no statistically significant change during CBZ monotherapy although the levels increased in eight out of the nine children examined. In addition there was no significant correlation between the percent change in γ -glutamyltranspeptidase activity and the per cent change in antipyrine clearance. Serum γ -glutamyltranspeptidase in two groups of epileptic children receiving various anticonvulsant drugs have been reported to be considerably higher than in children receiving no drug therapy (Bartels *et al.*, 1975; de Wolff *et al.*, 1980) but γ -glutamyltranspeptidase activity does not appear to be a reliable indicator of induction occurring during CBZ monotherapy. The influence of CBZ on antipyrine clearance and 6 β -hydroxycortisol excretion in adults has not been examined but the changes observed in study are comparable in magnitude with those seen in adults during induction with rifampicin, one of the most potent enzyme-inducing agents in man (Ohnhaus & Park, 1979). Branch & Shand (1979) proposed that hepatic drug clearance may be regarded as a product of liver size and specific drug metabolising enzyme activity per unit mass of liver and that larger responses to inducing agents would be expected in individuals with large livers. It has recently been confirmed that children have larger livers per unit body

weight than adults (Rylance *et al.*, 1982) and so would be expected to have a relatively greater mass of drug metabolising tissue to be induced.

The percentage decrease in antipyrine half-life in the children given CBZ therapy was correlated to the initial antipyrine half-life; individuals with long half-lives at the start of the study showed the greatest change in half-life after 5 weeks treatment. This agrees with a number of observations in adults given various enzyme inducing agents (Vesell & Page, 1969; Breckenridge *et al.*, 1971; Branch & Shand, 1979). However, there was no apparent relationship between the percentage change in antipyrine clearance and the initial clearance value, nor was there a correlation between the percentage changes in clearance and half-life. The latter finding is in contrast to the data of Roberts *et al.* (1976) reported by Branch & Shand (1979) who found a significant

relationship between the percentage changes in antipyrine clearance and half-life.

The clinical significance of these findings lies in the potential for drug interactions as a result of enzyme induction. A number of drugs administered to children reduce the levels of concomitantly administered drugs (Levy *et al.*, 1982) but confirmation that this actually occurs by induction of drug metabolising enzymes is missing in many instances. The present findings not only confirm that children metabolise drugs more rapidly than adults but also indicate that the potential for drug interactions involving induction is greater in paediatric practice.

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