Eur J Clin Pharmacol (1996) 50 : 411–415

B. Tomlinson · R. P. Young · M. C. Y. Ng
P. J. Anderson · R. Kay · J. A. J. H. Critchley

Selective liver enzyme induction by carbamazepine and phenytoin in Chinese epileptics

Received: 24 July 1995 / Accepted in revised form: 22 December 1995

Abstract *Objective*: Anticonvulsant drugs are known inducers of cytochrome P450 liver enzymes and it has been suggested that this induction increases susceptibility to paracetamol-induced hepatotoxicity.
Methods: We measured the percentage urinary recovery of paracetamol and its metabolites after a dose of 20 mg kg^{-1}, and the excretion of 6ß-hydroxycortisol as a ratio to urinary free cortisol(6ßOHF/F) in Chinese epileptic patients maintained on long term therapy with carbamazepine (n = 6) or phenytoin (n = 6).
Results: Compared to the healthy controls (n = 20), patients on phenytoin had significantly lower recoveries of mercapturic acid, cysteine and sulphate metabolites, but a higher recovery of glucuronide metabolites of paracetamol. The recoveries of paracetamol metabolites in patients on carbamazepine were not different from controls. In contrast, the 6ßOHF/F was significantly higher in patients on carbamazepine (3-fold) or phenytoin (2-fold) compared to controls.

Healthy control Chinese subjects metabolised paracetamol in a similar way to that reported in Caucasians, indicating that the risk for hepatotoxicity would be the same. Our findings in a group of Chinese patients on phenytoin were also similar to those previously reported in Caucasians on this drug. The apparent differences in the pattern of isoenzyme induction between the groups on phenytoin and carbamazepine require verification in larger studies. The data do not suggest an increased risk of paracetamol-induced hepatotoxicity in Chinese patients on anticonvulsants.

B. Tomlinson · R. P. Young · M. C. Y. Ng · P. J. Anderson
J. A. J. H. Critchley
Department of Clinical Pharmacology, The Chinese University of Hong Kong, Shatin, Hong Kong

R. Kay
Department of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong

Key words Anticonvulsants, Paracetamol; liver enzyme induction, cytochrome P450 induction, Chinese, 6ß-hydroxycortisol

Introduction

Anticonvulsant treatment induces certain liver enzymes, such as those producing glucuronidation and some of the cytochromes P450 (CYP). Some of the latter produce reactive intermediate drug metabolites and induction of those enzymes could lead to potentiation of paracetamol hepatotoxicity [1, 2]. Following therapeutic doses of paracetamol, measurement of its glutathione derived urinary metabolites, mercapturic acid (U_{merc}) and cysteine (U_{cyst}) conjugates, reflects the activity of the isoenzymes CYP 1A2 and CYP 2E1 [3], and, to a much smaller degree, CYP 3A4 [4]. Endogenous cortisol is also metabolised by hepatic cytochrome P450 to 6ß-hydroxycortisol (6ßOHF). The urinary output of 6ßOHF and its ratio to free cortisol (6ßOHF/F) (controlling for variation in endogenous cortisol production) reflect the activities of the isoenzymes CYP 3A3 and 3A4 [5].

Studies in Caucasian epileptics taking anticonvulsants have revealed induction of paracetamol glucuronidation and/or no increase in U_{cyst} or U_{merc} formation [6–8]. Caucasian patients taking anticonvulsants, such as phenobarbitone, carbamazepine and phenytoin, have elevated levels of 6ßOHF, suggesting induction of CYP 3A3 and CYP 3A4 acitivity [9–16]. However, none of these studies has compared different anticonvulsant drugs.

Oriental populations differ from Caucasians in having a lower frequency of slow oxidation of debrisoquine by CYP 2D6 and a higher frequency of impaired hydroxylation of several drugs (including s-mephenytoin) by CYP 2C19 [17–19]. In view of these ethnic differences, the lack of studies of individual inducing agents and the continuing belief in toxicology circles

that epileptics are more sensitive to paracetamol hepatotoxicity [2, 20], we have investigated if selective cytochrome P450 induction occurs in Chinese epileptic patients taking carbamazepine or phenytoin.

Subjects and methods

Subjects

Chinese epileptic out-patients with a diagnosis of grand mal epilepsy for at least 2 years and taking either carbamazepine or phenytoin were recruited. Their dose of anticonvulsant had been titrated according to clinical response with the aid of plasma level monitoring and had been unchanged for at least one month prior to study. They were on no other medication. Cigarette smokers and regular consumers of alcohol were excluded. Their full blood count, renal function and liver function tests, including γ-glutamyl transpeptidase and glucose, were normal. Controls were healthy non-smokers, non-drinkers taking no regular medication, who were recruited by advertisement. Approval from the Clinical Research Ethics Committee of the Chinese University of Hong Kong was obtained and all subjects gave informed consent.

Study design

Fasting subjects attended at 08.30 h and a dose of 20 mg kg^{-1} paracetamol syrup (Panadol, Sterling Winthrop) was given with 200 ml water, following which the patient remained fasting for 2 h. Morning anticonvulsant doses were omitted and coffee, tea and Chinese tea together with over the counter medicines were avoided for 12 h prior to and during the study. All urine was collected for the following 24 h into chloroform preservative and was stored at −20 °C until analysed.

Analytical methods

Urinary paracetamol and its metabolites were measured by HPLC with ultraviolet detection [21]. The urinary 6ßOHF was measured in duplicate by an ELISA method involving a two-step competitive enzyme immunoassay (Stabiligen, France). Intra-assay coefficients of variation (CV) were 14% and 10% for quality controls of 201 pg ml^{-1} and 793 pg ml^{-1} respectively (n = 17). Inter-assay CV's were 19% and 14% for quality controls of 170 pg ml^{-1} and 730 pg ml^{-1}, respectively (n = 15). The urinary free cortisol was measured in duplicate, after dichloromethane extraction, by an RIA method involving a competitive solid-phase radio-immunoassay (Coat-A-Count Cortisol, Diagnostic Products Corporation, USA). Intra-assay CV's were 5.4% and 5.8% for quality controls of 2.95 ug dl^{-1} and 39.5 ug dl^{-1}, respectively (n = 10), and inter-assay CV's were 5.5% and 7.5% for quality controls of 5.5 ug dl^{-1} and 24.8 ug dl^{-1}, respectively (n = 14). The output of urinary 6ßOHF and its ratio to urinary free cortisol were used as measures of hepatic cortisol metabolism. The results from the patients were compared with a group of healthy Chinese subjects of comparable age and sex not taking any medication.

Statistical methods

Patient and control group data were compared by two way Analysis of Variance (ANOVA) with All Pairwise Multiple Comparisons and using Bonferroni's t-test (Sigma Stat version 1.01, Jandel Scientific Software, San Rafael, California, USA). For comparative purposes, we have presented our results using parametric testing, although comparable differences were found using non-parametric tests.

Results

Six subjects (5F, 23–65 y, mean (SD) 33 (14) y; 45 (7) kg) on carbamazepine (mean dose 517 mg per day, range 300–1000 mg per day) and 6 subjects (3F, 17–40 y, mean (SD) 29 (8) y; 61 (8) kg) on phenytoin (mean dose 227 mg per day, range 100–300 mg per day) were studied. The higher proportion of women in the carbamazepine groups accounts for the lower mean weight (45 kg vs 61 kg. $P < 0.05$) and the lower creatinine clearance (88 ml min^{-1} vs 106 ml min^{-1}, $P < 0.05$), as the latter was not corrected for weight (Table 1). Plasma levels of the anticonvulsant drugs confirmed compliance at the time of the study and were

Table 1 General characteristics of control subjects and patients on carbamazepine and phenytoin. Mean (SD); *NR*, normal range. *NA*, not available

Characteristic	Controls n = 20		Carbamazepine n = 6		Phenytoin n = 6	
Age (y)	31	(11)	33	(14)	29	(8)
Male/Female	7/13		1/5		3/3	
Height (cm)	163	(7)	154	(3)	163	(10)
Weight (kg)	57	(9)	45	(7)	61	(8)*
Urea (mmol l^{-1}) NR 3.4–8.9	6.0	(3.0)	4.1	(1.0)	5.3	(1.3)
Creatinine (μmol l^{-1}) NR 44–107	75	(20)	64	(3)	75	(13)
Albumin (g l^{-1}) NR 36–48	46	(5)	43	(5)	45	(3)
Alanine transferase (IU l^{-1}) NR 0–58	10	(11)	22	(11)	39	(41)
γ Glutamyl transpeptidase (IU l^{-1}) NR 0–84	NA		50	(50)	75	(33)
Creatinine Clearance (ml min^{-1}) NR 80–140	92.3	(34.2)	87.8	(9.5)	106.1	(10.6)*
Plasma drug level (μmol l^{-1}) [Therapeutic range]			31.83 [34–51]	(6.36)	43.67 [40–79]	(23.69)

*$P < 0.05$ compared to carbamazepine group

near or within the recommended therapeutic range. Some patients with anticonvulsant drug levels below the usual therapeutic range had been free from seizures for some months, so it was not considered necessary to increase their dose. Plasma analysis for renal and liver function tests gave normal results, which were comparable between the two treatment groups and control subjects (Table 1). None of the patients or controls was taking oral contraceptives, which may cause an increase in the ratio of the urinary recoveries of glucuronide to sulphate conjugates of paracetamol [22].

The 20 control subjects (13F, 18–63 y, mean (SD) 31 (11) y; 57 (9) kg) had very similar fractional recoveries of paracetamol and its metabolites, and cortisol and 6ßOHF output to those reported in healthy Caucasians [9–16, 23, 24]. There was no significant difference between the three groups in the output of unchanged paracetamol (Table 2, Fig 1). Glucuronide recovery was greater in patients on phenytoin than in controls (70% vs 55%, $P < 0.05$) and tended to be greater in patients on carbamazepine. The fractional recovery of the sulphate metabolite was correspondingly reduced in patients on phenytoin compared to controls (23% vs 31%) and tended to be lower in those on carbamazepine.

Although there were no significant differences between the carbamazepine group and the controls in the output of the cysteine and mercapturic acid conjugates, the proportion of paracetamol undergoing metabolic activation was significantly reduced by about 50% in the phenytoin group.

There was no significant difference in daily free cortisol excretion between the three groups. However, in both anticonvulsant-treated groups, we found a significantly higher daily 6ßOHF output (1094 ug per day and 719 ug per day for carbamazepine and phenytoin, respectively) and 6ßOHF/F ratio (17.2 and 12.5 for carbamazepine and phenytoin respectively) compared to the controls, with a tendency to higher values in the carbamazepine group (Table 2, Fig 1).

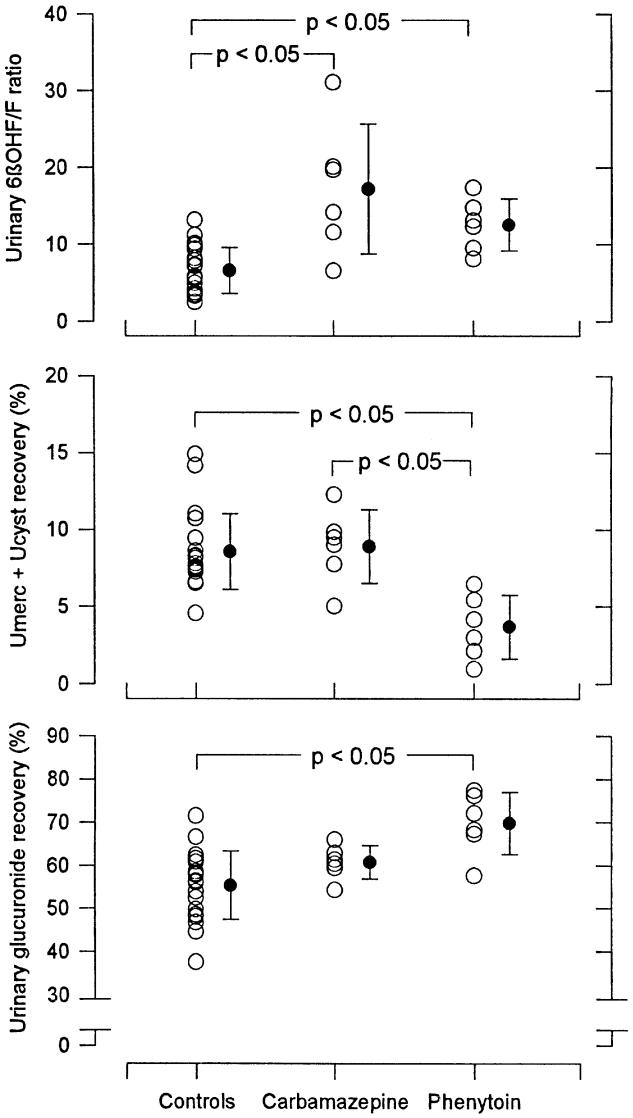

Fig. 1 Urinary recovery of percentage glucuronide, percentage U_{merc} + U_{cyst} and absolute 6ß-hydroxycortisol/free cortisol ratio in the three groups. Data shown are individual values with mean (SD)

Table 2 Mean 24 h urinary recoveries of metabolites of paracetamol and cortisol in the controls and patients on anticonvulsants. Mean (SD)

Urinary Metabolites	Controls $n = 20$	Carbamazepine $n = 6$	Phenytoin $n = 6$
1. Paracetamol Metabolites (% of total)			
– Glucuronide	55.4 (7.9)	60.7 (3.6)	69.8 (6.6)*
– Sulphate	30.8 (7.5)	24.6 (3.2)	22.9 (5.2)*
– Paracetamol (unchanged)	5.30 (2.08)	5.74 (1.69)	3.31 (0.78)
– Mercapturic acid (U_{merc})	4.10 (1.32)	3.49 (0.70)	1.79 (0.70)* **
– Cysteine (U_{cyst})	4.46 (1.32)	5.39 (1.93)	2.22 (0.90)* **
– U_{merc} + U_{cys} U_{cyst}	8.56 (2.48)	8.89 (2.20)	4.01 (1.51)* **
2. Cortisol and metabolites (total)			
– 6ßOHF µg·day^{-1}	306.1 (182.9)	1094.4 (586.5)*	718.8 (189.1)*
– Free cortisol µg·day^{-1}	48.4 (24.3)	69.3 (26.5)	59.4 (17.5)
– 6ßOHF/F ratio	6.61 (2.98)	17.2 (8.5)*	12.5 (3.4)*

* $P < 0.01$ (comparison with controls)
** $P < 0.05$ (comprison between patient groups)

Discussion

Chinese patients on either carbamazepine or phenytoin had a significantly higher urinary 6ßOHF daily output and urinary 6ßOHF/F ratio compared to controls, indicating induction of CYP 3A3/4. In contrast, for paracetamol metabolism we found no increased output of Umerc or Ucyst and thus no evidence of CYP 1A2 and CYP 2E1 induction.

A study in Edinburgh involving 15 Caucasians, of whom 12 were taking phenytoin, revealed almost identical changes to those in our Chinese patients, with increased glucuronidation and reduced sulphate conjugation compared to controls [6]. Indeed, although glucuronidation of certain drugs, such as codeine, may be reduced in Chinese people compared to Caucasians, this does not appear to be the case for paracetamol. A previous study in normal Chinese and Caucasian patients showed that recovery of the paracetamol glucuronide conjugate was similar in the two racial groups [23], and our Chinese control group gave a similar value to that. The Edinburgh study also showed a significant reduction in recovery of unchanged paracetamol (2% vs 5% in the controls), but this did not reach significance in our smaller group on phenytoin. These reductions in unchanged paracetamol output probably reflect the reduced areas under the plasma concentration-time curves associated with the expected shorter paracetamol half-life in patients with induced glucuronidation. Although the recovery of U_{merc} and U_{cyst} was not different between patients and controls in the Edinburgh study, they were reduced in those on phenytoin but not in those on carbamazepine monotherapy in the Chinese epileptics. This may reflect preferential metabolism of paracetamol to glucuronide conjugates. The study in 6 epileptics (3 on phenytoin, 2 on carbamazepine and one on the combination) by Miners et al. showed increased metabolic clearance of the glutathione-derived conjugates but no significant change in their percentage recovery compared to the control group, whereas both the clearance and percentage recovery of the glucuronide conjugate were increased [7]. Thus, there is no convincing evidence that the metabolic activation of paracetamol is enhanced in Caucasians or Chinese patients taking anticonvulsants, despite evidence of the induction of other enzyme pathways.

Previous studies in patients taking carbamazepine [9, 10] phenobarbitone [11–14], phenytoin [15], or combinations of these [16] have all reported a significant increase in urinary 6ßOHF or its ratio with free cortisol or 17-hydroxycorticosteroid compared to pretreatment or control groups. The magnitude of the increase varied from 3–9 fold, although differences in the ratio used and the type or combination of anticonvulsants taken (usually combined as a single group), makes between-study comparisons difficult. Furthermore, no study has attempted to compare the induction produced by the different anticonvulsant drugs. We found that carbamazepine and phenytoin tended to have differential effects on 6ßOHF and glucuronide formation. Thus, although carbamazepine appeared to be a stronger inducer of 6ß-hydroxylation, phenytoin seemed to be the more potent inducer of glucuronide conjugation. However, larger studies are required to reach definite conclusions. Despite evidence of CYP3A4 induction by anticonvulsants in our Chinese epileptic patients, we found no increase in the recovery of the glutathione-derived metabolites after paracetamol ingestion. This may be explained by the relatively small contribution (of the order of 1–20%) of CYP3A4 to the generation of these metabolites, as shown by Thummel et. al. [4].

It is possible that this pathway becomes more important when paracetamol is taken in overdose. There have been isolated case reports of severe or fatal liver damage in patients taking carbamazepine [25] or phenytoin [26] when the plasma paracetamol level appeared to be below the toxic range. In a retrospective review of patients presenting with fulminant hepatic failure, 18 patients on long-term anticonvulsant therapy, including 2 taking sodium valproate, an enzyme inhibitor, had a worse prognosis than other patients from the same unit [27]. However, this does not necessarily imply a greater susceptibility to paracetamol hepatotoxicity in patients on anticonvulsants, but may represent acceleration of the development of hepatic necrosis once toxicity has developed, as has been demonstrated in animal studies [28].

We conclude there was no evidence with either anticonvulsant drug for the significant induction of the isoenzymes responsible for the metabolic activation and potential hepatotoxicity of paracetamol given in therapeutic doses. The data do not support the suggestion that the toxicity of paracetamol in overdosage will be enhanced in patients taking anticonvulsants and we do not consider that the indications for N-acetylcysteine treatment in Chinese epileptics following paracetamol overdosage should be different from those for non-epileptic Chinese or Caucasians [1, 2].

Acknowledgements We thank Ms A. Wong and Ms K. Wong for their assistance with this study.

References

1. Prescott LF, Critchley JAJH (1983) The treatment of acetaminophen poisoning. Ann Rev Pharmacol Toxicol 23:87–101
2. Prescott LF, Critchley JAJH (1983) Drug interaction as a factor in analgesic toxicity. Am J Med 75:113–116
3. Raucy JL, Lasker JM, Lieber CS, Black M (1989) Acetominophen activation by human liver cytochromes P450IIE1 and P450IA2. Arch Biochem Biophys 271:270–283
4. Thummel KE, Lee CA, Kunze KL, Nelson SD, Slattery JT (1993) Oxidation of acetaminophen to N-acetyl-p-aminobenzoquinone imine by human CYP3A4. Biochem Pharmacol 45:1563–1569
5. Ged C, Rouillon JM, Pichard L, Combalbert J, Bressot N, Bories P, Michel H, Beaune P, Maurel P (1989) The increase

in urinary excretion of 6ß-hydroxycortisol as a marker of human hepatic cytochrome P450IIIA induction. Br J Clin Pharmacol 28:373–387
6. Prescott LF, Critchley JAJH, Balali-Mood M, Pentland B (1981) Effects of microsomal enzyme induction on paracetamol metabolism in man. Br J Clin Pharmacol 12:149–153
7. Miners JO, Attwood J, Birkett DJ (1984) Determinants of acetominophen metabolism: effect of inducers and inhibitors of drug metabolism on acetominophen's metabolic pathway. Clin Pharmacol Ther 35:480–486
8. Perucca E, Richens A (1979) Paracetamol disposition in normal subjects and patients treated with anti-epileptic drugs. Br J Clin Pharmacol 7:201–206
9. Moreland TA, Park BK, Rylance GW (1982) Microsomal enzymes induction in children: the influence of carbamazepine treatment on antipyrine kinetics, 6ß-hydroxycortisol excretion and plasma g-glutamyltranspeptidase activity. Br J Clin Pharmacol 14:861–865
10. Roots I, Holbe E, Hovermann W, Nigan S, Heinemyer G, Hildebrandt AG (1979) Quantitative determination by HPLC of urinary 6ß-hydroxycortisol, an indicator of enzyme induction by rifampicin and antiepileptic drugs. Eur J Clin Pharmacol 16:63–71
11. Desager JP, Dumont E, Harvengt C (1987) The urinary 6ß-hydroxycortisol excretion in man on inducers and inhibitors of the hepatic mixed function oxidase. Pharmacol Ther 33: 197–199
12. Saenger P (1983) 6ß-hydroxycortisol in random urine samples as an indicator of enzyme induction. Clin Pharmacol Ther 34:818–821
13. Ohnhaus EE, Park BK (1979) Measurement of urinary 6ß-hydroxycortisol excretion as an in vivo parameter in the clinical assessment of the microsomal enzyme-inducing capacity of antipyrine, phenobarbitone and rifampicin. Eur J Clin Pharmacol 15:139–145
14. Ohnhaus EE, Breckenridge AM, Park BK (1989) Urinary excretion of 6ß-hydroxycortisol and the time course measurement of enzyme induction in man. Eur J Clin Pharmacol 36:39–46
15. Saenger P, Forster E, Kream J (1981) 6ß-Hydroxycortisol: A non-invasive indicator of enzyme induction. J Clin Endocrinol Metab 52:381–384
16. Zhiri A, Wellman-Bednawska M, Siest G (1986) Elisa of 6ß-hydroxycortisol in human urine: diurnal variations and effects of anti-epileptic therapy. Clinica Chimica Acta 157:267–276
17. Lou YC, Liu Y, Bertilsson L, Sjoqvist F (1987) Low frequency of slow debrisoquine hydroxylation in a native Chinese population. Lancet ii:852–853
18. Wood AJJ, Zhou HH (1991) Ethnic differences in drug disposition and responsiveness. Clin Pharmacokinet 20:350–371
19. Ingelman-Sundberg M, Johansson I, Persson I, Yue QY, Dahl ML, Bertilsson L, Sjoqvist F (1992) Genetic polymorphism of cytochrome P450: Interethnic differences and relationship to incidence of lung cancer. Pharmacogenetics 2:264–271
20. Proudfoot AT (1993) Acute poisoning: diagnosis and management. 2nd ed. Butterworth-Heinnemann, Oxford p 175
21. Lau GSN, Critchley JAJH (1994) The estimation of paracetamol and its major metabolites in both plasma and urine by the same high performance liquid chromatography assay. J Pharma Bio Anal 12:1563–1572
22. Critchley JAJH, Nimmo GR, Woolhouse N, Prescott LF (1983) Ethnic differences in paracetamol metabolism – a comparative study in Scotland and Ghana. Br J Pharmacol 80:488P
23. Critchley JAJH, Nimmo GR, Gregson CA, Woolhouse NM, Prescott LF (1986) Intersubject and ethnic differences in paracetamol metabolism. Br J Clin Pharmacol 22:649–657
24. Osborne NJ, Tonkin AL, Miners JO (1991) Interethnic differences in drug glucuronidation: A comparison of paracetamol metabolism in Caucasians and Chinese. Br J Clin Pharmacol 32:765–767
25. Smith JA, Hine ID, Beck P, Routledge PA (1986) Paracetamol toxicity: is enzyme induction important? Hum Toxicol 5:383–385
26. Minton NA, Henry JA, Frankel RJ (1988) Fatal paracetamol poisoning in an epileptic. Hum Toxicol 7:33–34
27. Bray GP, Harrison PM, O'Grady JG, Tredger JM, Williams R (1992) Long-term anticonvulsant therapy worsens outcome in paracetamol-induced fulminant hepatic failure. Hum Exp Toxicol 11:265–270
28. Poulsen HE, Lerche A, Pedersen NT (1985) Phenobarbital induction does not potentiate hepatotoxicity but accelerates liver cell necrosis from acetaminophen overdose in the rat. Pharmacology 30:100–108