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Carbamazepine-hypersensitivity: assessment of clinical and *in vitro* chemical cross-reactivity with phenytoin and oxcarbazepine

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1 Seven patients clinically diagnosed as being hypersensitive to carbamazepine and one patient hypersensitive to both carbamazepine and oxcarbazepine have been identified. They have been compared with a control group (hereafter referred to as 'control subjects') comprising five patients on chronic carbamazepine therapy without adverse effects and 12 healthy volunteers who have never been exposed to anticonvulsants.

2 An *in vitro* cytotoxicity assay employing mononuclear leucocytes as target cells has been used first, to determine the ability of 10 different human livers to bioactivate carbamazepine to a cytotoxic metabolite, and secondly, to compare the cell defences of carbamazepine-hypersensitive patients and control subjects to oxidative drug metabolites generated by a murine microsomal system, using a blinded protocol.

3 With human liver microsomes, the metabolism-dependent cytotoxicity of carbamazepine increased with increasing microsomal protein concentration. At a protein concentration of 2 mg per incubation, the cytotoxicity of carbamazepine with human liver microsomes ($n = 10$ livers) increased from 7.2 ± 0.8% (baseline) to 16.4 ± 2.1% (with NADPH; $P = 0.002$).

4 In the presence of phenobarbitone-induced mouse microsomes and NADPH, the mean increase in cytotoxicity above the baseline with carbamazepine was significantly greater ($P < 0.001$) for the cells from the carbamazepine-hypersensitive patients (7.9 ± 0.8%) than from control subjects (2.6 ± 0.3%).

5 In the presence of phenobarbitone-induced mouse microsomes and NADPH, there was no significant difference in cytotoxicity between the cells from carbamazepine hypersensitive patients and from control subjects in the presence of either phenytoin or oxcarbazepine. However, one patient who was clinically hypersensitive to both carbamazepine and oxcarbazepine also exhibited marked chemical sensitivity to both compounds in the *in vitro* assay.

6 The chemical specificity of the test system was investigated by using synthetic metabolites of drugs unrelated to anticonvulsants: dapsone hydroxylamine and amodiaquine quinoneimine. Cytotoxicity with these two compounds was similar for hypersensitive patients and control subjects.

7 The results obtained suggest that predisposition to carbamazepine hypersensitivity reflects a reduced ability to detoxify epoxide metabolites, although enzyme induction by increasing the formation of reactive metabolites, may be a contributory risk factor.

Keywords toxicity microsomal metabolism carbamazepine phenytoin oxcarbazepine

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Introduction

Carbamazepine (CBZ) is widely used in the treatment of epilepsy, neuralgic pain syndromes and more recently, affective disorders.

It has been estimated that between 33% and 50% of patients on CBZ develop side effects (Pellock, 1987). Most of the side effects are mild and dose-dependent (type A reactions). Cutaneous reactions to CBZ are amongst the commonest adverse effects, estimated to occur in 3–16% of patients (Chadwick *et al.*, 1984; Crill, 1973). It has been suggested that the milder eruptions, which are relatively common, may be related to the initial CBZ dosage and, thus the serum concentrations (Chadwick *et al.*, 1984). The more severe reactions, such as Steven-Johnson syndrome, however, are independent of drug dosage and unpredictable from the known pharmacology of the drug (type B or idiosyncratic reactions; Shear *et al.*, 1988). These are relatively rare (estimated incidence 1:5000–10000) and may be accompanied by hypersensitivity manifestations such as fever, arthralgia and eosinophilia. Other idiosyncratic reactions reported with CBZ include aplastic anaemia (Gerson *et al.*, 1983), agranulocytosis (Pellock, 1987), hepatitis (Horowitz *et al.*, 1988) and pneumonitis (Stephan *et al.*, 1978)—these may occur either alone or in various combinations.

The pathogenesis of idiosyncratic reactions associated with CBZ is not known, but is thought to result from the formation (by the cytochrome P450 enzymes) of toxic, chemically reactive epoxides (Riley *et al.*, 1989; Shear *et al.*, 1988); such epoxides may act as haptens and lead to secondary immune reactions (Jerina & Daly, 1974; Park, 1986).

Shear *et al.* (1988) have shown that patients who have had hypersensitivity reactions to aromatic anticonvulsants (phenytoin, carbamazepine and phenobarbitone) can be distinguished from controls by exposing their respective lymphocytes *in vitro* to oxidative drug metabolites generated by a murine hepatic microsomal system. It has also been postulated that such patients may have a heritable deficiency in cellular detoxification processes (Shear *et al.*, 1988; Spielberg, 1984). Further, a high incidence of cross-reactivity (80%) between these three compounds was reported using the *in vitro* test system, suggesting a common type of reactive metabolite is responsible for the hypersensitivity reactions.

In the present study, we have identified seven patients clinically diagnosed as being hypersensitive to CBZ and one patient hypersensitive to both CBZ and oxcarbazepine (OXCZ) and investigated them using a similar *in vitro* test system (Riley *et al.*, 1988) to that employed by Spielberg and co-workers (Shear *et al.*, 1988; Spielberg *et al.*, 1981) in order to assess their *in vitro* sensitivity to CBZ and chemically related (phenytoin and oxcarbazepine) and unrelated (dapsone hydroxylamine and amodiaquine quinoneimine) compounds. In addition, to investigate the importance of the balance between activation and detoxication in predisposing an individual to idiosyncratic drug toxicity, the ability of 10 human livers to bioactivate CBZ to a cytotoxic metabolite, has been undertaken.

Methods

Carbamazepine-hypersensitive patients

The study was performed in eight patients (mean age 39 years): seven were hypersensitive to CBZ, while one patient (patient 8) was sensitive to both CBZ and OXCZ. Their clinical features are summarised in Table 1. Two patients (patients 3 and 4) had positive rechallenges to CBZ: patient 3 was deliberately rechallenged because of poor control of epilepsy and developed a rash and fever within 24 h. Patient 4 was schizophrenic with epilepsy who was inadvertently rechallenged on two occasions by separate general practitioners, both times requiring hospitalisation with a widespread erythematous rash and fever.

Patient 8 was prescribed CBZ for phantom limb pain and within 4 weeks developed an erythematous rash. This resolved after the CBZ was withdrawn. Subsequently, OXCZ was started several weeks later for the same indication and the patient re-developed a rash within a few days.

All patients (except patients 7 and 8) required hospitalisation during the acute phase and had relevant investigations to exclude other causes. In all patients, withdrawal of CBZ (and OXCZ in patient 8) resulted in clinical improvement.

Control subjects used for comparison with the hypersensitive patients

Two groups of controls (mean age 32 years; range 21–74; 3 females, 14 males) were used for experiments with the anticonvulsants: the first group ($n = 5$) were patients on chronic CBZ therapy without adverse effects. The second group ($n = 12$) were normal, healthy volunteers never exposed to any anticonvulsants. There was no difference in the *in vitro* responses of these two control groups to any of the compounds tested. Thus, the two control groups will hereafter be referred to as 'control subjects'.

Only normal healthy volunteers were used as controls for experiments with DDS-NOH ($n = 22$; mean age 26 years) and AQQI ($n = 9$; mean age 27 years). These are also referred to as control subjects.

All patients and control subjects gave their informed consent. The study was approved by the local ethics committee.

Subjects used for experiments with human liver microsomes

Mononuclear leucocytes isolated from normal, healthy male volunteers ($n = 7$, mean age 28 years) were used for experiments with human liver microsomes. All volunteers gave their informed consent.

Chemicals

5,5-diphenyl hydantoin (phenytoin), carbamazepine, 1,1,1,-trichloro-2-propane oxide (TCPO) and human serum albumin (HSA, fraction V) were obtained from

Table 1 A summary of the clinical features of hypersensitive patients. Patients 1–7 were hypersensitive to carbamazepine only, while patient 8 was hypersensitive to both carbamazepine and oxcarbazepine

Patient number	*Sex*	*Present age (years)*	*Age at time of reaction (years)*	*Time to onset of reaction*	*Rechallenge*	*Major manifestation*	*Other manifestation*	*Present treatment*
1	Male	18	18	3 weeks	–	Exfoliative dermatitis	Eosinophilia fever	Phenytoin
2	Male	16	8	5 weeks	–	Rash pneumonitis	Eosinophilia fever splenomegaly	Phenytoin Valproate
3	Female	21	13	6 weeks	Yes	Rash Hepatosplenomegaly	Fever eosinophilia lymphadenopathy	Phenytoin Primidone
4	Male	46	42	4 weeks	Yes	Rash	Fever	Valproate
5	Female	46	45	1 week	–	Steven-Johnson Syndrome	Fever Abnormal liver function tests	–
6	Female	78	78	4 weeks	–	Steven-Johnson Syndrome	Fever Abnormal liver function tests	Phenytoin Valproate
7	Male	16	11	2 weeks	–	Rash	Fever	Valproate Phenytoin Phenobarbitone
8	Female	76	75	4 weeks	Exposure to oxcarbazepine	Rash	Fever	Valproate

Sigma Chemical Co. (Poole, Dorset, UK). Oxcarbazepine was a gift from Ciba-Geigy Pharmaceuticals (Horsham, Surrey, UK). Reduced nicotinamide adenine dinucleotide phosphate (NADPH; tetrasodium salt) was obtained from BDH Chemicals Ltd (Poole, Dorset, UK). All solvents were of h.p.l.c. grade and were products of Fisons plc (Loughborough, Leics., UK).

Dapsone hydroxylamine (DDS-NOH) was synthesised using the method of Uetrecht *et al.* (1988). Amodiaquine quinoneimine (AQQI) was synthesised and characterised as described by Maggs *et al.* (1988).

Preparation of murine and human liver microsomes

Microsomes were prepared by the method of Purba *et al.* (1987) from histologically normal livers obtained from 10 kidney transplant donors (age range 6–66 years), ethical approval and informed consent from donors' relatives having been obtained, as previously indicated (Riley *et al.*, 1988). The livers from donors had been frozen within 1 h of removal and stored at −80° C until required for use. Microsomes were also prepared from 6–8 phenobarbitone pre-treated mice (60 mg kg^{-1} body weight day^{-1}, i.p. in 0.9% w/v saline for 3 days) by the same centrifugation procedure used to obtain washed human liver microsomes. The microsomes were stored at −80° C until use. Induction was confirmed by the measurement of the cytochrome P450 content by the method of Omura & Sato (1964) and microsomal protein content was determined by the method of Lowry *et al.* (1951).

Isolation of human mononuclear leucocytes

Peripheral blood mononuclear leucocytes (MNL) were isolated from fresh heparinised venous blood from patients and control subjects as described by Riley *et al.* (1988). The overall yield from 50 ml of blood ranged from 35×10^6 to 85×10^6 cells depending on the individual. Their viability upon isolation, as determined by trypan blue dye exclusion, was > 95%.

Comparative evaluation of direct and metabolism-dependent cytotoxicity in the hypersensitive patients and control subjects

MNL are used for this assay because they contain enzymes responsible for drug detoxification, including epoxide hydrolase and are easily available and isolated (Spielberg, 1984).

Four of the CBZ-hypersensitive patients and 8 control subjects were tested twice (at intervals of weeks to months). The rest of the patients and controls were tested once. The mean coefficient of variation for intra patient reproducibility of the test was 27%. Figures of 22% (Farrell *et al.*, 1985) and 23% (Larrey *et al.*, 1989) have previously been reported.

Whenever a patient was studied, two control subjects were tested simultaneously. In addition, a blind protocol was adopted such that none of the investigators knew which were patient or control subject lymphocytes.

MNL (1×10^6) in HEPES-buffered medium (1.0 ml; pH 7.4; Spielberg, 1980) were incubated under air with CBZ (50 μM), DPH (150 μM) or OXCZ (50 μM) and 0.5 mg microsomal protein in the presence or absence of NADPH (1 mM) for 2 h at 37° C. The concentrations of the drugs used were not directly toxic to the cells. The total incubation volume was 1 ml. The drugs were added in 10 μl h.p.l.c.-grade methanol, which, as a 1% solution (v/v) was non-toxic. Incubation of cells with methanol in the absence of drug provided the baseline cell death values.

In some experiments, the MNL were pretreated with the epoxide hydrolase inhibitor, TCPO (30 μM for 10 min), to characterise further the cytotoxic metabolite. This concentration of TCPO has previously been shown not to alter cell viability (Riley *et al.*, 1989).

To determine the direct toxicity of AQQI and DDS-NOH, 10 μM and 100 μM, respectively, of these compounds were incubated with 1×10^6 MNL for 2 h at 37° C in the absence of microsomes (final incubation volume 1 ml). AQQI and DDS-NOH were added in 10 μl methanol and acetone, respectively, which as 1% solutions (v/v), were non-toxic.

After 2 h, the cells were sedimented and resuspended in a drug-free medium (HEPES-buffered medium containing 5 mg ml^{-1} HSA). Incubations were continued for 16 h at 37° C. Cytotoxicity was assessed by trypan blue dye exclusion following the 16 h incubation (Riley *et al.*, 1988). The results are expressed as percentage of cells not excluding trypan blue.

Determination of metabolism-dependent cytotoxicity of carbamazepine with human liver microsomes

The method used to determine the bioactivation of CBZ (50 μM) by human liver microsomes was the same as that used for bioactivation with phenobarbitone-induced mouse microsomes (outlined above), except that a range of protein concentrations (0.5 mg–2 mg/incubation) were used with five human livers, and 2 mg protein/incubation was used for the other five human livers.

Statistical analysis

Each assay was set up in quadruplicate and the mean percentage of dead MNL was determined. The results are expressed as mean ± s.e. mean. Statistical analysis has been performed by one way analysis of variance, accepting $P < 0.05$ as significant. To analyse the difference between the hypersensitive-patient and control groups, the increase in cell death above the baseline, i.e. cell death in the presence of drug and the full metabolising system minus cell death in the absence of drug, was determined; the difference was then analysed by one way analysis of variance.

Results

Bioactivation of carbamazepine to a cytotoxic metabolite by human liver microsomes

In order to determine the optimum concentration of human liver microsomes required to bioactivate CBZ

(50 μM) in initial experiments with five human livers, a range of protein concentrations (0.5 mg, 1.0 mg, 1.5 mg and 2 mg per incubation) were used. This showed that the bioactivation of CBZ increased with increasing protein concentration (Figure 1), reaching statistical significance at protein concentrations of 1.0 mg ($P < 0.05$), 1.5 mg ($P < 0.005$) and 2.0 mg ($P = 0.005$) per incubation. Preincubation of cells with TCPO (30 μM) resulted in a non-significant increase in cytotoxicity at all protein concentrations (Figure 1).

At a protein concentration of 2 mg per incubation, CBZ was bioactivated to a cytotoxic metabolite by 9 of the 10 human livers tested (data not shown), the mean cell death value (for the 10 samples) increasing from 7.2 ± 0.8% (baseline) to 16.4 ± 2.1% in the presence of NADPH ($P = 0.002$).

Comparison of the metabolism-dependent cytotoxicity of carbamazepine, phenytoin and oxcarbazepine in hypersensitive patients and control subjects

The baseline cell death values, i.e. in the absence of drug, for the CBZ-hypersensitive patients and control subjects were 7.0 ± 0.6% ($n = 8$) and 5.7 ± 0.5% ($n = 17$) respectively.

Although a significant increase in cell death (in the presence of microsomes and NADPH) was seen with the three anticonvulsants in both the hypersensitive patients and control subjects, the mean increase in cytotoxicity above the baseline in the presence of CBZ was significantly ($P < 0.001$) greater in the carbamazepine-hypersensitive patients than in the control subjects (Figure 2). In contrast, with both DPH (Figure 3) and OXCZ (Figure 4), there was no significant difference in the cell death values of the two groups, with overlap in the individual values. Patient 8 who was hypersensitive to both CBZ and OXCZ had high cell death values with both these drugs (Figures 2 and 4) compared with the controls, while the cell death with phenytoin (Figure 3) was the same as that with control subjects.

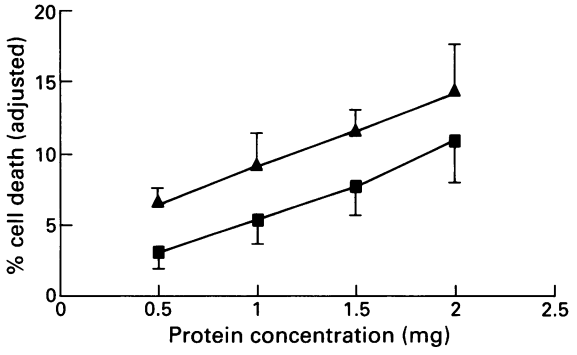

Figure 1 The effect of increasing the protein concentration of human liver microsomes on cytotoxicity of carbamazepine (50 μM) in the presence of NADPH (■), and after pre-incubation with trichloropropane oxide (▲; TCPO). The results are represented as increase in cell death above the baseline and represent the mean ± s.e. mean of 5 experiments with different human livers (incubations in quadruplicate).

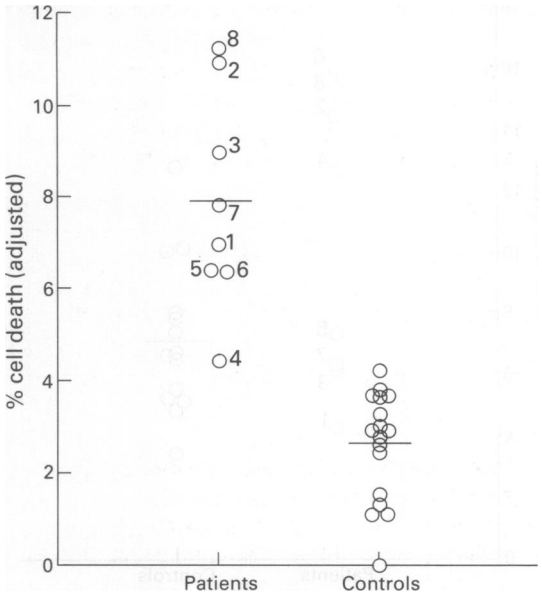

Figure 2 A comparison of the effect of carbamazepine in the presence of microsomes and NADPH in carbamazepine-hypersensitive patients and control subjects. The results for each individual are expressed as the increase in cell death above the baseline, i.e. cell death in the presence of carbamazepine, microsomes and NADPH minus cell death in the absence of carbamazepine. The patients are individually numbered as listed in Table 1. The horizontal bars indicate mean values.

With CBZ (50 μM), preincubation with TCPO, increased metabolism-dependent cytotoxicity in control subjects but not in patients (Table 2). TCPO increased the cytotoxicity of DPH (150 μM) in both groups but had no effect on the metabolism-dependent cytotoxicity of OXCZ (50 μM; Table 2).

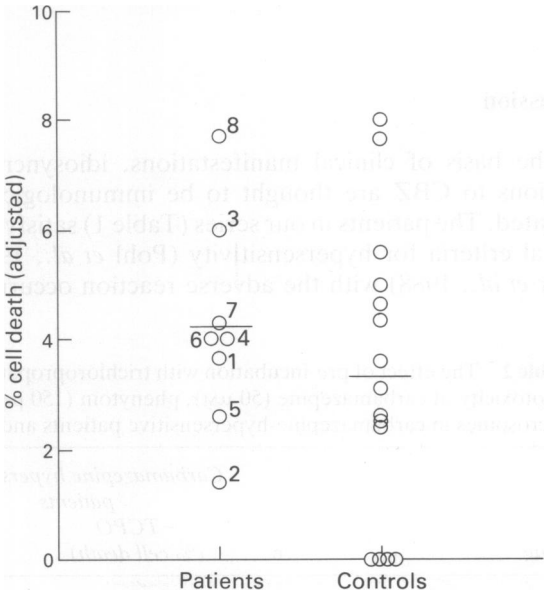

Figure 3 A comparison of the effect of metabolism-dependent phenytoin cytotoxicity in carbamazepine-hypersensitive patients and control subjects. The results are again expressed as increase in cell death above the baseline. The horizontal bars indicate mean values.

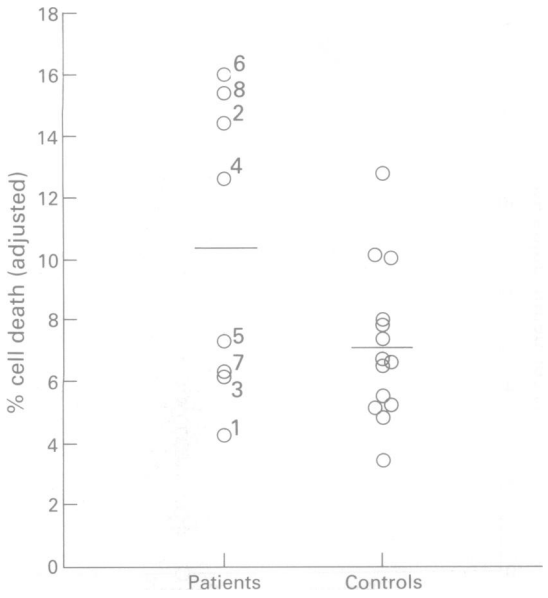

Figure 4 A comparison of the effect of metabolism-dependent oxcarbazepine cytotoxicity in carbamazepine hypersensitive patients and controls. The results are expressed as increase in the cell death above the baseline. The horizontal bars indicate the mean values.

Comparison of the direct cytotoxicity of dapsone hydroxylamine and amodiaquine quinoneimine in the hypersensitive patients and control subjects

Incubation of MNL from CBZ-hypersensitive patients and control subjects with DDS-NOH (100 μM) and AQQI (10 μM) resulted in a significant increase in cytotoxicity above the baseline in both patients ($P < 0.005$ and $P < 0.01$ for DDS-NOH and AQQI, respectively) and controls ($P < 0.001$ and $P < 0.005$ for DDS-NOH and AQQI, respectively; Figure 5). However, there was no significant difference in the cell sensitivity of patients and controls to DDS-NOH and AQQI.

Discussion

On the basis of clinical manifestations, idiosyncratic reactions to CBZ are thought to be immunologically mediated. The patients in our series (Table 1) satisfy the clinical criteria for hypersensitivity (Pohl *et al.*, 1988; Shear *et al.*, 1988) with the adverse reaction occurring after a mean of 25 days (range 8–42 days) and associated with a fever and rash in all patients. In addition, two of our patients had positive rechallenges to CBZ, with the reaction typically occurring much sooner on the second and subsequent re-exposures.

The unique susceptibility of certain patients to developing such idiosyncratic reactions may be due to a critical imbalance between the activation of CBZ to its postulated reactive epoxide metabolite and its detoxification. It is known that multiple epoxides are formed from CBZ (Lertratanangkoon & Horning, 1982), although only the 10,11-epoxide has been isolated, but this is not the metabolite responsible for toxicity (Riley *et al.*, 1989; Shear *et al.*, 1988). Consistent with the hypothesis that the toxic metabolite of CBZ is an epoxide, its toxicity was increased with TCPO (Riley *et al.*, 1989), an epoxide hydrolase inhibitor. Consequently, it has been suggested that a deficiency of the enzyme epoxide hydrolase (Gerson *et al.*, 1983; Riley *et al.*, 1989; Shear *et al.*, 1988) would lead to inadequate detoxification of the toxic metabolite, and hence the adverse reaction.

The *in vitro* cytotoxicity assay untilised in this study (Riley *et al.*, 1988) has been used to determine the ability of different human livers to bioactivate CBZ, and by using cells from CBZ-hypersensitive patients assess cell defence mechanisms against toxic metabolites formed by bioactivation. In the presence of either phenobarbitone-induced mouse microsomes or human liver microsomes, the cytotoxicity of CBZ required a full metabolising system, consistent with the hypothesis that the toxicity is metabolite-mediated. To assess the differences in cell defence mechanisms between CBZ-hypersensitive patients and control subjects, phenobarbitone-induced mouse microsomes, in line with previous studies (Riley *et al.*, 1989; Shear *et al.*, 1988), were used to generate the toxic metabolite(s). Thus, cells from the CBZ-hypersensitive patients were significantly more susceptible to the cytotoxic metabolite than the cells from control subjects (Figure 2)—this is in accordance with the findings of Shear *et al.* (1988). The *in vitro* susceptibility to the CBZ metabolite was independent of the time of the adverse drug reaction, as only two patients were tested acutely, while the rest were all investigated retrospectively. Preincubation with TCPO significantly ($P < 0.05$) increased cytotoxicity in the control subjects but had no effect in the hypersensitive patient group (Table 2)—this may reflect a lack of inhibitable enzyme in the

Table 2 The effect of pre-incubation with trichloropropane oxide (TCPO) on the metabolism (NADPH)-dependent lymphocyte cytotoxicity of carbamazepine (50 μM), phenytoin (150 μM) and oxcarbazepine (50 μM) with phenobarbitone-induced mouse microsomes in carbamazepine-hypersensitive patients and control subjects

	Carbamazepine hypersensitive patients			*Control subjects*		
Drug	n	*−TCPO (% cell death)*	*+TCPO (% cell death)*	n	*−TCPO (% cell death)*	*+TCPO (% cell death)*
Carbamazepine	8	14.8 ± 1.0	15.5 ± 2.0	16	8.2 ± 0.5	10.8 ± 0.8*
Phenytoin	8	10.4 ± 1.1	15.5 ± 1.6*	10	9.5 ± 0.7	12.6 ± 1.2*
Oxcarbazepine	8	17.6 ± 1.7	16.3 ± 2.8	13	12.7 ± 1.0	12.7 ± 1.2

The results are expressed as mean ± s.e. mean (performed in quadruplicate). Statistical analysis has been performed within the same group comparing incubations with and without TCPO (trichloropropane oxide): *$P < 0.05$.

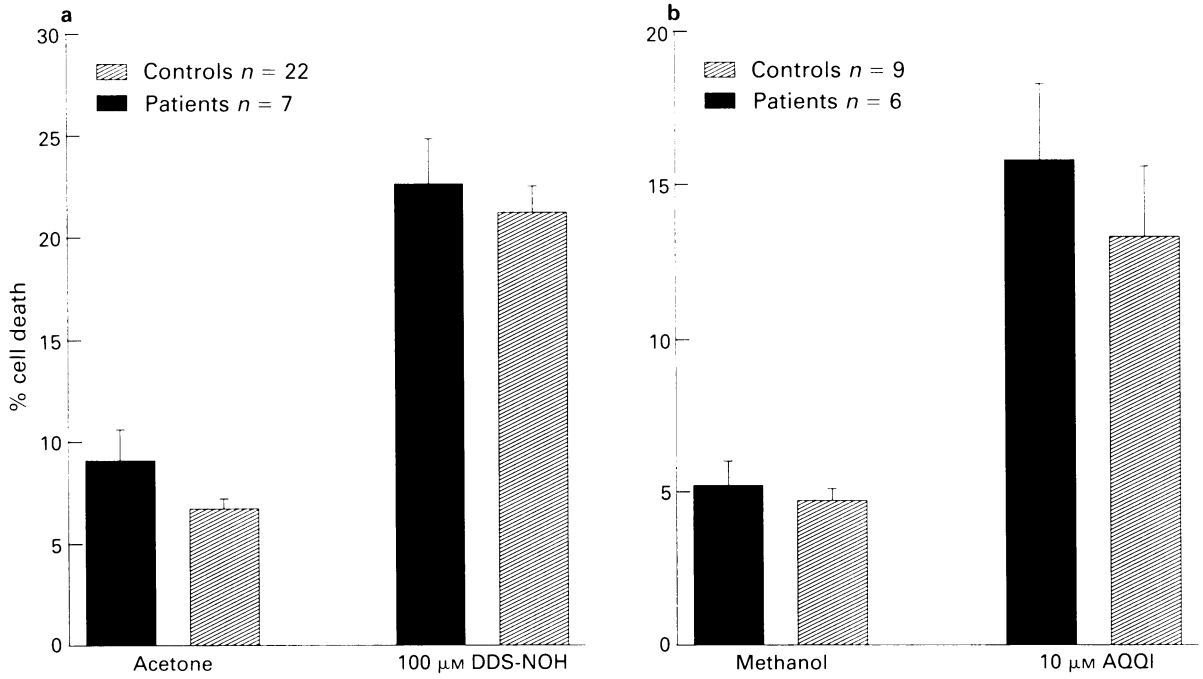

Figure 5 Direct cytotoxicity of a) dapsone hydroxylamine (100 μM) and b) amodiaquine quinoneimine (10 μM) in carbamazepine-hypersensitive patients and control subjects.

latter group. The importance of detoxification processes (or lack of them) in predisposing individuals to CBZ toxicity is further emphasised by the experiments with the human livers, which showed that they were capable of bioactivating CBZ to cytotoxic metabolites. In addition, enzyme induction by CBZ which would occur with continuous therapy, may enhance the conversion of CBZ to the toxic metabolite, thus further exacerbating the imbalance between activation and detoxification in predisposed individuals.

Phenytoin (DPH) which has a similar side-effect profile to CBZ (Pellock, 1987), may have a similar biochemical basis for its toxicity (Gerson *et al.*, 1983; Spielberg *et al.*, 1981). Clinical cross-sensitivity between CBZ and DPH has been reported (Gerson *et al.*, 1983; Reents *et al.*, 1989), although the exact incidence is unknown. Five of our CBZ-hypersensitive patients were on chronic DPH therapy without adverse effects and one patient (patient 4) had previously been treated with DPH without any problems. Exposure of these patient cells to DPH *in vitro* in the presence of microsomes and NADPH resulted in cell death cell values comparable to those of the control subjects (Figure 3); thus, the lack of chemical cross-reactivity *in vitro* corresponds to the lack of clinical cross-reactivity seen in our group of CBZ-hypersensitive patients. The lack of *in vitro* chemical cross-reactivity between phenytoin and CBZ in our group of eight patients contrasts with the findings of Shear *et al.* (1988), who reported an 80% incidence of *in vitro* chemical cross-reactivity between the aromatic anticonvulsants. The variability in the incidence of clinical cross-reactivity and, *in vitro* chemical cross-reactivity, between different groups of patients may be a reflection of the variation in the enzyme structure which could occur as a result of genetic heterogeneity of the epoxide hydrolase gene. Genetic heterogeneity, which has been demonstrated for adverse drug reactions associated with the debrisoquine/sparteine oxidation polymorphism (Brosen, 1990), could in theory, lead to clinical heterogeneity among anticonvulsant hypersensitive patients with subsets of patients being hypersensitive to one or more of the aromatic anticonvulsants.

Oxcarbazepine, a keto-analogue of CBZ, is widely used in Scandinavia. It is of comparable efficacy to CBZ (Reinikainen *et al.*, 1987) but is said to cause fewer allergic reactions than CBZ (Dam *et al.*, 1989). A clinical study in CBZ-hypersensitive patients showed that only about a quarter of these patients cross-reacted with OXCZ (Jenson *et al.*, 1986). In our study, exposure of MNL from CBZ-hypersensitive patients and control subjects to OXCZ (and its oxidative metabolites) *in vitro* did not result in a significant difference in the mean values of the two groups (Figure 4), although significant cytotoxicity was observed with cells from both groups. However, patient 8 who was sensitive to both CBZ and OXCZ had high cell death values to both these compounds (Figures 2 and 4).

It has been suggested by Shear *et al.* (1988) that *in vitro* cytotoxicity assays could be used prospectively to individualise drug therapy. Although the results obtained with CBZ in the present study are consistent with this hypothesis, the difference in cell death between the hypersensitive patient group and control subjects is small, suggesting that the test system does lack sensitivity. In addition, there is a great deal of variability in the cell death values to OXCZ in our CBZ-hypersensitive patients making it very difficult to predict which patients might cross-react with OXCZ. We therefore, feel that a more sensitive and direct measure of epoxide hydrolase deficiency, possibly involving genotyping, is required, not only to predict clinical

sensitivity to a drug but also to exclude cross-reactivity between related drugs.

To investigate further the specificity of the *in vitro* test system, we challenged MNL from CBZ-hypersensitive patients to toxic drug metabolites (DDS-NOH and AQQI) which were structurally and functionally unrelated to anticonvulsants, and more importantly, are not detoxified by the epoxide hydrolase enzymes. DDS-NOH is thought to be responsible for many of the adverse effects associated with chronic dapsone therapy (Coleman *et al.*, 1989; Uetrecht *et al.*, 1988), while AQQI has been implicated in causing amodiaquine-induced hepatotoxicity and agranulocytosis (Maggs *et al.*, 1988). Although there was an increase in cytotoxicity above the baseline with both compounds, there was no significant difference in the sensitivity of the patient and the control groups (Figure 4), reinforcing not only the chemical specificity of this *in vitro* test system, but also the unique sensitivity of the CBZ-hypersensitive patient cells.

In summary, the *in vitro* cytotoxicity assay which has been described shows a high degree of chemical specificity and does help in differentiating CBZ-hypersensitive patients and control subjects (healthy volunteers and patients on chronic CBZ therapy without adverse effects). Although it could be used for retrospective diagnosis of hypersensitive patients, and thus help define the chemical mechanism of drug toxicity, its lack of sensitivity will limit its use as a predictive test for drug hypersensitivity. All subjects may be capable of bioactivating CBZ to a toxic metabolite, and the critical factor in predisposing patients to hypersensitivity may be a deficiency in cellular detoxification, namely a deficiency of epoxide hydrolase. Further research is, therefore, required to establish the biochemical and genetic basis, so that patients who are particularly sensitive to serious adverse drug reactions to anticonvulsants can be easily identified.

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