

Induction of CYP1A2 activity by carbamazepine in children using the caffeine breath test

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Aims Carbamazepine is a known enzyme inducer. The aim of this study was to determine whether carbamazepine induces the metabolism of caffeine in children.

Methods Children due to receive carbamazepine for epilepsy were recruited into the study. The caffeine breath test was carried out prior to the administration of carbamazepine and after a minimum of 2–3 weeks therapy. Five children were studied and they received 200–600 mg carbamazepine daily.

Results The mean values of the 2 h cumulative labelled carbon dioxide were 3.47% before and 7.65% during carbamazepine. There was a significant increase in the percentage labelled caffeine exhaled as carbon dioxide during the administration of carbamazepine (Student's paired *t*-test, $P < 0.05$).

Conclusions The results suggest that carbamazepine induces the metabolism of caffeine by the CYP1A2 pathway in the children studied.

Keywords: caffeine, carbamazepine, children, caffeine breath test, enzyme induction, CYP1A2

Introduction

Carbamazepine has been shown to have an enzyme inducing effect on the metabolism of several drugs [1–3]. We decided to study the drug interaction between caffeine and carbamazepine in children. Studies of drug interactions usually require the collection of blood or urine samples, neither of which is easy in children.

We, and others, have used the caffeine breath test to study both drug interactions and the effect of disease on drug metabolism [4–7]. The test involves the use of a non-radioactive stable isotope (^{13}C on the 3-methyl group of caffeine). The caffeine is given orally and undergoes 3-*N*-demethylation which is a cytochrome P450 dependent reaction (CYP1A2). After *N*-demethylation the labelled methyl group enters the one carbon pool as it is converted to formaldehyde, formate, bicarbonate and then exhaled as carbon dioxide. We studied the effect of carbamazepine on caffeine metabolism in children utilising the caffeine breath test.

Methods

Plan of study

The study was approved by the local Ethics Committee. Informed consent was obtained from the children and their parents, prior to commencement into the study. Five children (aged 6–17 years) were studied both prior to and 2 to 3 weeks after commencing carbamazepine. All the children were patients with epilepsy at Alder Hey Children's Hospital and were prescribed carbamazepine as monotherapy.

Caffeine breath test procedure

All subjects abstained from caffeinated products for 20 h and fasted for at least 4 h prior to the caffeine breath test. All breath tests were commenced between 09.00 and 10.00 h. Carbamazepine was administered during the 2 h prior to commencement of the test. The subjects remained seated for 10 min prior to the collection of the first breath sample. They were kept occupied by the Research Nurse throughout the whole test in order to minimise physical activity which can affect carbon dioxide production and result in dilution of the labelled carbon dioxide [8]. The labelled caffeine (Cambridge Isotope Laboratories, Massachusetts, USA; chemical purity >98%) was given at a dose of 3 mg kg^{-1} dissolved in water and mixed with sugar-free squash to disguise the bitter caffeine taste. The solution was taken by mouth followed by a 20 ml water rinse of the container.

Breath samples were collected by getting the child to blow into a sample balloon (Alveosampler; Quintron, Menomonee Falls, U.S.A). Samples were collected at –20, –10, –1, 15, 30, 45, 60, 75, 90, 105 and 120 min after ingestion of caffeine. Expired air (10 ml) was removed from the balloon and transferred by syringe into a headspace analyser vial (Chromacol; London, U.K.) that had been pre-evacuated. Samples were unaffected by 8 weeks of storage in these vessels. The samples were sent by registered mail to The Scottish Universities Research and Reactor Centre for analysis.

Analytical methods

^{13}C enrichment of breath carbon dioxide was determined by continuous flow isotope ratio mass spectrometry [9]. Breath samples (10 ml) were injected automatically into the

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Table 1 Clinical details of patients and cumulative 2 h CO₂ output.

Patient	Sex	Age (years)	Height (cm)	Weight (kg)	Daily dose of carbamazepine (mg)	% of caffeine dose recovered before (B) carbamazepine therapy	% of caffeine dose recovered during (A) carbamazepine therapy	Change after carbamazepine (A-B)
53	M	6	120	20	200	1.86	3.12	1.26
55	M	7	118	21	300	3.67	8.85	5.18
54	F	10	146	39	400	1.49	10.02	8.53
56	M	10	137	30	400	4.63	9.64	5.01
57	M	17	176	67	600	5.70	6.60	0.90
Mean \pm s.d.						3.47 \pm 1.79	7.65 \pm 2.86*	4.18

*Significantly different from before carbamazepine, $P < 0.05$.

gas preparation device (Roboprep-G; Europa Scientific Ltd, Crewe, U.K.) where they were in turn, dried, resolved from interfering components by gas chromatography and passed, using helium as carrier, into the electron impact ion source of an isotope ratio mass spectrometer (Tracermass; Europa Scientific Ltd). The ion beams m/z 44, m/z 45 and m/z 46 were monitored continuously and used to calculate the partial pressure and [¹³C] enrichment of carbon dioxide, with reference to a 2% CO₂/98% N₂ gas supply which had been calibrated against a bicarbonate standard of known [¹³C] enrichment. Duplicates of each breath sample were analysed.

Calculations and statistical analysis

The [¹³C] enrichment of exhaled carbon dioxide was converted from delta units to atom % using the accepted atom fraction of the international bicarbonate standard [10]. [¹³C] enrichment was expressed as atom % [¹³C] excess, by subtracting the average pre-dose enrichment from each post-dose measurement. Cumulative ¹³CO₂ output was calculated from the measured [¹³C] enrichment of the eight breath samples taken during the first 2 h following administration of the [¹³C] caffeine dose and multiplying this by the average output of CO₂ over this period (assumed to be 24 mmol CO₂ kg⁻¹ body weight; [11]). This was expressed as a percentage of the caffeine dose.

There was no significant change in CO₂ partial pressure either in breath samples analysed during the breath test for each patient or in the group overall. The mean (\pm s.d.) CO₂ partial pressure was 3.06 (\pm 0.52)%.

The precision of a [¹³C] enrichment, derived from the average of 40 replicated breath analyses, was 0.00104 atom % ¹³C excess. This equates to 1.11% recovered of a typical caffeine dose.

Student's paired *t*-test was used to compare the data before and during carbamazepine. A sample size of 10 patients has a power of 90%, assuming a difference of 3 in the mean score (of the 2 h percentage dose recovered) to be significant at the 5% level [7]. The study was terminated when interim analysis of the data showed a significant effect.

Results

Table 1 shows the labelled cumulative 2 h CO₂ output for each patient both before and during carbamazepine expressed

as a percentage of the oral caffeine dose administered. The mean values of the caffeine dose exhaled as carbon dioxide in 2 h were 3.47% before and 7.65% during carbamazepine. There was a significant increase in the percentage labelled caffeine exhaled as carbon dioxide during the administration of carbamazepine (Student's paired *t*-test $P < 0.05$).

Discussion

Carbamazepine has an enzyme inducing effect on many different drugs including cyclosporin, warfarin, oral contraceptives, doxycycline, tricyclic antidepressants and anti-fungals such as itraconazole, as well as an inducing effect on its own metabolism [1–3]. The metabolism of cyclosporin, oral contraceptives and carbamazepine involve the CYP3A4 pathway. The induction of this pathway has been confirmed in human hepatocyte cultures [12].

The effect of carbamazepine on CYP1A2 activity has not been extensively studied. A previous study suggested that carbamazepine does not have an inducing effect on caffeine metabolism [13]. This study estimated caffeine clearance rather than partial metabolic clearance by CYP1A2, but as CYP1A2 is the major pathway of caffeine metabolism, [14] significant induction of CYP1A2 should have resulted in an increase in clearance. This study, however, used only two time points to determine the plasma clearance and half-life of caffeine and, therefore, its conclusions are open to question. Caffeine is accepted as the current gold standard for the assessment of CYP1A2 activity [14, 15].

The caffeine breath test and, in particular, the 2 h cumulative exhalation of labelled carbon dioxide is a measure of 3-*N*-demethylation which is catalysed by CYP1A2. The use of the 2 h cumulative exhalation of labelled carbon dioxide as an appropriate measure in the caffeine breath test has been well established in both adults and children [16, 17]. Our findings suggest that carbamazepine has an inducing effect on CYP1A2 as well as CYP3A4. This may result in clinically significant drug interactions involving drugs such as theophylline, imipramine and propranolol which undergo metabolism by CYP1A2 [15].

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