Br J clin Pharmac 1994; **38**: 573–576

Inhibition of caffeine metabolism by ciprofloxacin in children with cystic fibrosis as measured by the caffeine breath test

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The caffeine breath test was carried out in six children with cystic fibrosis, before and during a course of ciprofloxacin. There was a significant decrease in the 2 h cumulative labelled CO_2 exhaled during ciprofloxacin treatment, mean difference (s.d.) −5.2(3.3)%, $P < 0.02$. The results suggest an inhibition of 3-*N*-demethylation of caffeine (CYP1A2 enzyme activity) by ciprofloxacin. Ciprofloxacin may cause significant drug interactions in children with cystic fibrosis. The caffeine breath test can be used to study drug interactions involving CYP1A2 in children.

Keywords caffeine breath test children ciprofloxacin cystic fibrosis

Introduction

Ciprofloxacin has been shown to have an inhibitory effect on the metabolism of several drugs including theophylline and diazepam [1–3]. Theophylline has a narrow therapeutic index and inhibition of metabolism may result in toxicity. Patients with cystic fibrosis often receive bronchodilators and ciprofloxacin is often used for chest infections in these patients as it has antipseudomonal activity. We therefore decided to study this potentially dangerous drug interaction in children with cystic fibrosis. Studies of drug interactions usually require the collection of blood or urine samples. The collection of these samples is more difficult in children, especially in those with chronic illnesses who often require venepuncture for clinical reasons.

The caffeine breath test has been used to study both drug interactions and the effect of disease on drug metabolism [4, 5]. The test involves 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 have studied the effect of ciprofloxacin on caffeine metabolism in children with cystic fibrosis utilising the caffeine breath test.

Methods

Plan of study

Informed consent was obtained from the parents and, where appropriate, the child, and the study was approved by the local Ethics Committee. Six children (aged 4–15 years) were studied both before and 10 days after commencing a 14 day course of ciprofloxacin. All were patients attending the Regional Cystic Fibrosis Clinic at Alder Hey Children's Hospital and were receiving flucloxacillin, vitamin supplements and pancreatic enzymes. Clinical details, and the dosage of ciprofloxacin are shown in Table 1. The children were all receiving ciprofloxacin for a chest infection with radiological changes and a high suspicion of *Pseudomonas aeruginosa* as the causative organism. *Pseudomonas* was grown from sputum in five of the six children. The severity of the cystic fibrosis is illustrated by the respiratory function and the nutritional status which are shown in Table 1. Other medications received included inhaled budesonide (patients 1 and 6), colomycin (patients 4, 5, 6), tobramycin (patient 1) and salbutamol (patient 2).

Caffeine breath test procedure

All subjects abstained from caffeinated products for 20 h and fasted for at least 4 h prior to the caffeine

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This paper is dedicated to the memory of Björn Lindström, friend, scientist and colleague, who died suddenly last year.

Table 1 Clinical details of patients and cumulative 2 h CO_2 output

Patient	*Age (years)*	*Sex*	*Height (cm) Centile*	*Weight (kg) Centile*	*FEV_1 % Predicted FEV_1*	*Caffeine dose (mg)*	*Ciprofloxacin dose (mg kg^{-1} day^{-1})*	*%Caffeine dose before ciprofloxacin (B)*	*% Caffeine dose after 10 days ciprofloxacin (B)*	*B % of A*
1	4	M	103 20th	15.6 10th	83%	47	16.02	6.17	3.77	61.10
2	7	M	125 50th	22.15 20th	78%	66	22.57	12.23	1.96	16.03
3	12	F	159 80th	43.5 50th	80%	130	22.99	10.53	3.64	34.57
4	10	F	130 3rd	28.2 20th	85%	85	26.58	6.93	4.82	69.55
5	9	F	132 25th	27 25th	76%	81	27.78	8.36	5.46	65.31
6	15	F	162 60th	53.05 60th	86%	159	28.27	12.01	5.38	44.80
Mean ± sd	9.5	—	— —	— —	81	—	24.03	9.37 ± 2.60	4.17 ± 1.33	48.6
95% confidence intervals on differences									1.77–8.62	

breath test. All breath tests were commenced between 09.00 and 10.00 h. Ciprofloxacin 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 then remained seated throughout the whole test and were kept occupied by the Research Nurse in order to minimise physical activity which can affect carbon dioxide production and result in dilution of the labelled carbon dioxide [6]. The labelled caffeine 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. 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

The ^{13}C-enrichment of breath carbon dioxide was determined by continuous flow isotope ratio mass spectrometry [7]. Breath samples (5 ml) were injected manually into the on-line gas preparation device (Roboprep; 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 ion source of an isotope ratio mass spectrometer (MM602; VG Isotech, Middlewich, U.K.). The ion beams m/z 44 and m/z 45 were monitored constantly and used to calculate the partial pressure and ^{13}C-enrichment of carbon dioxide, with reference to a 10% CO_2/90% N_2 gas standard. Four replicates of each breath sample taken before, and duplicate samples taken after the caffeine dose, were analysed.

Calculations and statistical analysis

The enrichment of exhaled carbon dioxide was calculated from:

$$\text{Atom \% } ^{13}C = 100/(R + 1)$$

Where R = m/z 45/ m/z 44, corrected for ^{17}O after Craig [8]. ^{13}C-enrichment (Atom % Excess ^{13}C) was calculated by subtracting the natural abundance measured in samples taken before the caffeine dose. The ^{13}C natural abundance was 1.1048 atom %. Cumulative $^{13}CO_2$ output was calculated by averaging the measured ^{13}C-enrichment of the 8 breath samples taken during the first 2 h following administration of the [^{13}C]-caffeine dose, and summing with the carbon dioxide output calculated assuming an average output of 24 mmol CO_2 kg^{-1} body weight over this period [9]. This was expressed as a percentage of the caffeine dose. There was no significant change in CO_2 partial pressure either in breath sample analysis during the breath test for each patient or in the group overall (six subjects, eight samples each). The mean ± s.d. CO_2 partial pressure was 2.29 ± 0.64% prior to and 2.16 ± 0.32% during ciprofloxacin therapy. The coefficient of variation for the assay was 1.09% (n = 12).

Student's t-test was used to compare the data before and during ciprofloxacin.

Results

The results show a statistically significant decrease in the percentage labelled caffeine exhaled as carbon dioxide during the administration of ciprofloxacin (Student's t-test $P < 0.02$, 95% confidence intervals on difference 1.77–8.62%). Table 1 shows the labelled cumulative 2 h CO_2 output for each patient both before and during ciprofloxacin, expressed as a percentage of the oral caffeine dose administered. 9.37% of the caffeine dose was exhaled as CO_2 in 2 h before ciprofloxacin, and 4.17% during ciprofloxacin. Figure 1 shows the mean data of the six patients

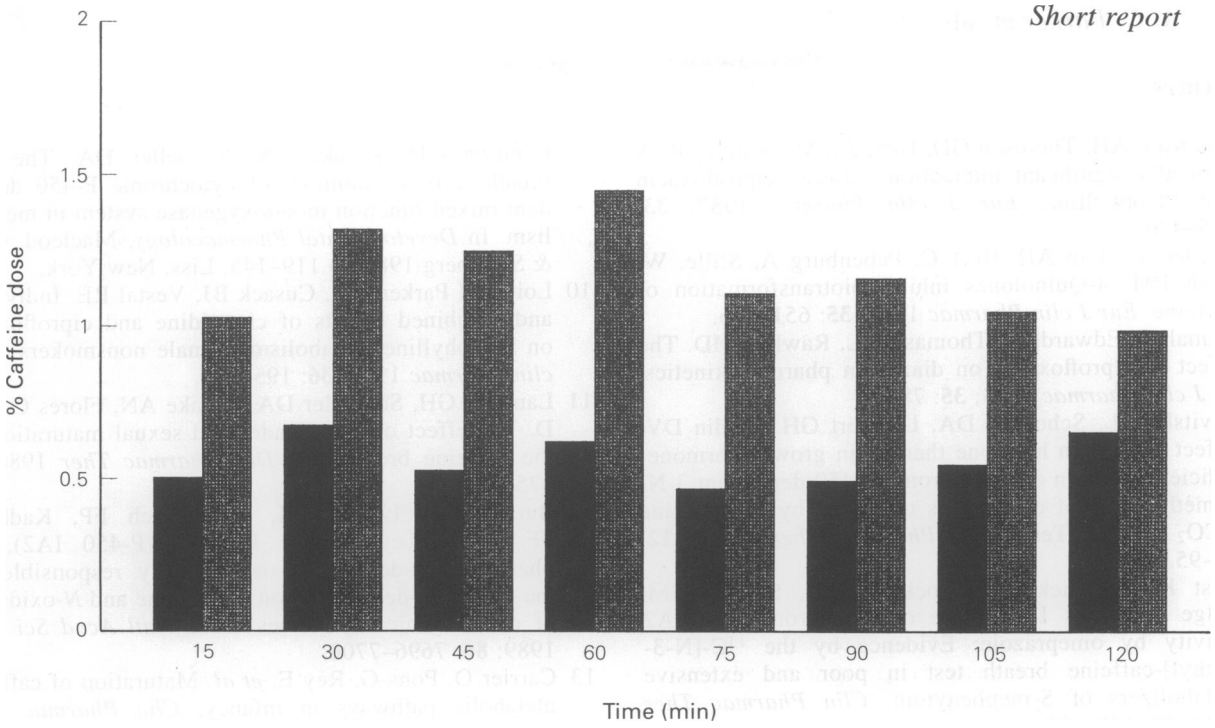

Figure 1 Mean % caffeine dose exhaled as labelled carbon dioxide in 15 min intervals after oral administration of 3 mg kg^{-1} caffeine to six children with cystic fibrosis before ciprofloxacin (▨) and during coadministration of ciprofloxacin (■).

studied at each 15 min time point and the percentage of the caffeine dose exhaled in the preceding 15 min. There was a decrease in the labelled carbon dioxide exhaled in all six patients and the extent of decrease ranged from 30–84%. The mean reduction was 49%. The cumulative carbon dioxide exhaled over a 2 h period is dependent on the 3-*N*-demethylation of caffeine and decrease in this activity suggests inhibition of CYP1A2.

Discussion

There are previous reports of an inhibitory effect of ciprofloxacin on the metabolism of caffeine, theophylline and diazepam in healthy volunteers [2, 3, 10]. A single case report in an adult patient suggested that ciprofloxacin inhibited the clearance of theophylline [1]. Our results show that ciprofloxacin inhibits the metabolism of caffeine in children with cystic fibrosis. Caffeine and theophylline are both xanthines and *N*-demethylation is a major pathway in the metabolism of both drugs. We would recommend strongly that plasma theophylline concentrations are monitored closely in children with cystic fibrosis who are due to receive ciprofloxacin. Further studies are required to determine whether the dose of theophylline should be lowered prior to the administration of ciprofloxacin.

The caffeine breath test was first used in children by Lambert *et al.* [11]. They showed that the time to peak rate of labelled carbon dioxide exhalation occurred in the first 2 h after administration of caffeine. It has been shown that the 2 h cumulative exhalation of labelled carbon dioxide reflects 3-*N*-demethylation and that this is a cytochrome P450 dependent reaction (CYP1A2) [12]. The use of the 2 h cumulative exhalation of labelled carbon dioxide as the appropriate measurement in the caffeine breath test has been well established in both adults and children [11, 12].

The same group subsequently showed that untreated growth hormone deficient children have enhanced 3-*N*-demethylation of caffeine [4]. Subsequent treatment with growth hormone decreased the 3-*N*-demethylation of caffeine. Other studies using urinary caffeine metabolite ratios [13] and the caffeine breath test [14], have shown that 3-*N*-demethylation of caffeine is low in infants under the age of 6 months. Therefore, studies of drug interactions on CYP1A2 activity should exclude infants in whom the enzyme is not fully developed, i.e. under the age of 6 months.

The advantages of the caffeine breath test, particularly when used in children, is that it is non-invasive. The caffeine breath test has not been used in children with lung disease. Children with cystic fibrosis have increased resting metabolic rates which result in increased carbon dioxide production. This will result in dilution of the labelled ^{13}C from the caffeine. By using subjects as their own controls we have shown that it is possible to study patients, with respiratory problems, with the caffeine breath test. Further studies are needed to determine the effect of respiratory disease on exhalation of carbon dioxide. We have developed a technique which is reproducible in children as young as 4 years of age.

We thank the Sir Jules Thorn Charitable Trust and the University of Liverpool for their financial support. We thank Dale Schoeller and George Lambert for their advice regarding the caffeine breath test. We also thank Gill Croxton for her help in preparing the caffeine and Mrs Alexandra Longworth for typing the manuscript.

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(Received 15 March 1994, accepted 4 August 1994)