Paediatric and Perinatal Drug Therapy, 2002; 5 (1)

The Caffeine Breath Test and CYP1A2 Activity in Children

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Abstract

The caffeine breath test is a non-invasive method of studying CYP1A2 activity that is suitable for children from the ages of 3 years upwards. It involves the oral administration of a stable isotope and the collection of breath samples for a period of 2 hours. Breath samples are analysed by continuous flow isotope ratio mass spectrometry. It has been used to study both drug interactions and the effect of disease on drug metabolism.

Paed Perinatal Drug Ther 2002; 5: 28–33

Key words: Caffeine breath test – drug metabolism

Introduction

Studies of drug metabolism in children are more difficult than studies in adults. For ethical reasons studies of drug metabolism in children are usually restricted to those children who are due to receive the drug in question for therapeutic purposes. Exceptions include the use of low dose probes with low toxicity e.g. caffeine, dextromethorphan[1]. This contrasts with the situation in adults where healthy adult volunteers take part in extensive studies of drug metabolism and pharmacokinetics on the basis of financial compensation. Such an approach in healthy children would quite correctly be considered unethical. An additional problem in children is that they are less likely to volunteer to have blood samples collected especially if it involves an additional venepuncture or the collection of a capillary sample.

The enzyme CYP1A2 is responsible for the 3-N demethylation of caffeine[2]. Caffeine has, therefore, been used as a probe to assess CYP1A2 enzyme activity in both adults and children[3]. The collection of blood, saliva and urine following the administration of an oral dose of caffeine have all been used to determine either plasma clearance or metabolite ratios[1,4,5]. These methods have been

used extensively in adults and to a limited extent in children. Children of different ages have however all shown their dislike for the collection of either blood, saliva or urine. Therefore, alternative non-invasive tests have been developed. The caffeine breath test (CBT) allows administration of an oral stable isotope and the collection of breath samples through either straws or balloons which most children find acceptable.

Stable isotopes

A stable isotope is a non-radioactive atom of the same chemical element, which differs only in the number of neutrons[6]. Stable isotopes occur naturally and approximately 1% of carbon occurs in the ^{13}C form as opposed to the more prevalent ^{12}C. Radioactive ^{14}C has previously been used to study drug metabolism in animals and adults. The use of radio-isotopes is impossible for ethical reasons in paediatric patients of all ages and also women of child bearing age.

Caffeine breath test

The test involves the use of a stable isotope of caffeine. The ^{13}C is 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[7].

Development of the caffeine breath test

The initial studies of the CBT were carried out by Dale Schoeller and his group in Chicago, USA in the 1970's[8]. They showed that the precision of the CO_2 breath test using ^{13}C is limited by the natural fluctuations in the ratio of $^{13}C/^{12}C$ in expired CO_2. These natural fluctuations were increased if the subject had anything to eat either immediately before or during the breath test. They made the following recommendations:

1. The subject should fast overnight before the test and during the test. If this was not possible, then the carbohydrate intake should be as low as possible.
2. The patient should not be physically active during the test as this would affect the endogenous CO_2 production.
3. Serial respiratory CO_2 samples should be collected prior to substrate administration to accurately determine the presence of ^{13}C.
4. The collection of CO_2 should be carefully standardised to avoid variation in isotope content due to fractionation effects.

The following year Dale Schoeller and Peter Klein described a simplified technique for the collection of breath samples which they showed to be stable for a period of three months[9].

Dose response

Several years later the same group determined the optimal conditions for performing the CBT[10]. They studied healthy adult volunteers who were smokers and non-smokers. They concluded that the 2 hour cumulative excretion of labelled CO_2 could be used to accurately predict the metabolic clearance rate of caffeine. They demonstrated that the smokers had twice the rate of labelled CO_2 excretion of non-smokers. They studied three different doses of caffeine (1,3 and 5 mg per kg). They found that increasing the dose beyond 3 mg per kg had no effect on CO_2 excretion during the first 2–3 hours of the tests.

The plasma clearance of caffeine was determined by the collection of blood samples at the same time as breath samples in 10 subjects who received 3 mg per kg of caffeine. The optimal correlation between the plasma clearance and the cumulative excretion of labelled CO_2 was at 2 hours ($r = 0.90$). This landmark paper identified the optimal dose of caffeine as 3 mg per kg and the 2 hour collection of breath samples allowing calculation of the cumulative excretion of labelled CO_2.

Initial studies

The first published studies of the CBT in children were by George Lambert, Dale Schoeller and co-workers in 1986[11]. They studied the effect of age, gender and sexual maturation in 62 subjects whose ages ranged from 3–20 years. Enzyme activity was greatest in prepubertal children and subsequently decreased in early puberty in females and late puberty in males.

Gerard Pons and co-workers in France studied the maturation of caffeine N-demethylation in infancy in 12 infants up to the age of 19 months[12]. The infants were all receiving caffeine therapeutically for apnoea. They showed minimal activity in infants in the first month of life. Breath samples were collected via a face mask attached to a 3 litre latex bag. This group showed a significant correlation between the 2 hour cumulative excretion and labelled CO_2 and plasma caffeine clearance in the patients studied ($r = 0.84$). The same group has carried out studies showing that the clearance of caffeine in children reaches adult rates by the age of 6 months[13].

Table 1 Drug interaction studies with the CBT						
Drug	**Age range (years)**	**Number of patients**	**Disease**	**Mean ± sd labelled 2 hour cumulative CO_2 output**		**Student's *t*-test**
				Before therapy	**During therapy**	
Ciprofloxacin	4–15	6	Cystic fibrosis	9.4 ± 2.6	4.2 ± 1.3	$P<0.02$
Cimetidine	2–15	11	Gastritis	5.6 ± 2.4	4.9 ± 3.2	$P>0.2$
Carbamazepine	6–17	5	Epilepsy	3.5 ± 1.8	7.6 ± 2.9	$P<0.05$

A year later the Chicago group carried out an elegant study in six children with growth hormone deficiency[14]. They demonstrated that, following one month of growth hormone therapy, there was a significant decrease in CYP1A2 enzyme activity using the CBT.

Studies in adults

Only a few groups have used the CBT to study aspects of drug metabolism in adults. Initial studies were carried out by the Chicago group. This group has studied the effects of dietary protein on drug metabolism[15]. They have also used the CBT as a marker of exposure to environmental pollutants[16,17].

Other groups in the USA and Canada have used the CBT in relation to studies of the drug tacrine, which is a reversible cholinesterase inhibitor for the treatment of patients with Alzheimer's disease[18]. Although one study has demonstrated a correlation between the clearance of tacrine and the CBT[18], another study showed that the CBT does not identify patients susceptible to tacrine hepatotoxicity[19].

We are only aware of one group outside of the North American continent who have used the CBT in adults. They studied the enzyme inducing effects of omeprazole on CYP1A2 activity[20,21]. There is significant inter-individual variation in CYP1A2 activity[3]. There have been no studies using the CBT to evaluate genetic differences in CYP1A2 activity. A review of the use of caffeine as a probe has confirmed the validity of the CBT as a method of studying CYP1A2 activity[3].

Drug interactions in children

We have used the CBT to study drug interactions in children. The scientific study of drug interactions in adults may involve the collection of a significant number of blood samples[22]. We have used the study design developed by Levitsky and colleagues whereby the CBT is used in children both prior to and during drug therapy. This involves the use of children as their own control. It involves relatively small numbers of children and as it involves children who are due to receive the medicine therapeutically it facilitates recruitment. The effect of age is excluded by only studying children aged at least 2 years. This ensures that full maturation of caffeine metabolism has already been reached.

We have used it to study both the inhibition and the induction of CYP1A2 enzyme activity. The initial study evaluated the effect of ciprofloxacin on CYP1A2 enzyme activity in children with cystic fibrosis [23]. There was a significant fall in labelled carbon dioxide excretion during therapy with ciprofloxacin. The mean ± standard deviation for labelled cumulative 2 hour CO_2 output is shown in Table 1.

A similar study was carried out in children due to commence carbamazepine[24]. This study showed a significant increase in labelled cumulative 2 hour CO_2 output. It is important to note that there is considerable inter-individual variation in CYP1A2 enzyme activity.

Cimetidine is a known enzyme inhibitor. A study of 11 children with presumed gastritis showed no significant inhibitory effect of cimetidine of CYP1A2 enzyme activity using the CBT [25]. Studies in adults have shown that smoking enhances the inhibition of theophylline metabolism by cimetidine[26]. This may have been a contributory factor to the lack of inhibition demonstrated. It is important to note that the CBT can be used to demonstrate no significant drug interaction.

Technique

The technique that we have used both in hospital and at home is as follows:

All subjects abstain from caffeinated products (eg coke and chocolate) for 20 hours and fast for at least four hours prior to the CBT. All breath tests

Figure 1. Child performing the caffeine breath test. The child is being kept occupied throughout the test by a friend in order to minimise physical activity so as not to increase carbon dioxide production.

Figure 2. Child blowing directly into a glass collecting vial via a straw.

are commenced in the morning between 0900 and 1000 hours. The subjects remain seated for 10 minutes prior to the collection of the first breath sample. The research nurse keeps the children occupied throughout the whole test in order to minimise physical activity so as not to increase carbon dioxide production (Figure 1).

The labelled caffeine (Cambridge Isotope Laboratories, Massachusetts, USA) is given at a dose of 3 mg per kg dissolved in water. The solution is taken orally followed by a 20 ml water rinse of the container.

Breath samples are collected by getting the child to blow directly into a glass collecting vial via a straw (Figure 2). Samples are collected at –20, –10, –1, 15, 30, 45, 60, 75, 90, 105 and 120 minutes after ingestion of the caffeine.

Analytical details

^{13}C enrichment of breath carbon dioxide is determined by continuous flow isotope ratio mass spectrometry[27]. Breath samples (10 ml) are injected automatically into the gas preparation device (Roboprep-G, Europa Scientific Ltd, Crewe, UK) where they are dried, resolved from interfering components by gas chromatography and passed using helium as a carrier into the electron ion source of an isotope ratio mass spectrometer (20–20, Europa Scientific Ltd). The ion beams *m/z* 44, *m/z* 45 and *m/z* 46 are monitored continuously and used to calculate the partial pressure and ^{13}C enrichment of carbon dioxide. A mixture of 3% CO_2 and 97% N_2 is used for calibration. This had been calibrated against a ^{13}C standard traceable to primary international standards. Duplicates of each breath samples are analysed. The technique has become more widely used with the advent of urea breath tests for *Helicobacter pylori*. Automated breath ^{13}C analysers are now available making the technique suitable for most laboratories.

The ^{13}C enrichment of exhaled carbon dioxide is converted from delta units to atom % using the accepted atom fraction of the international bicarbonate standard[28]. ^{13}C enrichment is expressed as atom % ^{13}C excess, by subtracting the average pre-dose enrichment from each post-dose measurement. A cumulative $^{13}CO_2$ output is calculated from the measured ^{13}C enrichment of the 8 breath samples taken during the first 2 hours following administration of the ^{13}C caffeine dose and multiplying this by the average output of CO_2 over this period (assumed to be 24 mmol CO_2 per kg body weight) [7]. This is expressed as a percentage of the caffeine dose.

Disease and drug metabolism

We carried out a study in young children (2–6 years) with cystic fibrosis to study the effect of this illness on CYP1A2 enzyme activity [29]. Using age matched controls we found a significant difference in enzyme activity in children with cystic fibrosis in comparison with the controls (Table 2). These findings are consistent with several other studies which have suggested

Table 2 Effect of cystic fibrosis on CYP1A2 enzyme activity				
Disease	**Age range (years)**	**Number of patients**	**Mean ± sd labelled 2 hour cumulative CO_2 output**	**Student's *t*-test**
Cystic fibrosis	2–6	8	7.8 ± 1.9	$P<0.02$
Controls	3–5	9	4.4 ± 2.8	

enhanced hepatic drug clearance in both adults and children with cystic fibrosis[30-32]. This study has shown that despite the inter-individual variation in CYP1A2 enzyme activity the CBT can be used to study the influence of disease on drug metabolism in children.

We are currently using the CBT to study the effects of therapeutic hypothermia and viral infections on drug metabolism in children. We have also carried out pilot studies of the effects of malnutrition on drug metabolism in children using the CBT. The CBT is ideally suited to studies in developing countries as it is non-invasive and the samples are stable at a tropical temperature. The fact that the samples do not require storing in a freezer is of considerable benefit in such countries.

Advantages of the CBT

Breath testing in children is a non-invasive way of collecting clinical samples, which children are happier to provide than blood, saliva or urine samples. It has the added advantage of being suited to the home environment which is familiar to the child.

Children learn to blow bubbles and blow out candles at an early age, usually around 2 or 3 years. This skill can easily be adapted later to games such as blow football, which can also be used to demonstrate how to provide breath samples into a vial via a straw for breath testing.

Children's nurses, who have previously made contact with the families, go to the homes of children to collect breath samples. They have experience in assessing child development and how best to help the child to continue to provide the samples over the 2 hour testing period.

During this period the child can continue playing or studying as usual within their familiar environment. The children's nurse can be prepared with a number of games or activities if needed, or the child could have a friend round to help pass the time. An electronic timer is useful, as it can be set to bleep just before each test. The child can be in control of this so that if they are in another room they know when to return for the next sample. The rest of the family can also continue with normal activities, which would be disrupted if the samples were collected in a hospital or clinic.

The main limitation of the CBT is the cost of the stable isotope. Previously the analysis of the breath samples was difficult and required specialised equipment, but this is now far more readily available.

Conclusion

Studies in both adults and children have shown that the CBT is useful to study both drug interactions and disease on drug metabolism. The test is acceptable to young children. It involves no blood samples and can be carried out at home. The caffeine, although expensive, is readily available and assays for the measurement of breath samples are becoming more readily available.

Acknowledgement

We thank Vanessa Peden, Astra Charnwood Research Nurse for her help in developing the technique for the collection of breath samples. Elizabeth Webster is funded by SPARKS.

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