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Drug metabolism studies in the newborn infant and children using an oral erythromycin breath test

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***Aims*: To develop an oral erythromycin breath test using a stable isotope-labelled form as a marker of CYP3A4 enzyme activity.**

***Methods*: $^{13}C_2$-labelled erythromycin (4 mg/kg) was administered orally to eight children with epilepsy due to commence carbamazepine. The children recruited completed the breath test after a minimum of four weeks therapy with carbamazepine. Breath samples were collected at 20 minute intervals for a period of 4 hours. Eight preterm neonates received 8 mg/kg of double labelled erythromycin solution down a nasogastric tube and had breath samples collected at 20 minute intervals for a period of 6 hours via the ventilator.**

***Results*: The median values of the 4 hour cumulative labelled carbon dioxide were 1.84% before and 3.92% during carbamazepine. There was no significant difference (Wilcoxon rank sum test $P>0.05$). The level of the 6 hour cumulative labelled carbon dioxide in the premature neonate showed considerable inter-individual variation (median value 0.06%, range 0–6.02%).**

***Conclusions*: The oral erythromycin breath test does not appear to be a suitable method of assessing the induction of CYP3A4 enzyme activity.**

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Introduction

Studies of drug metabolism in paediatric patients and neonates in particular are more difficult than studies in adults. We and others have used the caffeine breath test as a non invasive method of studying both drug interactions[1-3] and the effect of age and disease[4-7] on drug metabolism. The caffeine breath test involves the use of caffeine labelled with the non radioactive stable isotope ^{13}C, which is given orally[8,9]. The ^{13}C is on the 3 methyl group of caffeine. The 3 N-demethylation of caffeine in the liver is dependent upon CYP1A2 enzyme activity. 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. This can be collected by getting the children to blow into tubes using a straw. We have previously used the caffeine breath test in children as young as 4 years of age.

The caffeine breath test is an excellent method for studying activity of CYP1A2[10]. A far more important enzyme involved in drug metabolism is CYP3A4. Studies in adults involving an intravenous dose of radioactive erythromycin (^{14}C–N-methyl erythromycin) have shown the value of using erythromycin as a marker for CYP3A4 enzyme activity[11,12]. The aim of this study was to establish if a non radioactive stable isotope-labelled form of erythromycin could be used in an erythromycin breath test (EBT) as a marker of CYP3A4 enzyme activity in both children and neonates. The development of an oral EBT would have the advantage of being able to assess total CYP3A4 enzyme activity, i.e. both hepatic and intestinal.

In particular we wished to compare CYP3A4 enzyme activity in preterm and term infants. We also planned to use the EBT to study the effect of carbamazepine used as an anticonvulsant on CYP3A4 enzyme activity.

Methods

Healthy volunteer studies

Prior to using the EBT in children, we carried out studies in healthy adult volunteers. This involved the use of ^{13}C-erythromycin, both single labelled (^{13}C-N-methyl erythromycin) and double labelled ($^{13}C_2$-N, N-dimethyl erythromycin). It involved the administration of erythromycin lactobionate in a liquid form. The taste, however, was exceptionally bitter and therefore the erythromycin was subsequently administered in a capsule. Breath samples were collected at 15 minute intervals for up to 2 hours initially. Subsequently, they were collected for a period of 4 hours because of the low enrichment of ^{13}C in exhaled carbon dioxide. In order to neutralise the acidity of the stomach and hence minimise degradation of the erythromycin, maalox (magnesium and aluminium hydroxide) was administered 15 minutes prior to the erythromycin and, additionally, at the same time.

The initial dosage was 2 mg/kg labelled erythromycin lactobionate. This, however, resulted in a relatively low ^{13}C/^{12}C ratio. In order to increase this ratio and enhance ^{13}C amounts in exhaled breath, a dose of 4 mg/kg labelled erythromycin lactobionate was used. This was less than the dose used in adults (6–11 mg/kg) by others[13]. The use of a capsule and Maalox to facilitate absorption of the erythromycin was made following advice from the American group studying the use of erythromycin as a marker of CYP3A4 enzyme activity (personal communication from David A Wagner). The 4 hour collection period used in children is one hour longer than that used by the American group[13].

Paediatric patients

Children with epilepsy (aged 6–16 years) who were due to commence carbamazepine were considered for the study. If consent was obtained from the parent and the child, then they were asked to perform the EBT prior to and a minimum of four weeks after commencing therapy with carbamazepine.

Erythromycin breath test procedure

All breath tests were commenced in the morning. Children were allowed a light breakfast only. The child received 4 mg/kg (maximum dose 250 mg) of double labelled erythromycin. This was encapsulated in a gelatine capsule and administered in conjunction with Maalox suspension. 20 ml of Maalox was administered at –15 minutes and 10 ml at the same time as administration of the erythromycin. Thereafter, breath samples were collected at 20 minute intervals for a period of 4 hours. Breath samples were collected by getting the child to blow down a straw into a glass tube. Samples were sent by mail to the Scottish Universities Environmental Research Centre in Glasgow for analysis.

Neonatal study

Neonates who were ventilated were considered for inclusion into the study. If parental consent was given, they then received 8 mg/kg of double labelled erythromycin solution down a nasogastric tube. This was washed down by a 2 ml water rinse to ensure no isotope was left in the nasogastric tube. 10 ml aliquots of breath were collected from the ventilator in the expiratory phase via a manifold in the breathing circuit at –20, –10, –1 and thereafter at 20 minute intervals for 6 hours. A 6 hour collection period was used in neonates as it was anticipated that absorption of the erythromycin may be slower in children than in adults. Additionally, it was expected that biotransformation of erythromycin would be lower and hence the cumulative labelled CO_2 output would also be lower. For this reason a higher dose of 8 mg/kg labelled erythromycin lactobionate was used in neonates.

Analytical methods

[^{13}C] enrichment of breath carbon dioxide was determined by continuous flow isotope ratio mass spectrometry[14]. Breath samples (10 ml) were injected automatically into the gas preparation device (ABCA; Europa Scientific Ltd, Crewe, UK)

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 (20–20; Europa Scientific Ltd; Crewe, UK). The ion beams *m/z* 44, *m/z* 45 and *m/z* 46 were monitored continuously and used to calculate the partial pressure and [^{13}C] enrichment of carbon dioxide, with reference to a 3% CO_2/97% N_2 gas mixture which had been calibrated against a bicarbonate standard of known [^{13}C] enrichment. Duplicates of each breath sample were analysed.

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. ^{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 12 breath samples taken during the first 4 hours following administration of the labelled erythromycin (18 breath samples in 6 hours for neonates) and multiplying this by the average output of CO_2 over this period (assumed to be 24 mmol CO_2 per kg body weight). This is expressed as a percentage of the erythromycin dose. The precision of analysis of baseline ^{13}C abundance is $\pm$ 0.0005 atom % ^{13}C.

Calculations and statistical analysis

Wilcoxon rank sum test was used to compare the data before and during carbamazepine as the data were not normally distributed. We planned to use the same test for comparing data from preterm and term neonates. 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[2].

Ethical approval

The studies in patients were approved by Southern Derbyshire Ethics Committee. The studies in healthy volunteers were approved by Alder Hey Children's Hospital Research Ethics Committee.

Results

Paediatric

Eight children with epilepsy (ages 9–14 years) completed the study and concordance with carbamazepine was noted to be poor in patient 5. Only one of the eight children was receiving other medication during the study. Patient 4 was taking oral loratadine and nasal beclomethasone for hay fever as well as inhaled salbutamol, salmeterol and fluticasone for asthma. She received these medicines on each occasion that she completed the EBT. Baseline levels of ^{13}C in the children ranged from 10,814 to 10,859 ppm. Levels of ^{13}C at individual time points following administration of the labelled erythromycin increased by up to 26.06 ppm. The data for patient 6 while receiving carbamazepine is shown graphically in Figures 1 and 2. Figure 1 shows the individual time points and Figure 2 the cumulative data.

Table 1 shows the labelled cumulative 4 h CO_2 output expressed as a percentage of the oral erythromycin dose administered, both before and during therapy with carbamazepine (cbz).

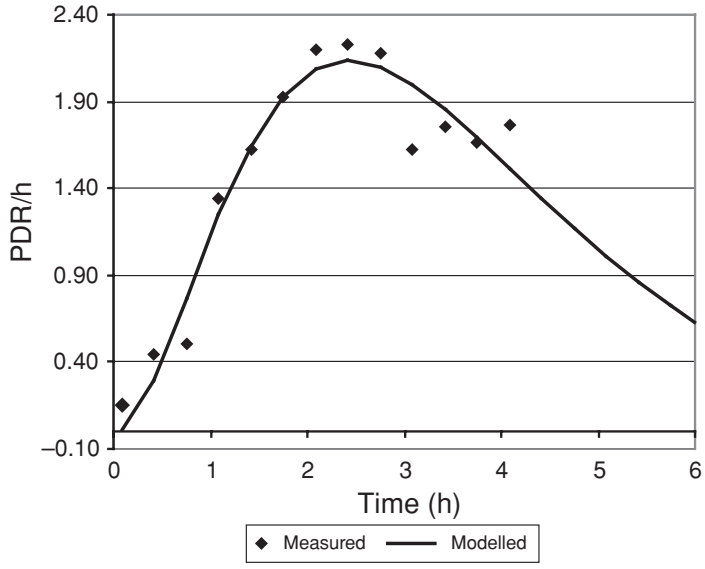

Figure 1 The amounts of labelled ^{13}C detected in the breath samples expressed as a percentage of the dose recovered (PDR) in patient 6 while receiving carbamazepine.

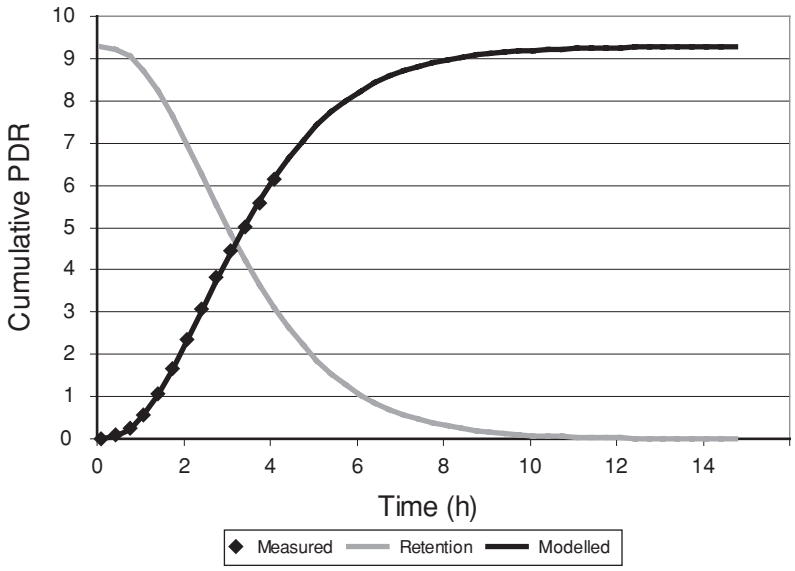

Figure 2 The cumulative percentage dose recovered (PDR) of labelled ^{13}C in breath samples over 4 hours in patient 6.

There was a minimum of four weeks between the two EBTs for each child. The median values were lower (1.84%) before than during (3.92%) carbamazepine therapy, but the differences were not statistically different, Wilcoxon rank sum test $P>0.05$.

Neonatal

Eight preterm infants were recruited for the EBT. Their ages ranged from 4-22 days and their gestation from 25 to 31 weeks. Baseline levels of ^{13}C in the neonates were higher than those in the children and ranged from 10,868 to 10,957 ppm. Levels of ^{13}C at individual time points following administration of the labelled erythromycin increased by up to 8.43 ppm. The labelled cumulative 6 h CO_2 output expressed as a percentage of the nasogastric erythromycin dose administered is shown in Table 2. The levels were not normally distributed, and the median level was 0.06%. The levels were extremely low in 5 of the 8 neonates. They were higher in the two neonates aged more than 7 days and one aged 4 days. Spearman's rank correlation was used to compare age and labelled CO_2 output, r_s 0.42 ($P>0.05$). Unfortunately, no term infants were recruited.

Discussion

Previous studies have suggested that carbamazepine induces CYP3A4 enzyme activity[15]. Our results in children with epilepsy show that the oral erythromycin breath test failed to demonstrate an enzyme inducing effect of carbamazepine on CYP3A4 activity. The individual data suggested that there was a trend to increased activity in some of the children but not in all. There was no obvious explanation for the low levels of labelled CO_2 during carbamazepine therapy in the two individuals where the levels were lower during therapy than prior to treatment.

Previous studies in adults involving an intravenous dose of radioactive erythromycin have suggested that this is a useful marker of CYP3A4 enzyme activity[11,12]. The EBT (involving IV radiolabelled erythromycin) has been thought to be correlated with clearance by some groups whereas other groups have questioned its value[11,12].

Table 1 Clinical trials in children with epilepsy and cumulative 4 h CO_2 output

Patient	Age (years)	Wt (kg)	Dose of cbz (mg BD)		% PDR 4 hours	
					Pre-cbz	During cbz
1	14 F	84.8	400		1.86	5.53
2	11 F	30.0	200		1.83	10.28
3	9 M	31.7	300		4.46	5.53
4	12 F	86.4	500		2.27	0.96
5	13 F	72.6	400		1.15	2.31
6	9 M	37.7	200		2.74	6.15
7	9 M	29.2	160		1.34	0.09
8	13 M	49.3	300		1.64	1.71
				Median	1.84	3.92
				Range	1.34–4.46	0.09– 10.28

Table 2 Clinical details in neonates and cumulative 6 h CO_2 output

Gestation (weeks)	Wt (kg)	Age (days)	% PDR 6 hours
25 M	0.85	9	3.20
30 F	0.90	4	2.86
31 F	1.24	5	0.04
30 M	1.68	6	0.07
31 F	1.29	4	0.05
30 F	1.67	5	0.00
28 F	1.15	22	6.02
29 M	1.53	4	0.10
		Median	0.06

There has only been one previous study using oral erythromycin and the subsequent collection of breath samples[15]. This study in adults involved both the use of a stable oral isotope and intravenous [^{14}C]. This group found that the oral EBT was not suitable as an *in vivo* probe for assessing the induction of CYP3A4 enzyme activity[13]. They had similar results to ours in that the labelled cumulative CO_2 output increased in the majority of individuals following treatment with an enzyme inducer (rifampicin). Several individuals, however, did not show an increase in the labelled CO_2 output. Interestingly, this group found that the oral EBT was suitable as an *in vivo* probe for assessing the inhibition of CYP3A4 enzyme activity by troleandomycin[13].

All carbon contains approximately 1% of the stable isotope of carbon (^{13}C). Well-known small differences in its ambient concentration (natural abundance) occur. For instance, our diet contains components that are derived from tropical grasses (maize and sugar cane) which have a natural abundance of 1.11 atom % ^{13}C and temperate grasses (wheat, barley and rice) have a natural abundance of 1.08 atom % ^{13}C. There are also differences in dietary fat which is ^{13}C depleted with respect to carbohydrate even from the same plant source. These differences mean that one tries to control diet during and immediately prior to the breath test. One also controls physical activity by undertaking tests at rest in order to minimise the likelihood of changes in the fuel being oxidised by the body. We have published a survey of the ^{13}C natural abundance in the British diet to aid the design of ^{13}C breath tests[16]. When designing ^{13}C breath tests with relatively expensive tracers, it is common to choose dosages that on full recovery in breath CO_2 raise the ambient baseline by 0.01 atom % ^{13}C or 100 ppm ^{13}C. The discrimination of this small excess of ^{13}C is well within the capability of modern mass spectrometers. However, should baseline variations be uncontrolled, tracer oxidation be impaired or ^{13}C abundance measurement be compromised by low CO_2 partial pressure in the samples, then one's ability to estimate tracer recovery with accuracy will be compromised. The current test was designed with a generous quantity of tracer to counteract poor recovery due to slow oxidation. Even so, tracer recovery was minimal, especially in the neonates.

Our studies in premature infants showed very low levels of labelled CO_2 being exhaled over a 6 h period in five of the infants studied. Unfortunately we were unable to study a comparative group of ventilated term infants. One therefore cannot be certain that the low level of exhaled CO_2 confirms low enzyme activity as we have been unable to validate the method of collection of exhaled air in term infants. Samples having low CO_2 partial pressure give rise to poorer results due both to poor analytical precision and also to the fact that the sample is likely to represent an admixture of exhaled breath and ambient air. Although CO_2 concentration in air is low, its natural ^{13}C signature is more enriched than exhaled breath. As such, this compromises analytical accuracy and our ability to discern low levels of CYP3A4 enzyme activity in premature infants. Previous studies have suggested that CYP3A4 enzyme activity is very low in the first few days of life[17]. This is compensated for by increased CYP3A7 activity. Our results suggest that this increased CYP3A7 activity does not result in increased biotransformation of erythromycin.

The caffeine breath test has shown itself to be an excellent method for studying CYP1A2 enzyme activity. It has proved useful in studying both drug interactions and the effect of age and disease on drug metabolism[1-7]. We had hoped that the oral EBT would prove to be a suitable way of studying CPY3A4 enzyme activity in both neonates and children. Unfortunately, our findings suggest that the oral EBT is not suitable as a marker of the induction of CYP3A4 enzyme activity. Our findings are similar to those of the other group that has tried to develop an oral EBT[13].

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