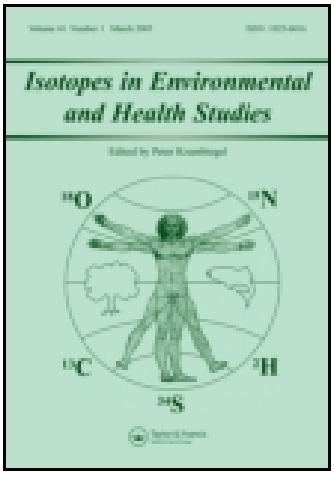

Isotopes in Environmental and Health Studies
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gieh20

[^{13}C]Aminopyrine and [^{13}C]Caffeine Breath Test: Influence of Gender, Cigarette Smoking and Oral Contraceptives Intake

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Published online: 04 Jan 2007.

To cite this article: M. S. Caubet , A. Laplante , J. Caillé & J. L. Brazier (2002) [^{13}C]Aminopyrine and [^{13}C]Caffeine Breath Test: Influence of Gender, Cigarette Smoking and Oral Contraceptives Intake, Isotopes in Environmental and Health Studies, 38:2, 71-77, DOI: 10.1080/10256010208033314

To link to this article: http://dx.doi.org/10.1080/10256010208033314

Isotopes Environ. Health Stud., 2002, Vol. 38(2), pp. 71–77

Taylor & Francis
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[^{13}C]AMINOPYRINE AND [^{13}C]CAFFEINE BREATH TEST: INFLUENCE OF GENDER, CIGARETTE SMOKING AND ORAL CONTRACEPTIVES INTAKE

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(Received 22 June 2001; In final form 7 November 2001)

The [^{13}C]aminopyrine breath test ([^{13}C]ABT) measures the global activity of cytochrome P450 *in vivo* and is a sensitive indicator of liver metabolic dysfunction. The present study aims to determine whether gender and cigarette smoking influence the results of [^{13}C]ABT as well as to confirm the effect of oral contraceptive steroids (OCS) intake on this metabolic test. Hundred and ten healthy subjects, including men and women, smoker and non-smoker, women taking OCS or not, were phenotyped for CYP1A2 using the [^{13}C]caffeine breath test and underwent a [^{13}C]ABT. Both tests showed large inter-individual variations in accordance with that of CYP450 liver content. [^{13}C]ABT was sensitive enough to point out a significant induction or inhibition related to cigarette smoking habits or OCS. The combined effect of smoking and OCS resulted in an overall unchanged metabolic activity. Consequently, the impact of the studied conditions on the [^{13}C]ABT parameters must be considered by clinicians or clinical investigators.

Keywords: [^{13}C]Aminopyrine; breath test; [^{13}C]caffeine; Carbon 13; CYP450; induction; inhibition

INTRODUCTION

Various *in vivo* methods are used to assess the liver state and its capacity to metabolize substrates. Among them are breath tests using molecules labelled with stable isotopes as metabolic probes for various enzyme systems [1–3].

The [^{13}C]aminopyrine breath test ([^{13}C]ABT) is used world wide for measuring the activity of cytochrome P450 (CYP450) *in vivo* and assessing a degree of disorder of the liver [4–6]. The contribution of the CYP450 enzymes to the metabolic pathways of aminopyrine is not yet well known but seems close to the antipyrine's metabolism. The later involves CYP1A2, and CYP2C9, CYP2E1 to a lesser extent and also CYP2C19, CYP2D6 and CYP3A [7]. The metabolism of aminopyrine shows two sequential oxidative *N*-demethylations, which involve different CYP450, especially CYP1A2 and CYP3A3/4 [8]. No correlation between [^{13}C]aminopyrine breath test ([^{13}C]ABT) results and CYP1A2 or CYP3A4

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ISSN 1025-6016 print; ISSN 1477-2639 online
DOI: 10.1080/10256010290005801

activities have been demonstrated but [^{13}C]ABT has been correlated to the amounts of CYP450 [9] and the functional hepatic mass [10]. Like antipyrine, aminopyrine clearance is a global measure of CYP450 oxidative capacity because of the multiplicity of the enzymes involved in the formation of their metabolites with a slight to moderate weight toward CYP1A2 [11].

Nevertheless, the influence of potential inhibitors or inducers of the aminopyrine metabolism has still not been evaluated on the [^{13}C]ABT results, except for oral contraceptive steroids (OCS). In 1978, Herz *et al.* [12] studied the effect of OCS on the rate of demethylation of [^{14}C]aminopyrine using the [^{14}C]ABT. Later, Opekun *et al.* [13] demonstrated that the intake of low-dose OCS could affect the score of the ABT but no assays have mentioned the impact of other factors such as the gender or smoking habits. In this context, it is highly relevant to study factors that could affect the results and the clinical interpretation of the [^{13}C]ABT.

The aim of this study was to analyse the influence of gender, cigarette smoking and OCS intake on the [^{13}C]ABT, in healthy subjects.

Furthermore, caffeine is mainly metabolised by CYP1A2 [14] and has been applied as a substrate for phenotyping CYP1A2 in the liver [15–17]. The non-invasive [^{13}C]caffeine breath test ([^{13}C]CBT) is able to point out the influence of inhibitors or inducers on the CYP1A2 activity, via caffeine metabolism [18, 19].

Since CYP1A2 participates to aminopyrine metabolism, [^{13}C]CBT was performed together to [^{13}C]ABT in order to check the CYP1A2 activity of the selected subjects. The use of these two tests was compared for phenotyping specific and non-specific CYP450 enzyme activities.

METHODS

Chemicals

[1,3,7-trimethyl-$^{13}C_3$]caffeine (99% ^{13}C) and [*N*,*N*-dimethyl-$^{13}C_2$]aminopyrine (99% ^{13}C) were obtained from Isotec Inc., Ohio, USA. Helium and SFC grade CO_2 were purchased from Prodair Canada, Quebec, Canada.

Subjects and Drug Administration

The protocol was approved by the Research and Ethical Committee of Algorithme Pharma Inc. (Montreal, Canada), in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. An informed written consent was obtained from each subject. The trial was

TABLE I Subjects included in the Trial.

Groups	*Subjects*	*Number of subjects*	*Weight (kg) Mean ± SD*
A	Men non-smokers	24	75 ± 8
B	Men smokers	22	74 ± 8
C	Women non-smokers taking no oral contraceptives steroids	24	62 ± 9
D	Women non-smokers taking oral contraceptives steroids	20	60 ± 6
E	Women smokers taking oral contraceptives steroids	20	62 ± 10

carried out in 5 groups of healthy adult subjects, aged from 18 to 40 (Tab. I). These groups were chosen as characteristic groups to evaluate the basal level of general drug metabolizing enzyme activity (A and C), the effect of induction of CYP1A2 (B), the effect of inhibition of CYP1A2 (D), the effect of combined exposition to inducers and inhibitors of CYP1A2 (E) and the influence of sex (A and C). All subjects had normal total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase and alkaline phosphatase. Women were not pregnant.

Pre-requisites required 7 days without medication, 48 h without alcohol and a ten-hour fast before the tests. After this period of washout, the subjects underwent a [^{13}C]CBT and 2 days after a [^{13}C]ABT. For both tests, subjects ingested an oral dose of the labelled metabolic probe as an aqueous solution (50 ml). According to body weight ranging from 45–60 kg, 60–75 kg and 75–90 kg [1,3,7-methyl-^{13}C]caffeine doses were 155 mg, 200 mg and 245 mg respectively and [4-dimethyl-^{13}C]aminopyrine doses were 105 mg, 135 mg and 165 mg respectively. These doses were chosen to simplify the usual doses per kg of the body weight, with a view to eventually develop a simple commercial test. Breath samples were collected in duplicate, 5 min before beginning the test, every 15 min for the first 90 min and then every 30 min during the last 90 min.

Measurements

Breath samples were collected in duplicate into glass tubes (Vacutainer®). Isotope ratio measurements were carried out using a AP 2003 gas chromatography – isotope ratio mass spectrometer (GC-IRMS), from Analytical Precision (Northwich, Cheshire, UK). The mass spectrometer was calibrated for ^{13}C and ^{18}O with an online internally calibrated CO_2 reference gas.

The results were expressed as $\Delta‰ = \delta‰_{(t)} - \delta‰_{(t=0)}$, where $\delta‰_{(t=0)}$ is the isotope abundance prior to the administration of the labelled probe and $\delta\ ‰_{(t)}$ is the isotope abundance measured at "t" time during the test, with respect to the Pee Dee Belemnitella, the international ^{13}C reference.

The area under the $\Delta‰ = f(t)$ curve (AUC) was calculated according to the trapezoidal rule. As the doses of labelled caffeine and aminopyrine were administered according to the body weight, isotope abundances were expressed using the data without normalization according to the body weight.

Statistical Analysis

The results are presented as the mean AUC ± standard deviation (SD) for each groups of subjects, for both the [^{13}C]CBT and [^{13}C]ABT. Student's t test was used for comparison between groups. Similar results were obtained when using AUC between 0 and 60 min, 0 and 90 min, 0 and 120 min and 0 and 180 min. Therefore, only calculations and comparisons performed with AUC between 0 and 180 min (AUC_{180}) are reported.

RESULTS

[^{13}C]CBT

The inter-individual variations of the AUC_{180} for the [^{13}C]CBT, expressed as the coefficients of variation of the AUC_{180}, ranged between 33% and 58%, for the 5 different groups.

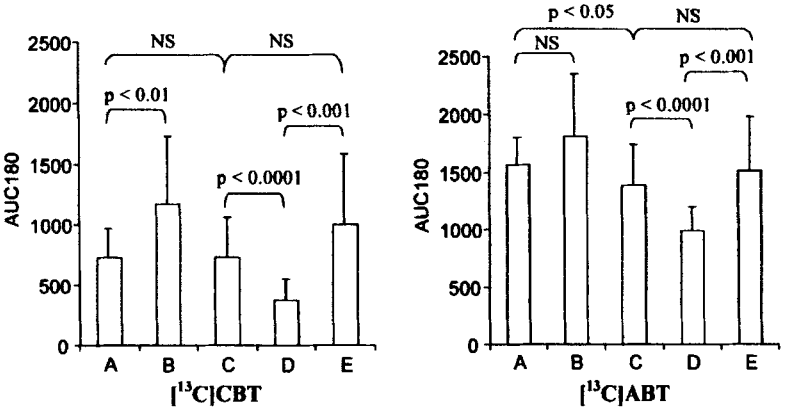

FIGURE 1 Mean $AUC_{180} \pm$ standard deviation (SD) for [^{13}C]CBT and [^{13}C]ABT. A: men non-smokers, B: men smokers, C: women non-smokers taking no oral contraceptives, D: women non-smokers taking oral contraceptives, E: women smokers taking oral contraceptives, NS: not significant.

As shown in Figure 1 lefthand, the smoking habit in men significantly increased the AUC_{180} ($p < 0.01$ for A and B). The intake of OCS in women decreased significantly the AUC_{180} ($p < 0.0001$ for C and D). Moreover, smoking habit increased significantly the AUC_{180} in women taking OCS ($p < 0.001$ for D and E). Nevertheless, the AUC_{180} calculated from the [^{13}C]CBT were not significantly different in men and women.

[^{13}C]ABT

The results obtained from the [^{13}C]ABT showed large inter-individual variations too. The values of the coefficients of variation of the AUC_{180} values ranged between 15% and 31%, for the 5 different groups. Figure 2 shows the mean $\Delta‰ = f(\text{time})$ curve for the [^{13}C]ABT, for men non-smokers. It can be observed that the ^{13}C abundance increases during the first 60 min of the test and then decreases progressively. The abundance of the expired CO_2 ($\Delta‰$) shows large SD among subjects.

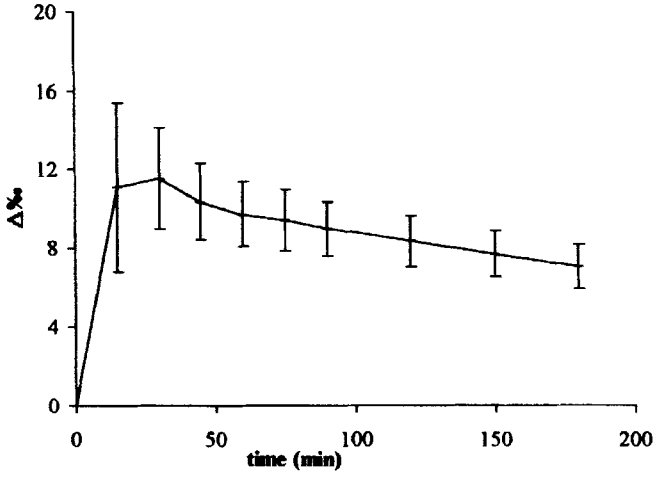

FIGURE 2 Mean $\Delta‰ = f(\text{time})$ for group A, during the [^{13}C]ABT.

When comparing [^{13}C]ABT from [^{13}C]CBT results in Figure 1, [^{13}C]ABT also showed an increase, but not significant, of AUC_{180} for men smokers and a significant decrease of AUC_{180} for women taking OCS ($p < 0.0001$ for C and D). Smoking habit increased significantly AUC_{180} for women taking OCS for either [^{13}C]ABT or [^{13}C]CBT. However, unlike [^{13}C]CBT, the parameters calculated from the [^{13}C]ABT show a significant gender difference ($p < 0.05$ for A and C)

The values of the biochemical parameters measured for the hepatic function (bilirubin, aminotransferase, etc.) showed no significant difference between the 5 groups of subjects, except between men (A) and women (C), and no correlation with the [^{13}C]CBT or [^{13}C]ABT results.

DISCUSSION

The high inter-individual variations for both tests were in accordance with what is known on the individual variability of the CYP450 and CYP1A2 content in the human liver (about 40% for CYP1A2) [20].

[^{13}C]CBT results confirmed the CYP1A2 enzyme induction and inhibition related respectively to cigarette smoking habits and OCS intake. Actually, previous studies using either [^{13}C]CBT or urinary metabolic ratios of caffeine demonstrated that CYP1A2 activity is induced by the polycyclic aromatic hydrocarbons contained in cigarette smoke and inhibited by oral steroids contraceptives [18, 19, 21–23]. As mentioned in literature [24], [^{13}C]CBT was not able to differentiate men from women.

The comparisons between the groups of subjects showed that gender, smoking and OCS had an influence on the [^{13}C]ABT results. The significant inhibitory effect of OCS confirms the results obtained from Herz *et al.* [12] and Opekun *et al.* [13]. Differences between smokers and non-smokers observed with [^{13}C]ABT were due to the participation of CYP1A2 to the metabolism of aminopyrine. However, the contribution of this enzyme is not important enough to show a significant induction.

The present studies allowed to evaluate the influence of smoking habit in women taking OCS on the enzymatic liver activities. [^{13}C]CBT and [^{13}C]ABT showed that the inductive effect of smoking combined with the inhibitory effect of OCS results in an overall metabolic activity which is not different from that of women non-smokers not taking OCS.

In contrast to [^{13}C]CBT, [^{13}C]ABT results show significant differences when comparing men and women. This can be explained by the fact that aminopyrine metabolism is less specific for CYP1A2 than caffeine metabolism and involves various other liver enzymes. Hormonal fluctuations within the menstrual cycle may be a primary cause of gender differences. Since aminopyrine can be used as a probe to assess non-selective P450 liver function [3, 10], the [^{13}C]ABT is appropriate to point out differences in the enzymatic liver activities between men and women.

The comparison between the values of biochemical parameters and the [^{13}C]ABT score confirms that there is no relationship between the conventional static biochemical tests, which are routine examinations of the liver condition, and the dynamic test used for checking drug metabolizing enzyme activity [25]. Therefore, static biochemical liver function tests must not be used as indicators of potential modifications of drug metabolizing enzymes. The non-specific [^{13}C]ABT and/or other specific breath tests ([^{13}C]CBT or [^{13}C]erythromicin breath test [26]) are more relevant to measure, in a non-invasive way, changes in global or specific metabolic activities.

The present studies shows that, in addition to medical applications designed to assess liver diseases (diagnosis or prognosis), [^{13}C]ABT could be used for other application where a global assessment of drug metabolizing activities is required. As example, clinical trials, especially biopharmaceutical trials (bioavailability and bioequivalence studies), require selection of healthy subjects with biochemical and pharmacokinetic characteristics as homogeneous as possible, in order to minimise the inter-individual variations of the measured pharmacokinetic parameters. In that context, the [^{13}C]ABT could be useful to give information on the liver detoxification and metabolism capacities.

Today, the cost of molecules labelled with stable isotopes and their analysis decreases regularly. The feasibility (oral ingestion) and non-invasive procedure should participate to the development of tests using labelled molecules combined with breath tests. Depending on the purpose of their application it is useful to make a choice between non-specific tests such as [^{13}C]ABT or [^{13}C]methacetin or [^{13}C]phenylalanine breath test [27] and specific tests as [^{13}C]CBT or [^{13}C]erythromycin breath test.

Therefore, it is important for clinicians and investigators to recognise factors and medications that can influence test results and what kind of information they are able to afford.

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