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Original article

Evaluation of the impact of CYP1A2 induction by charbroiled meal on metabolic phenotype

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SUMMARY

Background & aims: The process of grilling food items often generates polycyclic aromatic hydrocarbons which are established inducers of CYP1A2, a human drug metabolising enzyme, known to activate some procarcinogens. The impact of such induction on CYP1A2 metabolic phenotype has been the subject of some discordant findings. This study, while considering some limitations in previous study designs, evaluated the effect of CYP1A2 induction by the consumption of charbroiled meal on its metabolic phenotype.

Methods: Caffeine was administered to 17 healthy subjects before, and after, four consecutive days of charbroiled beef ingestion. Blood and spot urine samples were subsequently collected at the 4th and 6th hour post caffeine-administration, respectively, for the assessment of CYP1A2 activity. An additional caffeine administration and sample collection was repeated 48 h after the cessation of charbroiled-beef intake. CYP1A2 activity, derived as the log-transformed molar ratios of caffeine and its metabolites, was statistically analysed for changes in metabolic phenotype.

Results: Urinary and plasma metrics of CYP1A2 activity had mean reference values of 1.53 and 0.38, respectively, in the study subjects. CYP1A2 metabolic phenotype before and after the ingestion of charbroiled meal was not significantly different. However, urinary and plasma metrics of CYP1A2 activity decreased by about 19% (1.53 *vs* 1.24) and 65% (0.38 *vs* 0.14), respectively, 48 h after the cessation of charbroiled meal ingestion.

Conclusions: The induction of CYP1A2 by the consumption of charbroiled meals may not portend increased rate of CYP1A2-activation of procarcinogens in humans. However, a potentially significant CYP1A2 inhibition which might result in increased-exposure for drugs predominantly metabolised by this enzyme is likely.

1. Introduction

CYP1A2, whose expression is predominantly hepatic in humans, mediates the metabolism of some clinically relevant drugs. Its activity is, however, known to be influenced by genetic, non-genetic, environmental and epigenetic factors [1]. The complex interplay of these factors has been shown to result in a highly varied activity of CYP1A2 in humans, with potential consequences for certain drugs. For example, adverse events in users of clozapine, a substrate-drug, have been linked with reduced CYP1A2 activity [2]. Conversely, the increased clearance of theophylline in some individuals, a likely consequence of increased CYP1A2 activity, has been reported to impact negatively on the success of therapy [3]. The modification of CYP1A2 activity by the various established factors is also of toxicological significance, especially because CYP1A2 metabolises and activates several procarcinogenic substances.

General interest in the induction or inhibition of CYP1A2 by environmental substances has been particularly fuelled by several reports linking its activity with the susceptibility to certain cancers, especially lung carcinogenesis [4]. Such cancers are thought arise in humans from an increased exposure and activation of potential carcinogens which include dietary heterocyclic amines, certain mycotoxins, tobacco-specific nitrosamines, and aryl amines [5]. The inducibility of CYP1A2 by exposure to polycyclic aromatic hydrocarbons (PAHs), often emanating from the incomplete combustion

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of organic matter, found in smoke or other environmental sources such as charbroiled meals have been studied for this among other reasons. This is because PAHs have been observed to induce CYP1A2 expression by binding aryl hydrocarbon receptor (AhR) response elements which upregulate the transcription of the *CYP1A2* gene [6].

The induction of CYP1A2 arising from the ingestion of charbroiled meals, and its implication for the metabolism of some drugs and procarcinogens, was the subject of several studies [7–10]. While the induction of CYP1A2 reportedly led to the increased clearance of theophylline and antipyrine [10], a similar effect was not seen with caffeine [8,9]. The most recent of these studies by Larsen et al. [8] did, however, opined that the discrepancies in previous results may have been due to the weak CYP1A2 activity index used. These studies had determined CYP1A2 activity by administering caffeine, a CYP1A2 probe, and thereafter quantified caffeine and its metabolites from urine as against other strongly correlated CYP1A2 activity indices which required monitoring caffeine and its metabolite in plasma or saliva over specific periods [11,12].

Resolving the discrepancies in the previous studies would define a clearer impact of PAHs in charbroiled meals on CYP1A2 metabolic phenotypes in humans. In addition, a reliable approach for studying the effect of CYP1A2 induction on the exposure of relevant substrates might also be provided. This study, having considered the limitations of previous study designs, assessed the impact CYP1A2 induction by PAHs in charbroiled meals on metabolic phenotype. The study also tested the hypothesis that the onset of such changes in metabolic phenotype may be detectable at a period different from those monitored in previous studies.

2. Materials and methods

2.1. Chemicals

1,7-dimethylxanthine (17X) and 1,7-dimethyluric acid (17U) were purchased from Carbosynth Limited (Berkshire, UK). Caffeine tablets were from ProLab (Chatsworth, CA, USA), while phenacetin and glacial acetic acid were from BDH chemicals Ltd. (Poole, UK). Caffeine standard and HPLC-grade acetonitrile were from Sigma–Aldrich (Steinheim, Germany) and diethylether was purchased from Scharlau Chemicals (Barcelona, Spain).

2.2. Subjects

The study was approved by the Ethics committee of the Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria. The study sample size, estimated as described by Faul et al. [13] using G*Power (version 3.0.10), was expected to have a minimum power of 80% to detect a 20% change in CYP1A2 activity index. Volunteers ($n = 17$) were healthy unrelated subjects, non-smokers and non-users of contraceptives, comprising 11 males and 6 females with a median age (range) of 21 y (19–26) and median body weight (range) of 58 kg (52–86). These subjects were from the *Yoruba* ethnic group in Nigeria and they all provided written informed consent for participation in the study.

2.3. Experimental

Volunteers were required to stay off charbroiled meals, caffeinated substances and vegetables for a week prior to the study and for the entire duration of the study. Spot urine samples were collected for confirmation of caffeine-free diet compliance immediately before the study. Subsequently, caffeine tablet containing 200 mg of caffeine was administered to each subject (period 1). Blood samples were collected at the 4th hour post-administration, while spot urine samples were taken 6 h after caffeine ingestion. After a three-week period, all subjects were fed daily for four consecutive days with 50 g of charbroiled beef which was prepared by grilling fresh, previously boiled beef on coal for 20 min. On the fifth day, 200 mg of caffeine was administered to all subjects and spot blood and urine samples were collected as described in the first period (period 2). The administration of 200 mg caffeine was again repeated on the seventh day (48 h after the last caffeine ingestion) and samples collected as earlier described (period 3). All blood samples collected throughout the study were processed immediately for plasma, and both plasma and urine samples were refrigerated until further analysis.

Caffeine and some of its metabolites (17U and 17X) were thereafter extracted from plasma and urine samples. Briefly, 250 μL of the biological sample was added to 10 μL of the internal standard (200 μg/mL phenacetin). The mixture was vortexed for 30 s and thereafter, 1 mL of diethylether was added. Extraction of caffeine and its metabolites was then carried out by vortexing the resulting mixture for 5 min, followed by centrifugation at 5000 × g for 5 min. The organic phase was aspirated and left to dry at room temperature in a fume cupboard for 3 h. The extracted components were reconstituted in 100 μL of 0.002% acetic acid/acetonitrile (90:10). Subsequently, 50 μL was injected for HPLC analysis.

The HPLC (1100 series from Agilent technologies, Palm Alto, CA, USA) employed for analysis was fitted with a quaternary pump, a Diode array detector, and a reverse phase Eclipse XDB-CN (4 μm, 150 × 4.6 mm i.d.; Agilent Technologies, Palo Alto, CA, USA) column. Sample injection was done using a Rheodyne model 7725i valve (Agilent technologies, Cotati, CA, USA) fitted with a 20 μL loop, and chromatograms were recorded with a HP chemstation software (Agilent technologies, Palm Alto, CA, USA). The resolution of caffeine and its metabolites was done at a column temperature of 27 °C with a mobile phase comprising 0.002% acetic acid and acetonitrile in the ratio 90:10. Flow rate was set at 0.8 mL/min, and column effluent was monitored at 274 nm. The run time for each analysis was 5 min.

Calibration curves were constructed in the range of 5–150 μM for 17U and 17X, while a range of 2.5–75 μM was used for caffeine (137X). Molar ratios of (17U + 17X)/137X was used as the CYP1A2 activity index in urine [11] while 17X/137X served as the index for activity in plasma [12]. The calibration curves derived were linear in the range studied with coefficients of determination greater than 0.99 for 17U, 17X, and 137X. Deviations from nominal values of the back calculated concentration of the calibration standards were < ±13% for 17X, < ±8 for 17U and < ±11 for 137X in urine, while values of < ±8% for 17X, < ±14% for 137X were derived in plasma. The limits of quantification were 5.78 μM and 4.19 μM for 17X and 137X in plasma, respectively, while the corresponding values for these analytes in urine were 4.38 μM and 4.34 μM. All peaks were well-resolved with retention times of 3.01 min for 17U, 3.42 min for 17X, 3.71 min for internal standard, and 3.95 min for 137X.

2.4. Statistical analysis

CYP1A2 activity values were log-transformed prior to statistical analysis. A one-way analysis of variance with repeated measures, coupled with a post hoc analysis (STATA version 12, StataCorp LP, TX, USA), was used to assess differences in CYP1A2 activity across the studied periods. Statistical significance was inferred at a $P < 0.05$.

3. Results

An interval plot of mean log-transformed CYP1A2 activity derived from urinary indices across the three study periods is

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