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A Potential Case of Fluvoxamine-Induced Inhibition of Caffeine Metabolism

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INTRODUCTION

Because of the potential for abuse of caffeine (1,3,7-trimethylxanthine) as a stimulant or diuretic, consumption of atypically large doses is banned by many sports organizations. The concentration of caffeine in urine is generally measured using HPLC with UV absorbance detection. If the urinary caffeine concentration is confirmed to exceed an administrative cutoff [e. g., 12 $\mu\text{g/mL}$ for the International Olympic Committee (IOC)], the sample is reported as a caffeine positive. An athlete who consumes ordinary dietary quantities of caffeine-containing beverages and foods, or one who takes recommended doses of over-the-counter medications, generally does not produce a caffeine positive urine. While routine caffeine drug tests measure the concentration of unmetabolized caffeine only, it must be noted that human urine may also contain 17 or more caffeine metabolites (1). It has been estimated that about 80% of the caffeine consumed by a normal individual undergoes N3-demethylation to produce paraxanthine, or 1,7-dimethylxanthine (2). The liver enzyme cytochrome P4501A2 (CYP1A2) catalyzes this N-demethylation. However, CYP1A2 can be inhibited by the selective serotonin reuptake inhibitor fluvoxamine (3), an antidepressant used to treat attention deficit hyperactivity disorder (ADHD).

Our laboratory recently confirmed a caffeine positive sample from an athlete who was being treated with fluvoxamine for ADHD. The hypothesis that fluvoxamine-induced inhibition of caffeine metabolism was responsible for her abnormally high urinary caffeine concentration was supported by a previous study (3) that showed a marked inhibition of CYP1A2 during administration of fluvoxamine as evidenced by increased caffeine concentrations in the plasma and urine. Clearly, an analytical approach for substantiating or disproving claims of altered caffeine metabolism would be useful. We developed a method to assess the inhibition of CYP1A2 via measurement of the concentrations of caffeine and its principal physiological metabolite, paraxanthine. A diagnostic paraxanthine/caffeine concentration ratio was computed, and a value of

< 2 was taken to indicate probable metabolic inhibition (3). A liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (LC/APCI-MS) method was used for analysis; sample preparation employed a liquid/liquid extraction procedure. β -hydroxyethyltheophylline, which is structurally similar to caffeine, was used as an internal standard.

One female subject agreed to participate in a simple, single-trial metabolic study. The subject was given a small (about 200 mg) dose of caffeine while maintaining their normal fluvoxamine dosing schedule. A urine sample was collected immediately before administration of the caffeine, and collection was continued until 7 h after administration. In this report, we present the caffeine and paraxanthine concentrations in each of these urine samples. We provide evidence that the use of caffeine with fluvoxamine may result in an abnormally high urinary caffeine concentration, and we discuss the relevance of these findings with regard to routine caffeine testing of athletes.

METHODS

Metabolic Study. The subject, while taking regular doses of fluvoxamine, was given about 200 mg of caffeine. Urine samples were collected immediately before caffeine administration, and at the following post-dose times: 1 h, 3 h, 5 h, and 7 h. Samples were analyzed for caffeine and paraxanthine by LC/MS as described below.

Sample Preparation and Analysis. Caffeine and paraxanthine were extracted from urine samples by liquid/liquid extraction, using a method based on that of Grant et al. (4) Briefly, a 0.2 mL sample of urine was placed in a screw cap glass tube and was spiked with β -hydroxyethyltheophylline to a concentration of 12.5 $\mu\text{g/mL}$. The sample was treated with 120 mg of ammonium sulfate. After approximately 5 s of vortex mixing, 6 mL of 85:15 chloroform isopropanol (v/v) was added. The vessel was shaken for 2 min and centrifuged for 5 min (1000 x g). The organic layer was removed and dried on a vacuum centrifuge (Savant SpeedVac; Farmingdale, NY, USA). The sample was reconstituted in 100 μL of a 9:1 (v/v) mixture of mobile phase A-B (see below for mobile phase compositions). A 10 μL sample was injected on a Hewlett-Packard (Little Falls, DE) Model 1100 LC/MSD with an APCI interface. The reverse-phase column (3.0 x 150 mm) was obtained from YMC (Wilmington, NC, USA) and contained C₁₈ ODS-AQ (5 mm, 120 Å) silica particles. The flowrate was 0.5 mL/min and the column was maintained at 40 °C. Gradient elution was employed; mobile phase A was 10 mM aqueous ammonium acetate (pH 4.4) and mobile phase B was acetonitrile. The program began with a

composition of 95:5 A-B (5 min hold) followed by a linear ramp to 30:70 A-B over a 15 min period. The MS source was operated using the following settings: drying gas flow, 4 L/min; drying gas temperature, 300 °C; nebulizer pressure, 60 psig; vaporizer, 325 °C; capillary voltage, 4000 V; corona current, 4 mA; fragmentor, 80 V. Using these conditions, minimal fragmentation of the pseudomolecular ions was observed. Mass calibration was performed according to the manufacturer's instructions. The quadrupole was scanned from m/z 100 to 400 using a step size of 0.1 unit. Caffeine and paraxanthine were quantitated using the peak heights of extracted ion chromatograms (EICs) corresponding to the (M+H)⁺ ions of caffeine, m/z 195.1; paraxanthine, m/z 181.1; and β -hydroxyethyltheophylline, m/z 225.1. Quantitative calibration was performed using a multi-point curve based on the internal standard.

RESULTS AND DISCUSSION

The metabolic study described here was performed with an athlete who had recently tested positive for caffeine, but had argued that fluvoxamine-induced inhibition of CYP1A2 metabolism was responsible for her high urinary caffeine concentration. To investigate this claim, we designed an LC/MS method suitable for quantitation of a variety of caffeine metabolites in urine. The analysis of paraxanthine was of particular interest in our metabolic inhibition study because it is the major product of CYP1A2-catalyzed metabolism of caffeine. The concentrations of caffeine and its metabolites were sufficiently high that the limit of quantitation of the method was not a concern; accordingly, full scan mass spectra (i.e., m/z 100 to 400) were acquired. EICs were then used to quantitate both caffeine and paraxanthine, based on the internal standard, β -hydroxyethyltheophylline. The EICs shown in Figure 1 correspond to the (M + H)⁺ pseudomolecular ions of caffeine and paraxanthine. Similarly, an EIC was obtained for the internal standard (not shown), which eluted between paraxanthine and caffeine.

Urine samples were obtained from the athlete immediately before (i.e., at "0 h") administration of about 200 mg of pharmaceutical-grade caffeine, and at several timepoints after the administration. The subject did not interrupt their regular fluvoxamine dosing schedule. The caffeine and paraxanthine concentrations for each urine sample are shown in Table 1. Post-dose, the concentration of caffeine increased to a level of about 7 $\mu\text{g/mL}$, which is significantly higher than expected from consumption of this small dose of caffeine. The paraxanthine concentration and the paraxanthine/caffeine concentration ratio are also given in Table 1. Previous studies (3) of caffeine metabolism have demonstrated that the paraxanthine/caffeine concentration ratio can be used to assess the metabolic capacity of CYP1A2. Specifically, a paraxanthine/caffeine ratio of < 2

indicates probable inhibition of caffeine metabolism. The subject's post-dose paraxanthine/caffeine ratio of < 2 is consistent with drug-induced CYP1A2 inhibition.

We did not perform the logical control experiment, in which our subject's fluvoxamine treatment would have been discontinued prior to administration of caffeine. Quantitation of the caffeine and paraxanthine in the corresponding urine samples would have provided experimental evidence as to whether fluvoxamine was the causal agent of the abnormal metabolism. Because our subject was an amateur athlete who used fluvoxamine according to their physician's prescription, they could not participate in the proposed control study. However, the data presented in this report indicates that the subject did not metabolize caffeine normally while under the influence of fluvoxamine. Considering previous studies that clearly demonstrate fluvoxamine's inhibitory effect on caffeine metabolism, we suspect that the athlete described here exhibits fluvoxamine-induced metabolic inhibition. Regardless of the specific causal agent, determination of the urinary paraxanthine/caffeine concentration ratio in caffeine positive samples should be useful for investigating cases in which inhibition of caffeine metabolism is suspected.

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