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Elimination of ephedrines in urine following multiple dosing: the consequences for athletes, in relation to doping control

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Aims
To study the elimination of ephedrines with reference to the International Olympic Committee (IOC) doping control cut-off levels, following multiple dosing of over-the-counter decongestant preparations.

Methods
A double-blind study was performed in which 16 healthy male volunteers were administered either pseudoephedrine or phenylpropanolamine in maximal recommended therapeutic doses over a 36-h period. Urine was collected every two hours between 08:00 and 24:00 h and at 04:00 h throughout the testing period of three days. Urine drug levels were quantified using high performance liquid chromatography. Side-effects were assessed, including heart rate and blood pressure, every four hours between 08:00 and 20:00 h.

Results
Mean (95% CI) total phenylpropanolamine and pseudoephedrine eliminated unchanged was 75 (88, 61) and 81 (92, 71)%, respectively. Maximum urine concentrations of phenylpropanolamine and pseudoephedrine were 112.1 (164.2, 59.9) and 148.5 (215.0, 82.1) $mg.l^{-1}$, respectively. A peak in drug urine concentration occurred four hours following the final dose. There were no adverse cardiovascular effects and only mild CNS stimulation was evident.

Conclusions
Following therapeutic, multiple dosing, drug levels remain above the IOC cut-off levels for a minimum of 6 h and 16 h following final doses of phenylpropanolamine and pseudoephedrine, respectively. Athletes require informed advice on this from their healthcare professionals.

Introduction

Sympathomimetics are included in the International Olympic Committee (IOC) list of banned substances, under the class, stimulants. For years, athletes have tested positive for these substances, but many have claimed they took the drugs inadvertently in over-the-counter (OTC) cough and cold preparations. In recent years, the IOC has introduced cut-off values for urinary concentrations of these drugs below which athletes are not subject to sanctions. The current IOC cut-off concentrations for both phenylpropanolamine and pseudoephedrine are 25 $mg{\cdot}l^{-1}$, whereas that for

norpseudoephedrine is 5 mg·l^{-1}[1]. However, the previous cut off value was less lenient in the case of phenylpropanolamine and pseudoephedrine, being set at 10 μg ml^{-1} [2]. The rationale for the selection of these cut-off concentrations has not been published by the IOC and does not appear to be based on experimental evidence.

The pharmacokinetic parameters for pseudoephedrine and phenylpropanolamine administered in liquid formulations such as solution or syrup, have been established [3, 4]. Findlay and colleagues [5] have examined the plasma pharmacokinetic parameters or pseudoephedrine following administration of multiple, therapeutic doses. However, in immediate release tablet form no studies have examined the urine elimination of pseudoephedrine and phenylpropanolamine following therapeutic, multiple dosing of immediate release tablet formulations, a regime likely to be used by athletes.

Methods

Subjects

Sixteen healthy male subjects volunteered to take part in this study and provided written, informed consent. Individuals on medication or suffering any cardiac complaint were not allowed to participate in the study. Subjects were divided into two groups ($n = 8$), matched for age and body mass, according to the drug administered. The pseudoephedrine group were aged 26.1 ± 3.1 year with a body weight of 75.5 ± 7.6 kg and the phenylpropanolamine group were aged 25.0 ± 2.7 year with a body weight of 75.3 ± 7.0 kg. The study was approved by the Human Ethics Committee of Liverpool John Moores University.

Study design

The study was double blind and data collection took place over a 64-h period involving drug administration during the initial 28 h. Urine was collected and heart rate and blood pressure were monitored throughout the whole 64 h.

Subjects were asked to refrain from using drugs (including alcohol and nicotine) and foods containing caffeine the day prior to and throughout the period of testing. A balanced diet was maintained throughout the study. Water was provided in 200 ml quantities and consumed every two hours between 08:00 h and 24:00 h to provoke diuresis and allow sufficient urine flow during sampling. Vigorous exercise or work was prohibited on the day prior to testing and throughout the period of testing.

Urine collection

On 'day one', following an overnight fast, subjects provided a urine sample at 08:00 h for use as a blank sample and also to void the bladder. Subsequent samples were collected every two hours between 08:00 and 24:00 h and also at 04:00 h throughout the three days. Urine volumes were recorded and approximately 20 ml transferred to a labelled sterile specimen container. Following cooling in a refrigerator the pH of the sample was measured using a pH meter with temperature compensation (Philips, PW 9418, Cambridge, UK) and combination pH electrode (Russell pH Limited, Fife, UK). Samples were stored at −70 °C until analysis.

Drug administration

Following the initial urine collection, subjects were given six envelopes each containing two gelatin capsules to be administered orally. The capsules contained either pseudoephedrine or phenylpropanolamine mixed with dextrose monohydrate powder. Drugs were present in their commercial formation, namely pseudoephedrine hydrochloride (Decongestant tablets, Boots, Nottingham, UK) and phenylpropanolamine hydrochloride combined with paracetamol (Mu-Cron tablets, Novartis, Horsham, UK). The drugs were administered in maximal therapeutic doses (as recommended by manufacturers) over a 36-h period. This involved administration of two capsules every four hours between 08:00 and 20:00 h on day one and between 08:00 and 12:00 h on day two. In total, drugs were administered six times in individual doses of 60 mg and 25 mg for pseudoephedrine and phenylpropanolamine, respectively.

Monitoring of side effects

Side-effects were monitored by questionnaire, completed every four hours, between 08:00 and 24:00 h throughout the testing period. Heart rate and blood pressure were monitored prior to testing to establish baseline data for each subject and to confirm their suitability for inclusion into the study, and then every four hours, following urine collection between 08:00 and 20:00 h. Subjects maintained a supine position during measurement, using a Dinamap Compact Monitor (Critikon, Tampa, Florida, USA), and for 15 min prior to measurement.

Urinalysis

Pseudoephedrine and phenylpropanolamine urine concentrations were determined using the high performance liquid chromatography method described by Chester [6]. A 1-ml urine sample [including 50 mg·l^{-1} of alpha-

(methylaminomethyl) benzyl alcohol (internal standard)] was adjusted to pH 9 using a 0.1-m ammonium hydroxide solution. A Bond Elut C_{18} solid phase extraction cartridge (1 ml/100 mg) from Varian Limited (Walton-on-Thames, UK) was conditioned by successively passing 1 ml of methanol and 1 ml of water (adjusted to pH 9 using a buffer solution made by adding 18.3 ml of 0.1 M NaOH to 50 ml of 0.025 M Borax) through it. The urine sample was then applied to the cartridge, which was rinsed with 1 ml of water (pH 9). The cartridge was then dried under suction and the elution step was performed using 2 ml of methanol containing 1% v/v HCl. The eluate was evaporated to dryness at 40 °C under a gentle flow of nitrogen. The residue was reconstituted with 1 ml of water of which 10 µl was injected on to the HPLC column (3.9 x 150 mm Symmetry Shield RP8 column, 5 µm particle size) from Waters Limited (Watford, UK). Chromatography was performed using a Waters 2960 separations module connected to a Millennium 32 data system and a 996-photodiode array detector (Waters Limited, Watford, UK). Two mobile phases (A) acetonitrile with 0.1% trifluoroacetic acid and (B) water containing 0.1% trifluoroacetic acid were passed through the column according to the following gradient (A vol%/B vol%), at start: 3/97, at 10 min: 15/85, at 11 min: 50/50 and at 12 min: 3/97. The flow rate was 1 ml·min^{-1} and the column temperature was 30 °C. The column effluent was monitored at 206 nm. Calibration curves were linear over the range 1 mg·l^{-1}–300 mg·l^{-1} with correlation coefficients between 0.999 and 1.000. The limits of determination were 0.42, 0.36 and 1.0 mg·l^{-1} for pseudoephedrine, phenylpropanolamine and norpseudoephedrine, respectively. At drug urine concentrations of 5 mg·l^{-1} and 100 mg·l^{-1} the coefficient of variations were 6% and 7% for pseudoephedrine, 3% and 6% for phenylpropanolamine and 14% and 1% for norpseudoephedrine.

Data analysis

All results were calculated as mean ± standard deviation and represented graphically as scatter plots.

Results

Three subjects, two from the phenylpropanolamine group and one from the pseudoephedrine group failed to complete the study.

The pharmacokinetic data obtained from the urinalysis are summarised in Table 1. Mean urinary pH was 6.5 (± 0.4) and 6.3 (± 0.3) in the pseudoephedrine and phenylypropanolamine groups, respectively. The mean total

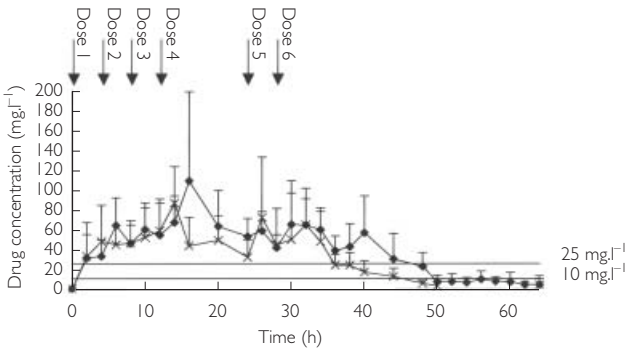

Figure 1

Mean urine pseudoephedrine (◆) and phenylpropanolamine (×) concentrations (+ s.d.) *vs* time following multiple dosing of immediate release capsules

phenylpropanolamine eliminated unchanged was 75 (± 13)% of the dose and that for pseudoephedrine was 81 (± 11)%. Mean (+ s.d.) urinary concentration *vs* time date are shown in Figure 1. The maximum drug concentrations in urine ($^{u}C_{max}$) throughout the testing period are displayed in Table 1. The mean $^{u}C_{max}$ for phenylpropanolamine was 112 (± 50) mg·l^{-1} and that for pseudoephedrine was 149 (± 72) mg·l^{-1}. Following the final dose at 12:00 h on day 2, the final peak in urine drug concentration was recorded and is represented by $^{u}C_{max}$ POST. The time to this peak is represented as $^{u}T_{max}$ POST in Table 1. The median $^{u}T_{max}$ POST for both drugs, was 4 h after the final dose.

The mean total excretion of norpseudoephedrine, following pseudoephedrine administration was 6 (± 2)% of the dose. The maximum concentration of norpseudoephedrine in urine ($^{u}C_{max}$) was greater than the IOC limit of 5 mg·l^{-1} (Table 1). An indication of the total elimination of pseudoephedrine was achieved through the addition of the parent drug and the metabolite and was found to be 87%. With the two drugs present in the urine the detection limit would assume the higher IOC limit (25 mg·l^{-1}) [2], however, when both drug concentrations were added together, the very low levels of norpseudoephedrine meant that overall concentrations were not substantially affected.

Significant elevations in heart rate and blood pressure were not evident following phenylpropanolamine and pseudoephedrine administration. Other side-effects including headache, dry mouth, anxiety, nausea, dizziness, palpitations, sleeplessness and loss of appetite, were reported infrequently by five subjects on 39 occasions but none were severe enough to warrant withdrawal from the study.

Table 1
Pharmacokinetic parameters of phenylpropanolamine, pseudoephedrine and norpseudoephedrine

Subject	^{u}pH	% Eliminated	$^{u}C_{max}$ (mg.l^{-1})	$^{u}C_{max}$ POST (mg.l^{-1})	$^{u}T_{max}$ POST (h)
Phenylpropanolamine					
1	6.3	92.5	162.5	71.2	4
2	6.2	52.4	155.2	36.7	2
3	6.7	74.3	75.9	75.9	4
4	6.1	73.0	72.7	34.0	4
5	5.8	75.7	53.5	53.5	6
6	6.5	78.8	152.6	117.8	4
Mean (± s.d.)	6.3 (± 0.3)	75 (± 13)	112 (± 50)	65 (± 31)	4*
Confidence Interval					
Lower	5.9	61	60	32	–
Upper	6.6	88	164	98	–
Co. of Var. (%)	4.5	17	44	48	–
Pseudoephedrine					
1	6.6	84.5	110.2	110.2	4
2	6.8	90.7	154.1	98.2	2
3	6.2	83.7	136.8	83.4	4
4	7.2	82.8	294.4	68.9	4
5	6.0	84.3	123.8	41.5	4
6	6.1	85.7	157.7	51.1	4
7	6.3	56.7	62.8	39.6	6
Mean (± s.d.)	6.5 (± 0.4)	81 (± 11)	149 (± 72)	70 (± 28)	4*
Confidence Interval					
Lower	6.1	70	82	45	–
Upper	6.9	92	215	96	–
Co. of Var. (%)	6.8	14	48	40	–
Norpseudoephedrine					
1	–	2.0	6.1	6.1	4
2	–	5.2	9.6	9.6	20
3	–	5.2	7.0	6.0	4
4	–	7.2	7.2	7.2	10
5	–	6.3	6.1	6.1	2
6	–	3.7	5.3	5.3	12
7	–	9.7	8.8	8.8	6
Mean (± s.d.)	–	6 (± 2)	7 (± 2)	7 (± 2)	6*
Confidence Interval					
Lower	–	3	6	5	–
Upper	–	8	9	8	–
Co. of Var. (%)	–	44	22	24	–

**, Median; ^{u}, the 'u' prefix refers to urinary measurements.*

Discussion

In this study 75% of phenylpropanolamine was excreted unchanged over the study period, which is comparable to previous studies that have reported recoveries of 63% to 97% [4, 7–11]. In the studies cited, the urine collection period varied between 14 h and 48 h, which may account, to some degree for the variability in recoveries. Only about 4% of a dose of phenylpropanolamine is excreted as metabolites.

Eighty-one percent of pseudoephedrine was excreted unchanged, compared to previously reported values of between 43 and 96% within 24 h [12–18]. Total drug recovery calculated by parent plus metabolite was 87%. The sample collection period does not appear to be the only factor influencing drug recovery. Brater and colleagues [12] collected samples over a 48-h period and reported the proportion of unchanged drug excreted in urine to be only 45%, whereas Lai and colleagues [18]

collected samples over a 12-h period and reported 89% unchanged drug was excreted.

A small percentage of pseudoephedrine (~1%) is metabolized to norpseudoephedrine through N-demethylation [19]. In the present study 6% of the dose was recovered as norpseudoephedrine following administration of pseudoephedrine. However, recovery is partially dependent on urinary pH. In alkaline urine the increased reabsorption would result in prolonged retention of drug in the body, thus allowing more extensive metabolism to occur [20]. However, Lai *et al.* [18] found that the total amount of pseudoephedrine excreted was independent of urinary pH, probably because the quantity of metabolite excreted is too small (~1% of the dose) for any changes to be detected within the narrow range of urinary pH studied (5.7–6.5).

The dosing regimen used in this study was designed to simulate the likely use by athletes of OTC medicines for the management of upper respiratory tract (URT) conditions, prior to competition. Our results show that an individual taking the maximal therapeutic dose over a relatively short time period can greatly exceed the IOC cut-off concentrations. We found that the mean urinary drug concentration was greater than 4 times the IOC cut-off value for phenylpropanolamine and almost 6 times that for pseudoephedrine. The period over which the urinary drug concentration was above the IOC limit was 16 h following the final dose of pseudoephedrine and 6 h after that of phenylpropanolamine. After the first dose, urine drug concentrations were substantially above the IOC limit. Lefebvre *et al.* [21] found that following a single therapeutic dose of ephedrine administered intranasally, six out of eight subjects attained urine concentrations above the original cut-off value of 5 $mg \cdot l^{-1}$ set by the IOC. However, no subjects produced concentrations above the new IOC cut-off value for ephedrine of 10 $mg \cdot l^{-1}$, after a single dose. However, subsequent doses resulted in five subjects attaining concentrations considerably higher than 10 $mg \cdot l^{-1}$.

When both pseudoephedrine and norpseudoephedrine are detected in urine, concentrations are added, to gain an overall measure of drug exposure. The higher IOC limit of 25 $mg \cdot l^{-1}$ for pseudoephedrine plus norpseudoephedrine is used in such cases [2]. In the present study there was no significant difference in the concentration of pseudoephedrine plus metabolite compared to that of the parent drug alone.

Peak drug concentration following the final dose occurred at four hours. This has implications for the drug testing of athletes, because participation in competition may well fall within this time scale.

Despite evidence suggesting no CNS stimulatory effects after relatively low doses of ephedrines [13, 22], it is conceivable that multiple dosing may induce stimulation. In the present study, subjects were found to suffer from a number of side-effects suggesting mild CNS stimulation, such as headache, dry mouth, sleeplessness, anxiety, nausea, dizziness, palpitations and loss of appetite. Adverse cardiovascular effects in response to drug administration were not evident. There have been conflicting reports regarding the effect of ephedrines on heart rate and blood pressure. Bye and colleagues [23] reported significant increases in heart rate and systolic blood pressure following pseudoephedrine ingestion in therapeutic and supratherapeutic doses, but no effect on heart rate and blood pressure was found by Bright *et al.* [24] who administered similar doses.

Conclusions

In conclusion, multiple therapeutic dosing of pseudoephedrine or phenylpropanolamine over a 36-h period to healthy subjects produced urine drug concentrations above the drug cut-off values set by the IOC (constituting a doping offence) for as much as 16 h following the final dose. The highest urine drug concentrations occurred, post administration four hours after the final dose. Clearly, athletes need careful advice from their medical practitioner or other health professional, regarding the use of these drugs.

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