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Effect of oxidizing adulterants on human urinary steroid profiles

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ABSTRACT

Steroid profiling is the most versatile and informative technique adapted by doping control laboratories for detection of steroid abuse. The absolute concentrations and ratios of endogenous steroids including testosterone, epitestosterone, androsterone, etiocholanolone, 5α-androstane-3α,17β-diol and 5β-androstane-3α,17β-diol constitute the significant characteristics of a steroid profile. In the present study we report the influence of various oxidizing adulterants on the steroid profile of human urine. Gas chromatography–mass spectrometry analysis was carried out to develop the steroid profile of human male and female urine. Oxidants potassium nitrite, sodium hypochlorite, potassium permanganate, cerium ammonium nitrate, sodium metaperiodate, pyridinium chlorochromate, potassium dichromate and potassium perchlorate were reacted with urine at various concentrations and conditions and the effect of these oxidants on the steroid profile were analyzed. Most of the oxidizing chemicals led to significant changes in endogenous steroid profile parameters which were considered stable under normal conditions. These oxidizing chemicals can cause serious problems regarding the interpretation of steroid profiles and have the potential to act as masking agents that can complicate or prevent the detection of the steroid abuse.
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1. Introduction

Steroid profiling is one of the most commonly adapted methodologies in doping control laboratories for the detection of steroid abuse in sport drug testing [1,2]. A steroid profile is made up of concentrations and ratios of various endogenous urinary steroidal species. The most characteristic endogenous steroids that define the steroid profile are testosterone (T), epitestosterone (E), androsterone (A), etiocholanolone (Etio), 5α-androstane-3α,17β-diol (Adiol) and 5β-androstane-3α,17β-diol (Bdiol) with their structures shown in Fig. 1 [3]. As evidenced by several studies, the steroid profile is naturally well balanced and does not show much intra-individual variations especially with regard to the ratios utilized for doping control purposes such as T/E, A/Etio, A/T and Adiol/Bdiol [4]. These ratios are demonstrably not influenced by intense and/or sustained exercise, menstrual cycle and circadian and annual rhythms [2,5]. In contrast, administration of endogenous or exogenous steroids impairs the sensitively balanced system of steroid biosynthesis and alters one or more steroid profile parameters. Consequently, the steroid profile has been utilized by doping control laboratories to detect steroid abuse and to help assign a urine specimen to a particular person [4,6].

Due to the vital importance of the steroid profile in the fight against doping in sports, researchers have investigated several factors which influence the steroid profile such as application of pharmaceutical preparations, technical parameters like inhibition of hydrolysis, incomplete derivatization, matrix problems, bacterial activities and side reactions during sample preparations [2]. However no studies on the influence of various oxidizing adulterants on urine steroid profile has been reported so far.

Manipulations of urine samples by urine substitution, dilution and adulteration with highly oxidative chemicals to escape detection in doping control analysis have been reported several times in the past [3,6–8]. Even though WADA has imposed stringent regulations on sample collection process, recent reports of suspected and substantiated manipulation outline the complexity and diversity of tampering options [3]. Recently a method based on the introduction of protease granules into the urethra to impede common urine drug test has been identified [7]. Incidences where doping control officials were involved in sample manipulation have also been reported [3]. Access to unethical health professionals and other advisors has greatly increased the sophistication of both doping and masking strategies [9]. A battery of adulterants ranging from common household chemicals like chlorine bleach, liquid drain cleaner to commercial adulterants such as Urine Luck™ (pyridinium chlorochromate), Stealth™ (peroxidase), Klear™ (potassium nitrite), Whizzies™ (sodium nitrite), and Instant Clean™ (gluteraldehyde) are being used to render false negative results during urinanalysis [10–15]. Several studies have investigated the effectiveness of these adulterants in masking detection

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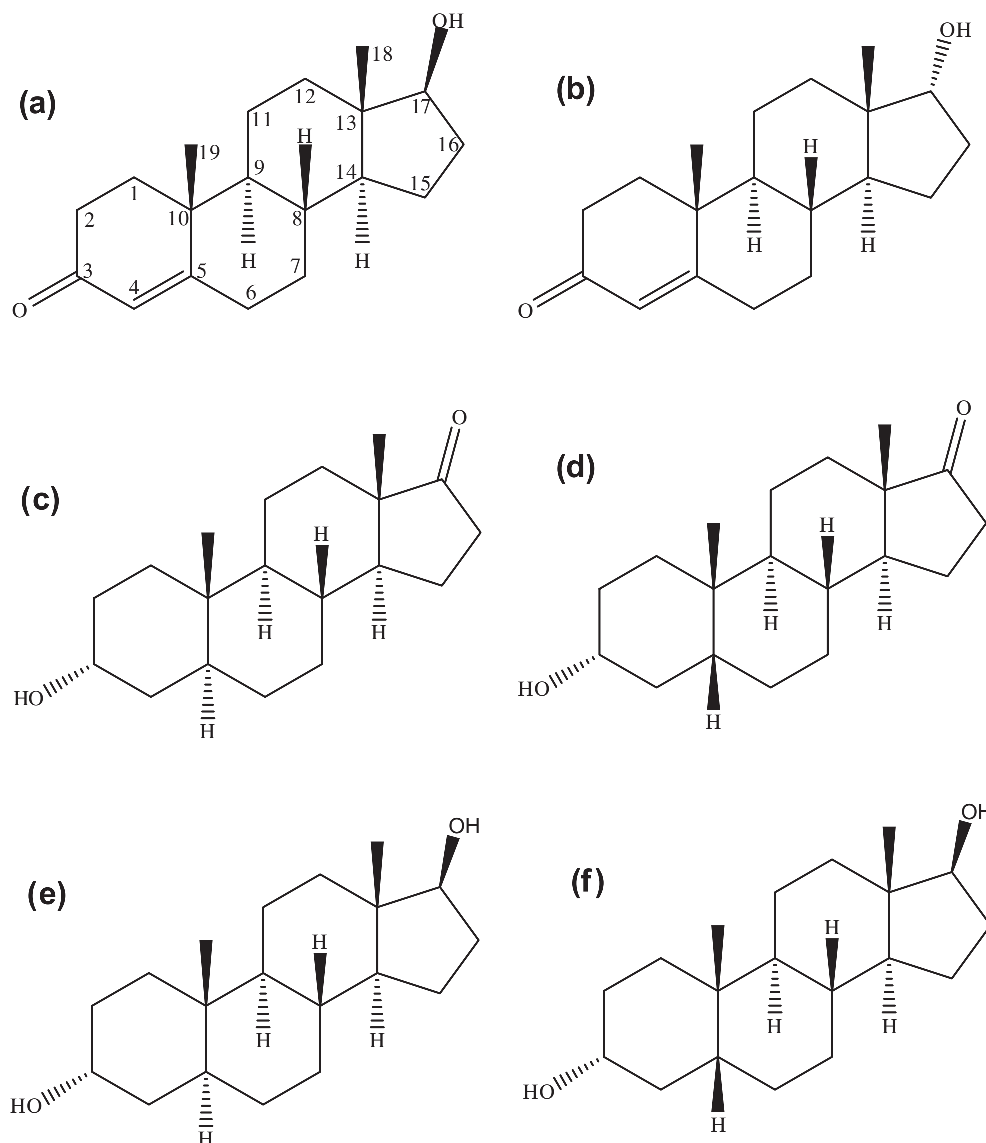

Fig. 1. Chemical structures of (a) testosterone (T), (b) epitestosterone (E), (c) androsterone (A), (d) etiocholanolone (Etio), (e) 5α-androstane-3α,17β-diol (Adiol) and (f) 5β-androstane-3α,17β-diol (Bdiol).

of cocaine metabolites, amphetamines, opiates, phencyclidine, and cannabinoids [11,16–21]. These adulterants act by either interfering with immunoassay procedures or by converting the target drugs to other compounds [11]. The current study aimed to identify the effect of nine oxidizing adulterants namely, sodium nitrite, potassium nitrite, sodium hypochlorite, pyridinium chlorochromate, potassium dichromate, cerium ammonium nitrate, potassium permanganate, sodium metaperiodate and sodium perchlorate on the steroid profile of human male and female urine.

2. Experimental

2.1. Reagents, chemicals and standards

Reference standards T, E, A, Etio, Adiol, Bdiol and their corresponding deuterated analogues (16,16,17-d_3)-testosterone (d_3-T), (16,16,17-d_3)-epitestosterone (d_3-E), (2,2,4,4-d_4)-androsterone (d_4-A), (2,2,4,4-d_4)-etiocholanolone (d_4-Etio), (16,16,17-d_3)-5α-androstane-3α,17β-diol (d_3-Adiol), and (2,2,3,4,4-d_5)-5β-androstane-3α,17β-diol (d_5-Bdiol) were obtained from the National Measurement Institute (Pymble, NSW, Australia). Sodium hypochlorite, potassium nitrite, sodium nitrite, pyridinium chlorochromate, cerium ammonium nitrate, potassium perchlorate, potassium permanganate, sodium metaperiodate, potassium dichromate, mercaptoethanol, ammonium iodide and β-glucuronidase from Helix pomatia (Type H-3) were purchased from Sigma Aldrich (Sydney, NSW, Australia). *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from UCT (Bristol, PA, USA). Surine (synthetic urine devoid of human urinary steroids) was purchased from Ceriliant (Austin, TX, USA). Potassium bicarbonate, potassium carbonate, sodium acetate and glacial acetic acid were obtained from Univar (Ingleburn, NSW, Australia). All other reagents were of analytical grade and obtained from Lab Scan (Seacliff, SA, Australia).

2.2. Collection of urine

All volunteers were asked to provide informed consent, in compliance with the requirements of the National Health and Medical Research Council (NHMRC) and Australian Vice Chancellors' Com-

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