

“Non-banned drugs as metabolic modulators: effect of antifungals on the urinary steroid profile”

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Project overview:

In the doping control field, the concept of urinary steroid profiling was first introduced in the '80s by Donike et al. to reveal the misuse of testosterone and its precursors. The kinetics of excretion in urine of the target compounds that are part of the steroid profile may be affected by various factors, primarily among them sex, age, ethnicity, physical activity, diet, alcohol consumption, as well as other physiological or pathological conditions. We are focusing our attention on another possible cause of alteration of the urinary steroid profile, that is on the consequences of drug-drug interactions. Examples of agents affecting the urinary steroid profile that are included in the WADA list of banned substances and methods are mainly represented by the masking agent probenecid and by the whole class of the aromatase inhibitors. In addition to those drugs, several classes of compounds not included in the WADA list have been reported to play a significant role on testosterone synthesis and metabolism. An example is represented by the antifungal ketoconazole that acts as inhibitor of the cytochrome P-450-dependent enzymes involved in the hydroxylation during steroid hormone synthesis and in the oxidative/reductive reactions during steroid hormone phase I metabolism.

Even if the effects of ketoconazole on the steroids hormone synthesis are well known, very little information is available about its impact on the effectiveness of the screening and confirmation strategies routinely followed by the WADA-accredited antidoping laboratories to detect the abuse of androgenic anabolic steroids that are normally present in the human body. This project aims to evaluate the alterations caused by the intake of azole antifungal drugs and to verify whether these effects could in some way affect the reliability and the accuracy of the analytical strategies currently followed to detect doping by testosterone and precursors.

Results and Conclusions:

The objective of this project was to investigate the effects of azole antifungals administration on the strategies currently adopted by the antidoping laboratories to report an adverse analytical finding for testosterone related steroids. More specifically, we have considered the effects of the administration of miconazole and fluconazole by different routes and doses on the physiological circadian fluctuation of both the parameters reported in the TD2016EAAS and the endogenous reference compounds reported in the TD2016IRMS.

In the first part of this project, the circadian fluctuations of the parameters of the steroid profile selected were evaluated in four male Caucasian subjects for at least five days, and basal ranges of each parameter in each subject were established. These basal ranges were then utilized to evaluate the potential effects of azole antifungals administration on the determination of the parameters selected to detect doping by testosterone related compounds. Finally analytical methods to screen and confirm azole antifungals in human urine were set up and validated.

The results obtained show that both miconazole and fluconazole are able to alter significantly the key parameters of the steroid profile. More in details, the imidazole antifungal miconazole causes after oral and buccal administration (i) a significant increase of the 5 α -androstan-3 α ,17 β -diol/5 β -androstan-3 α ,17 β -diol ratio, (ii) a significant decrease of androsterone, etiocholanolone, androsterone/etiocholanolone ratio, androsterone/testosterone ratio, and 5 α -androstan-3 α ,17 β -diol/epitestosterone and (iv) a moderate decrease of the 5 β -androstan-3 α ,17 β -diol and 5 α -androstan-3 α ,17 β -diol. Limited effects were instead registered after dermal intake. These finding can be primarily explained by the ability of miconazole and its metabolites in altering the kinetic/efficacy of de-glucuronidation of the endogenous steroids by β -glucuronidase. The oral intake of the triazole antifungal fluconazole, instead, increase the urinary levels of androsterone, etiocholanolone, androsterone/etiocholanolone ratio, androsterone/testosterone ratio, 5 α -androstan-3 α ,17 β -diol, 5 β -androstan-3 α ,17 β -diol, 5 α -androstan-3 α ,17 β -diol/5 β -androstan-3 α ,17 β -diol ratio, and 5 α -androstan-3 α ,17 β -diol/epitestosterone ratio. This finding is not linked to the efficacy of the enzymatic hydrolysis by β -glucuronidase, but might be explaining considering the putative anti-aromatase activity of the triazole antifungal agents.

These evidences underline the importance to screen for the azole antifungals in the athlete urines collected in occasion of doping control test, to reduce the risk of reporting uncorrected results.