"Origin production of 19-norandrosterone in human urine samples and doping analysis"

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Project Overview

In extremely rare circumstances the presence of 19-norandrosterone (19-NA) in human urine can be explained by the "instability" or "activity" of the urine specimens. This is most likely due to the sample transportation and storage in the laboratory and is based on the 19- demethylation of abundant endogenous steroids in the urine samples.

Tests for the assessment of the activity of the urine samples have been established. The hypothesis is that 19-NA will be produced by 19-demethylation of androsterone, the main androgen metabolite present in the urine. Briefly, an aliquot of sample to be tested for activity will be incubated in the presence of deuterated androsterone. The formation of deuterated 19-NA will be the proof of urine activity.

From the data collected until now, it appears that the instability of these urine samples is due to enzymatic activity expressed (from exogenous origin) in the sample. It seems unlikely that the 19-demethylation would be the result of a pure, unassisted chemical reaction. If this were the case, this phenomenon would be reproducible at any time, and is not. The growth of microorganisms in the urine samples is not unlikely. It is reasonable to link this enzymatic activity to the presence of a microorganism growing in the urine. The expression of aromatase is restricted to the gonads and brain in many vertebrates, from aquatic and avian species to mammals. The removal of the methyl group in the 19 position seems not linked to an aromatization process since no microorganism expresses such an enzyme. An important demethylase enzyme present in some microorganisms is the 14-demethylase (CYP51A1). The activity of this enzyme is crucial to the life of these microorganisms since it is the responsible for the formation of ergosterol from lanosterol. The hypothesis is that CYP51 uses androsterone as "substrate" consequently producing 19-NA.

Results and Conclusions

The present was focused on the hypothesis that the CYP51 (14α-demethylase) of fungal origin may be the cause of the alteration of the metabolic profile found in the so-called active urines. In these cases a concentration of 19-NA beyond the limits allowed, but with a ratio 19-NA/19-NE reversed compared to that A / E (usually the concentration of 19-NA in urine is greater) are observed.

To check if the CYP51 is able to demethylate androsterone and / or etiocholanolone, yeasts such as S. cerevisiae and C. albicans were chosen. As substrates of of the yeasts, androsterone, etiocholanolone and androstenedione were selected as being the most probable substrates, based on their structure and on the amount present in routine anti-doping samples.

The following experiments were performed:
1. S. cerevisiae and C. albicans were incubated in the presence of androsterone, etiocholanolone, and androstenedione in culture medium and in synthetic urine (in sterile conditions). The products of fungal metabolism were found both in the supernatant and in cell lysate.
2. S. cerevisiae and C. albicans were incubated in human urine in non-sterile conditions. Alterations of the hormonal profile in urine were evaluated according to the protocols of the anti-doping laboratory of Rome, used in the screening of banned substances.

The main conclusions of the present project are:
· The demethylation process of steroids produced in the so-called active urines is not originated chemically, at least under the common laboratory conditions during the sample processing of the urine samples in doping control.
· The aromatization process that is the responsible of the in-vivo formation of 19-norandrosterone is not the responsible for the 19-demethylation ex-vivo during sample storage of the urine samples.
· In cultured C. albicans and S. cerevisiae conducted in SDB medium and in the presence of steroid hormones, any relevant reaction of demethylation was observed. By-products of fungal metabolism on these hormones have been detected.
· In human urine inoculated with the same microorganisms, changes in the hormonal profile were detected, that can be attributed to the activity of C. albicans. These observations were previously detected in culture medium under sterile conditions. Even in these experiments the formation of 19-narandrosterone and 19-noretiocholanolone was not detected.
· Although the formation of 19-norsteroids object of this study was not detected, the changes in urinary steroid profile as a result of contamination with fungi are considered to be relevant for the correct interpretation of the data in doping control.

Under the experimental conditions described, where the functional growth of fungi has been demonstrated, the transformation of androsterone and etiocolanolone to their respective 19-norderivatives, as happens in the case of active urine, was not observed. With the current obtained knowledge, we can say that the ex-vivo process of demethylation is complex and involving several actors. The proposed test to demonstrate the “activity” of a particular sample provided that deuterated androsterone or etiocholanolone were transformed in their corresponding 19-nor deuterated products. This is not the case with the presence of the tested fungi alone. The process of demethylation implies the presence of an unsaturation in alpha with respect to the methyl group to be

removed, absent in androsterone and etiocholanolone. Our experimental evidence supports the hypothesis that the process is a multistep process consisting in a first dehydrogenation of A ring, most probably in the C1 position, followed by the multistep oxidation or the methyl group.