

PROJECT REVIEW

“Distinction Between Systemic and Topic Use of Xenobiotic Glucocorticoids in Urine: Pilot Study-CORTICOTOPIC”

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Glucocorticoids are potent anti-inflammatory and immunosuppressive agents used to treat a broad variety of medical conditions. Due to their ability to alleviate pain and enhance the athlete's concentration capacity during strength and endurance competitions, these drugs have become doping substances. Consequently, the systemic administration of these steroids is forbidden by WADA, and their use requires a therapeutic use exception approval. However, topical preparations when used for dermatological, auricular, nasal, ophthalmic, buccal, gingival and perianal disorders are not prohibited and do not require any form of therapeutic use exemption. Since some glucocorticoids are marketed in both systemic and topical forms, the distinction between different routes of administration through the analysis of urine samples is necessary. Currently, no methodology is available to address this discrimination.

Recently, it has been discovered that the misuse of a testosterone gel can be distinguished from an oral or intramuscular testosterone administration through the changes in the steroid profile. In particular, a characteristic increase in the excretion of the 5 α -metabolites of testosterone versus their 5 β counterparts has been detected.

There are two genes encoding two distinct isoenzymes of 5 α -reductase that are differentially expressed in human tissues. The type 1 isoenzyme is transiently expressed in newborn skin and scalp, and permanently expressed in skin from the time of puberty. Type 2 is the predominant isoenzyme detectable in male accessory sex glands and in the prostate. Both enzymes are expressed in the liver. No difference between the ability to reduce cortisol or testosterone, to 5 α -tetrahydrocortisol, or androsterone respectively, has been proved for 5 α -reductase enzymes. In that sense, patients affected by a 5 α -reductase deficiency (type 2 isoform deficient) can be easily diagnosed by finding either low 5 α -tetrahydrocortisol/5 β -tetrahydrocortisol or low androsterone/etiocholanolone urinary ratios. Thus, it is to be expected the topic use of a corticosteroid will produce an increase in the excretion of the 5 α /5 β ratios similarly at what it has been observed for testosterone. As a consequence, a distinction between topical and systemic use of corticosteroids could be accomplished.

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Results and Conclusions

The application of corticosteroids from a potential doping point of view in athletes is considered different based on the different routes of application. Systemic use is considered prohibited but topical use is permitted. This dichotomic situation, easy from regulatory side, is very difficult to face by antidoping laboratories, as no analytical distinction so far has been approved to distinguish both routes of application.

The purpose of the present project was to afford insight into the possibility to discriminate between prohibited and permitted forms of use of corticosteroids based on metabolic findings in urine. Initial hypothesis was based on the differential appearance of reduced metabolites. Primary focus was directed to 5 α and 5 β reduced metabolites based on findings for other steroids. This study needed the synthesis of authentic standards for proper chromatographic identification, which was carried out by reaction with sodium borohydride. However, when actual excretion urines after administration of corticosteroids were studied, none of these metabolites were detected. Focus was then moved to other forms of metabolic reduction, especially on C20. Also the possibility to study metabolites originated by oxidative metabolism had to be considered.

In order to detect as much metabolites of corticosteroids as possible, a series of innovative methodologies based on LC/MS were developed, based on precursor ion scan and neutral fragments losses. An exhaustive methodology was proposed. When it was applied to different corticosteroids administered orally, they were able to detect the presence of some known but many unknown new metabolites. In fact, as much as 28 metabolites were detected for prednisone, 20 for triamcinolone and 28 for methylprednisolone.

The structure of many of those metabolites, however, was not fully identified with the data afforded by the LC/MS approach alone. Taking methylprednisolone as the target compound, an experimental approach combining data from LC/MS and new data generated by GC/MS (methyl-oxime trimethyl-silyl derivatives), it was possible to ascertain the structure of up to 15 metabolites (many unknown so far). Main routes of metabolism identified after oral application were those based on reduction of C20, on oxidation of C6, on C11, on C16, on methyl linked to C6, and the formation of double bond between C6 and C7.

When methylprednisolone was administered topically in a relatively high dose (5g), none of the metabolites were present in urine. Only in one patient receiving repetitive massive doses of topical methylprednisolone, some metabolites were present but in very low amounts. There is no reason for an active athlete to receive those massive administrations.

Thus, in conclusion, to differentiate a systemic from a topical administration of corticosteroids in sport, the establishment of a threshold concentration in urine appears as the decision of choice. It is worth to summarize that the present project, in addition to contribute to the analytical distinction between different routes of administration of corticosteroids in sport, has afforded fundamental information previously unknown regarding the human metabolism of the family of synthetic corticosteroids.

Publications

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- Pozo O, Marcos J, Ventura R, Segura J. Using complementary mass spectrometric approaches for the determination of methylprednisolone metabolites in human urine. *Rapid Communications in Mass Spectrometry* 2011, to be submitted