PROJECT REVIEW

”Synthesis of main metabolites of several aromatase inhibitors and antiestrogens as reference compounds for doping analytics”

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Inhibitors of aromatase (estrogen synthetase) and antiestrogens have been developed mainly as treatment for menopausal breast cancer. The purpose of these drugs is the same, to reduce effects of estrogen. Aromatase inhibitors (letrozole, anastrozole, exemestane) inhibit the production of estrogen, whereas antiestrogens (tamoxifen, toremifene, clomiphene) block the estrogen receptors and thus prevent the interaction of estrogen with the receptors. Abuse of these drugs in sports stems from the fact that they can be used to counteract the adverse effects of an extensive use of anabolic androgenic steroids (gynaecomastia), and to increase testosterone concentration by stimulation of testosterone biosynthesis.

All of the previously mentioned drugs are included in the World Anti-Doping Agency’s list of substances and methods that are prohibited in sports. In doping analysis, the analytical data obtained from the sample are compared to the data obtained from a negative and positive reference. According to the WADA’s international laboratory standard and ISO 17025 standard, well characterized reference materials are recommended to be used as references in the analysis.

Presently, the metabolites of aromatase inhibitors (letrozole, anastrozole, exemestane) and antiestrogens (clomiphene, toremifene) are not commercially available as reference substances. Therefore, we propose to develop a high-yielding and selective synthesis method for the preparation of these metabolites or synthesize some of the metabolites according to the published methods. Although tamoxifen metabolite is commercially available, it will be synthesized for comparison purposes. The synthesized metabolites would then be available as reference substances to the world’s antidoping community. Fully characterized reference substances would enable the reliable and legally defensible confirmation analysis of the drugs. The synthesized substances could also be used in quality assurance and in development of new analytical methods.

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Report and Conclusion

Inhibitors of aromatase (estrogen synthetase) and antiestrogens have been developed mainly as treatment for menopausal breast cancer. The purpose of these drugs is the same, to reduce effects of estrogen. Abuse of these drugs in sports stems from the fact that they can be used to counteract the adverse effects of an extensive use of anabolic androgenic steroids (gynaecomastia), and to increase testosterone concentration by stimulation of testosterone biosynthesis.

According to the WADA’s international laboratory standard and ISO 17025 standard, well-characterized reference materials are recommended to be used as references in the analysis. Presently, the metabolites of aromatase inhibitors (exemestane) and antiestrogens (fulvestrant, toremifene, raloxifene) are not commercially available as reference substances.

In this WADA-funded research project we synthesized 17-hydroxyexemestane and 17-ketofulvestrant, the metabolites of exemestane and fulvestrant, respectively. GC-MS, LC-MS/MS, LC-TOFMS and NMR methods were developed to characterize the synthesized exemestane metabolite in urine samples and to test its stability. The methods can be used also as screening or confirmation methods in doping analysis. The structure of the 17-ketofulvestrant metabolite was confirmed by ^{1}H NMR and LC-MS using both accurate mass measurement and multiple reaction monitoring. Synthesis of raloxifene metabolites and 4-hydroxytoremifene was also studied.

The metabolites synthesized in this project have been delivered to WADA-accredited anti-doping laboratories.

Publications of the project

Vahermo M, Leinonen A, Suominen T, Kuuranne T, Kolmonen M, Yli-Kauhaluoma J. Synthesis of 17-dihydroexemestane as a reference compound in doping control. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, editors. Recent Advances in Doping Analysis (17): Proceedings of the Manfred Donike Workshop, 27th Cologne Workshop on Dope Analysis 1st to 6th March 2009. Cologne: Sportverlag Strauß; 2009. p. 327-330.

Vahermo M, Leinonen A, Yli-Kauhaluoma J. Synthesis of three hydroxyraloxifenes as reference compounds in doping analysis. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, editors. Recent Advances in Doping Analysis (29): Proceedings of the Manfred Donike Workshop, 29th Cologne Workshop on Dope Analysis 13st to 18th March 2011. Cologne: Sportverlag Strauß; 2011. p. 300-303.