"Detection of Testosterone Esters in Blood Sample"

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Project Summary

Testosterone is still regarded as the major contributor to steroid doping world-wide. Among the most common forms of application are injections of different sorts of esters. State-of-the-Art detection of Testosterone doping includes the quantification of the Testosterone /Epitestosterone – Ratio (T/E – ratio) as well as subsequent Isotope ratio mass spectrometry (IRMS). Previous published studies of our research group have demonstrated that a comparably high percentage of testosterone preparations do not significantly differ from endogenous values of testosterone and markers of testosterone doping. Consequently when such preparations with endogenous – like 13CVPDB values are applied, IRMS - technology fails to detect testosterone doping. Direct detection of the testosterone ester leads to an unequivocal proof of doping with testosterone preparations, because such esters are not built endogenously. Previous studies indicate that a direct detection of testosterone esters in both hair and plasma is possible.
Aim of the proposed project is the investigation and optimisation of the direct detection of testosterone esters in body fluids like serum, whole blood and stabilized blood with an already developed detection method using modern and sensitive technology. The project will gain information on diagnostic windows for detection of doping using testosterone esters and proper sampling conditions. Additional aim of the proposed project is to evaluate the suitability of already collected blood samples in doping control (e.g. samples collected for blood parameter measurement or growth hormone detection) for a possible reanalysis for testosterone esters.

Results and Conclusions

Injections of synthetic esters of testosterone are among the most common forms of testosterone application. In doping control, the detection of an intact ester of testosterone in blood gives an unequivocal proof of the administration of exogenous testosterone. The aim of the current project was to investigate the detection window for a number of testosterone esters in blood. Furthermore, the suitability of different types of blood collection devices was evaluated.
A clinical study with six participants was carried out, comprising a single injection of a testosterone ester preparation. The enrolled subjects were randomly assigned to receive either a single intramuscular injection of testosterone undecanoante 1000 mg (Nebido®) or a single intramuscular injection of a mixture of four testosterone esters: testosterone propionate 30 mg, testosterone phenylpropionate 60 mg, testosterone isocaproate 60 mg and testosterone decanoate 100 mg (Sustanon®). Three subjects were assigned to each study group and blood was collected throughout a testing

period of 60 days. At each sampling, the blood was collected into three different blood collection tubes: Tube A: Vacuette 9 ml serum tube with clot activator and gel separator (Greiner bio-one Vacuette®, Kremsmünster, Austria). Tube B: Vacuette 6 ml plasma tube with sodium fluoride, potassium oxalate, and no gel separator (Greiner bio-one Vacuette®, Kremsmünster, Austria). Tube C: Vacutest 3.5 ml plasma tube with sodium fluoride, disodium ethylenediaminetetraacetic acid (Na2EDTA), and gel separator (Vacutest Kima, Arzergrande, Italy). Additionally, a study on the in vitro degradation of testosterone esters in blood was performed, using the same collection tubes as described above. The applied analytical method included liquid-liquid extraction and preparation of oxime derivatives, prior to TLX-sample clean-up and LC-MS/MS detection.

In the clinical study, the elimination half-lives and detection times depended on the type of testosterone ester administrated. The depot effect of an intramuscular testosterone ester preparation increases in proportion to the length of the ester side chain. This is because the half-life of the absorption increases with longer chains.

As expected, the shortest chained ester, testosterone propionate, showed the most rapid elimination and shortest half-life. Nevertheless, the ester could still be detected for 4-5 days in serum and plasma of all study participants receiving the drug. Testosterone phenylpropionate and testosterone isocaproate were detected for at least 8 days in serum and plasma, whereas testosterone decanoate showed a detection time of 18 days. Testosterone undecanoate was detectable in all post-administration blood samples collected during the whole study period (60 days), thereby giving the longest detection time of the esters investigated.

The stability studies showed that the shorter chained testosterone esters were hydrolysed more rapidly in blood collection tubes not stabilized with NaF (tube A) compared to stabilized tubes (tube B and C). The rate of hydrolysis seems to be dependent on storage temperature. In the clinical study, though, the testosterone ester detection window was not affected by the applied blood collection tube.

The work has been submitted to Drug Testing and Analysis. Special issue "Recent Advances in Doping Control Analysis – 33rd Cologne Workshop".