Project Review

"Improvement of a Myostatin Imperacer assay towards a high-sensitive test system for the detection of anabolic manipulations, including gene doping strategies"

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Inhibition of the growth factor myostatin is a promising pharmacological strategy to increase muscle mass. Several companies are currently developing myostatin inhibitors. In addition, there are concerns that inhibition of myostatin could also be achieved by gene doping strategies including viral-based gene overexpression and antisense technologies. Especially the latter possibility is highly relevant with respect to doping abuse because the technology is relatively easy to apply even in small underground labs. In the last two years we have developed a highly sensitive immuno-PCR-based detection system (Imperacer® technology) to analyse expression ratios of several members of the myostatin signaling family. Assays have been developed for Myostatin, Myostatin Propeptide, Follistatin, Follistatin-related protein and Activin receptors (Myostatin Imperacer®). We were able to demonstrate that Imperacer® is a highly sensitive and robust test system that allows detection in extremely small quantities of blood and saliva. A key finding of the project was that serum ratios of the indicated proteins were very stable and were not affected by regular physical training. However, analyzes of blood samples of body builders abusing anabolic steroids indicate significant changes in the pattern of Myostatin Imperacer®. This fits to recent observations from our laboratory, indicating that the Myostatin gene is regulated by androgens and anabolic steroids. Based on these data we have developed the hypothesis that Myostatin Imperacer® technology could be a general tool for the detection of attempts leading to an unphysiological increase of muscle mass.
In the current project we want to further enhance the sensitivity and reliability of the Myostatin Imperacer® assay. We will systematically test if abuse of anabolic steroids is detectable by the established Myostatin Imperacer® assay. We are going to compare ratios measured in venous and capillary blood of abusing and non-abusing body builders to ratios measures in saliva. In addition, in an animal experimental study, mice will be treated with myostatin antisense oligonucleotides and rats with different anabolic steroids. The Myostatin Imperacer® assay and Western Blotting will be used to analyze ratios of relevant proteins in blood and skeletal muscle tissue.
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Results and Conclusions

Our recent research has demonstrated that monitoring the fingerprint expression of members of the myostatin signalling pathway is a promising tool to detect manipulations of myostatin. An important result of this project was the development of a sensitive ACT IIB Imperacer. This is a very essential step into the fight against the abuse of myostatin inhibitors because a soluble form of the activin type IIB receptor (ACT IIB), as a strategy for myostatin inhibition, is already examined in clinical trials (Acceleron). In our opinion our assay is applicable to detect the abuse of such myostatin inhibitors. First results demonstrate that it also works in capillary blood.

As an alternative to antibody based Imperacers we also have started to develop Imperacer assays based on receptor ligand interactions. For example we could demonstrate that myostatin is detectable in the serum via binding to a recombinant ACT IIB receptor linked to DNA. As a basis for an indirect detection of myostatin inhibition ratios of FOLLI, MYPORO and ACT IIB were determined in long term studies with male volunteers and in females in different phases of the menstrual cycle. The results show notably inter-individual variations, however the distinct individual ratios were stable and not affected by training, menstrual cycle and circadian rhythms.
To determine whether the analysis of FOLLI, MYPORO and ACT IIB ratios are suitable to detect anabolic effects of steroids, their expression was analysed in untrained males, bodybuilders abusing anabolic steroids and “clean” bodybuilders. Our data demonstrate a tendency for a lowered FOLLI/MYOPRO ratio in the serum of natural bodybuilders. However, in our opinion, these variations cannot be used to decide whether somebody has abused anabolic steroids or not.

These data are in agreement to data from matching animal experiments. Fortunately, in these experiments we found that the endocrine profile and IGF-1 expression is strongly affected by anabolic steroid abuse. A WADA funded pilot project based on this observation is ongoing in the moment.

Finally we have conducted an animal experiment with myostatin siRNA. The results of this experiment, in agreement to data obtained from Myostatin Knockout (KO) mice, indicate that manipulation of myostatin signalling, even by siRNA, is indeed detectable by comparing ratios of FOLLI, MYOPORO and ACT IIB. Interestingly in these experiments we recognised that fat mass was affected by myostatin siRNA

much stronger than muscle mass. This knowledge is very helpful for identification of new biological markers for indirect detection of myostatin inhibition.

In summary we succeeded in the development of a high sensitive ACT IIB Imperacer, which can be used for the direct detection of specific myostatin inhibitors. This assay seems to be functional in capillary blood samples. Long term studies in males and females indicate that the ratios of FOLLI, MYPORO and ACT IIB are individually very stable and not effected by training, menstrual cycle and anabolic steroids. The use of specific myostatin inhibitors, in our studies specific siRNA to inhibit myostatin in mice, resulted in a significant shift in these patterns. This knowledge is very helpful for indirect detection of myostatin inhibition.