PROJECT REVIEW

"High sensitive detection of genetically and pharmacological manipulations of the myostatin signal transduction pathway by multiplex immuno PCR fingerprint analysis"

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Great progress has been achieved over the past years by means of innovative molecular techniques which has led to the discovery of new growth factors involved in the regulation of muscle development. These findings provide new starting points to understand the mechanisms involved in the adaptation of skeletal muscle to exercise training. One of these newly identified growth factors is myostatin, a member of the transforming growth factor-β family of proteins that has been demonstrated to play a fundamental role in the regulation of skeletal muscle growth during embryogenesis. Blocking of the myostatin signalling transduction pathway by specific inhibitors and genetic manipulations has been shown to result in a dramatic increase of skeletal muscle mass. Drugs or genetic manipulations with the ability to modulate myostatin signalling may have the potential to enhance physical performance in athletes and therefore probably represent a new class of doping substances. To identify manipulations of myostatin signalling, a promising strategy is the analysis of ratios of factors and molecules associated with the myostatin signal transduction pathway, a so-called molecular fingerprint. Manipulations either by application of a factor or by inhibition of its signalling will change these ratios, resulting in a different fingerprint. A methodological strategy to analyse simultaneously the expression of a variety of these factors and relevant signal transduction molecules with high sensitivity is the detection by Immuno

PCR (iPCR).
The presented project aims to establish Real time iPCR as a new tool for highly sensitive detection of members of the myostatin signal transduction pathway in skeletal muscle, blood, oral mucosa, and urine. As targets we will choose myostatin itself, myostatin propeptide, follistatins, the follistatin related gene (FLRG), the activin receptors ActRIIA and ActRIIB, the family of activin receptor –interacting proteins (APRIPs) and gasp-1. The technique of iPCR will allow to analyse these parameters simultaneously using multiplex real time PCR.
During the initial phase of our project this technique will be established and validated using multiplex Real Time iPCR for the detection of the mentioned factors in tissue, blood, oral mucosa and urine in close cooperation with our partner, who has a wide experience using this technique.
In the second phase of the project the technique will be applied to study the expression of myostatin and members of the myostatin signal transduction pathway under training conditions. Such information is very limited but essential to develop test systems to identify manipulations of myostatin signalling either by pharmacological or genetic approaches. Muscle biopsies, blood, oral mucosa, and urine will be investigated. In detail we will determine specific ratios between the different members of the signal transduction pathway (fingerprints) in the different matrices, the effect of physical activity, and inter-individual variations.
In a future phase of the project we will try to analyse if the application of myostatin blocking agencies (antibodies, follistatin peptide) will change such fingerprints. For ethical reasons, as long as myostatin inhibitors are not licensed for therapeutical applications, this will be only possible in animal models.

High sensitive detection of genetically and pharmacological manipulations of the myostatin signal transduction pathway by a multiplex Immuno-PCR (Imperacer™) fingerprint analysis

Results and Conclusions

In the 24 months of the funding period of the project we succeeded in the development of functional ELISAs and immuno-pCR assays (Imperacer™) for MYO, MYOPRO, FOLLI, FSTL and the ACT IIA. The Imperacer™ assay detected the respective proteins with significantly higher sensitivity than the corresponding ELISAs.

To analyze the effects of training on the ratio of MYOPRO, FOLLI and FSTL in serum, training experiments were performed. A single bout of resistance training did not affect the serum levels of the proteins. To analyze long-term training effects, 30 young male volunteers were divided into a control, a strength and an endurance-training group and were trained for three months. The serum levels of the respective proteins in serum before and after training were determined and correlated to the tissue expression of the m. vastus lateralis. Skeletal muscle biopsies were taken and the expression of MYO was analyzed by real time PCR. Serum concentrations of all proteins analyzed displayed a moderate inter-individual variability. More important was the finding that the individual ratios of the analyzed proteins were very constant and were not affected by any kind of training. To get an impression whether skeletal muscle mass or intake of anabolic steroids affect the ratios of the chosen proteins, serum concentrations in non-trained, healthy young males, paraplegic patients and body builders abusing anabolic steroids were determined. There were no significant differences in the average serum concentrations off all proteins analyzed between healthy young man and paraplegic patients. Interestingly, the average serum concentration of MYO propeptide was significantly elevated in the body builder group compared to the other groups. Our data provide evidence that bodybuilders abusing anabolic steroids have different MYOPRO/FOLLI or MYOPRO/ACTIIA ratios than untrained controls or paraplegic patients

As a step towards a suitable routine test system, we developed a multiplex Imperacer™ assay that is able to simultaneously detect MYOPRO and ACTIIA ratios with high sensitivity in a single well. MYOPRO and ACTIIA ratios were not only successfully determined in venous blood, but also saliva and capillary blood.

Summarizing our results, we believe that the data provides evidence that the determination of expression profiles of members of the MYO signaling pathway is a promising strategy to detect potential abuse of MYO inhibitors. We believe that any manipulation of myostatinMYO signaling will be detectable by determination of the ratios of MYO propeptide to other factors involved in MYO signaling. Interestingly this may imply that not only the use of MYO inhibitors but also many other anabolic manipulations (treatment with anabolic steroids including SARMS, GH administration, IGFI and MGF) may be detectable analysing such ratios.

Publications

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