PROJECT REVIEW

"Development of pharmacological in vitro test systems for the structure independent identification of anabolic substances."

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The classical methodology to detect anabolic steroids or other anabolic substances in doping analytics is GC-MS. However, last year, the example of THG has demonstrated that even substances with a chemical structure typical for this class of substances, are sometimes not identified during routine screening by GC-MS if their exact chemical structure is unknown. Obliviously there is the danger that anabolic steroids with unknown chemical structure can be misused without being detected in routine screening. In addition, beside classical anabolic steroids, also substances which interfere with growth factor associated signal transduction pathways may induce anabolic effects. Examples are inhibitors of metalloproteases like TAPI or BB-3103, which have been demonstrated to modulate myostatin synthesis, and provoke myotube hypertrophy. At present it is completely unknown if such substances are being misused for doping. In addition, problems may arise with nutritional supplements for which purity and efficacy are largely unknown.

To detect anabolic substances, independently of their chemical structure, pharmacological in vitro test systems are promising alternatives and supplement the established systems used in routine doping analytics. In vitro test systems are used routinely in the development of pharmaceuticals and for the detection of poisons or endocrine disruptors in the environment. They are easy to handle, reliable and easy to adapt for high capacity screening. Anabolic steroids and growth factor interfering compounds are known to effect cell proliferation and differentiation of a myoblast cell line. In our project we want on the one hand to establish a test system for anabolic substances based on the analysis of cell cycle distribution and differentiation status of myoblast cells by flow cytometry. This test system would also allow identifying nonsteroidal substances with anabolic properties. In addition, a yeast-based test system has been established for the identification of androgenic compounds in the environment (endocrine disruptor identification) and plant extracts. Because of its sensitivity and robustness, this is a promising tool for the detection of anabolic steroids and metabolites in urine. New designer steroids (synthesized in the Institute of Biochemistry, Deutsche Sporthochschule, Köln) will be used together with well known compounds (DHT, THG etc.) to validate and characterize our bioassays.

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Results and Conclusions

The classical methodology to detect anabolic steroids or other anabolic substances in anti-doping analytics is GCMS. However, the example of THG has demonstrated that even substances with a chemical structure typical for this class of substances, are sometimes not identified during routine screening by GCMS if their exact chemical structure is unknown. Obviously there is the danger that anabolic steroids with unknown chemical structure can be misused without being detected in routine screening. In addition, substances which interfere with growth factor associated signal transduction pathways may induce anabolic effects. At present it is completely unknown if such substances are being misused for doping.

To detect anabolic active substances, independent of their chemical structure, pharmacological in vitro test systems are promising complementary techniques to the established systems used in routine anti-doping analytics. In the current project we have successfully established pharmacological in vitro test systems to detect anabolic substances based on their bio effects. Two strategies were followed. On one hand we tried to establish a test system where anabolic effects of substances could be detected because they affect the biological behaviour of C2C12 myoblast cells. On the other hand we tried to establish a test system for the detection of anabolic steroids in the urine, based on a stable transfected yeast androgen receptor (AR) transactivation system. Whereas the C2C12 system allows to detect all substances with the ability to modulate myotube formation, the yeast AR system will be able to detect all substances with affinity to the AR, but independent of a knowledge of the chemical structure.

The results of our experiments demonstrate that analysing the differentiation of C2C12 cells is a promising strategy to detect the anabolic properties of substances. Treatment of C2C12 cells with anabolic steroids including THG, resulted in an induction of differentiation which could be quantitatively detected by measuring the activity of the creatine kinase. However, the system would not be suitable to detect misuse of such substances in athletes' urine.

Our results obtained with the yeast reporter gene system demonstrate that this test system is a powerful tool to characterize substances with affinity to the AR pharmacologically. Furthermore we could demonstrate in first preliminary experiments that the system detects anabolic steroids and corresponding metabolites with high sensitivity even in urine of athletes who have abused steroids and have been identified by GCMS to be positive. Therefore we believe that this system can be developed towards a powerful (pre) screening tool and complement the established anti-doping tests. It is characterized by its easy handling without need of high tech equipment, a high robustness, clearness of results and finally by its good cost efficiency. Because the test system is independent of the chemical structure, it is most suitable to be used as a pre-screening system to identify the misuse of anabolic steroids including selective androgen receptor modulators (SARMs), especially in training out-of-competition controls. Our future aim is now to develop the yeast assay as a suitable and reliable test system applicable to routine doping analytics. Ultimately we aim to

obtain an easy to handle and high sensitive assay with, which can after adoption be used in routine anti-doping analyses.

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