Project Review

“Yeast transactivation systems to support (routine) doping analytics”

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In the last years the WADA has founded studies of our groups aiming to develop in vitro test systems for the structure independent identification of anabolic substances. Our test system is a stable transfected yeast transactivation system (SC) for the identification of substances with affinity to the androgen receptor. Using this test system we could identify and characterise several designer steroids. In the last funding period we further characterized SC and started with the construction of a new reporter gene system in Schizosaccharomyces pombe (SP). SC was able to detect anabolic steroids and their metabolites with a high specificity and sensitivity in urine of abusers (Zierau et al. 2008). Even selective androgen receptor modulators (SARMs) could be detected with SC. In excretion studies with Methyltestosterone in close cooperation with the doping control lab cologne SC was able to detect 1-Testosterone abuse up to 307 hours (GCMS detection limit was 118 hours). Treatment of the urine (concentration, purification) further increases the sensitivity of SC. Using new reporter gene plasmids we could reduce the duration time of the test from 2 days down to 18 h. In addition SP was successfully generated and is now ready to be further characterized. Reaching these milestones, our future aim is to use the SC to supplement GCMS techniques in routine doping analytics. Therefore we want to develop a standard routine procedure protocol to use the system in routine analysis. We also want to further enhance the sensitivity of the system by validation the newly generated SP system. In addition our SC system will be used to identify new long-term metabolites of anabolic steroids. So SC will in addition further improve the sensitivity of the GCMS detection systems

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Results and Conclusions

The classical methodology to detect anabolic steroids or other anabolic substances in anti-doping analytics is MS. However, in particular the example of THG has demonstrated that even substances with a chemical structure typical for this class of substances are sometimes not detected during routine screening by MS, if their exact chemical structure is unknown. Moreover a great number of substances have been developed since the fifties and nowadays many pharmaceutical companies are working on non-steroidal androgen receptor modulators (SARMs) which have a completely different chemical structure and metabolism than classical anabolic steroids. In 2005 and 2006 WADA has funded pilot studies of our groups aiming to develop *in vitro* test systems for the structure independent identification of anabolic substances.

In these funding periods we have successfully established and validated the use of yeast reporter gene systems (SC) for the detection of substances with the ability to bind to the androgen receptor in urine. Besides that we could demonstrate that SC is able to detect anabolic steroids and SARMS in urine. Samples from athletes abusing anabolic steroids were successfully uncovered using SC. The primary aim of our ongoing project was to use the SC system to supplement MS techniques in routine anti-doping analytics. The system is easy to perform, without high tech equipment, cheap and the results are conclusive. Therefore it is most suitable to be used as a pre-screening system to identify the misuseof anabolic steroids, independent of the chemical structure, especially in training controls. Similar systems are successfully in use to identify anabolic misuse in other fields as for example application for enhancement of meat production in livestock. For this we aimed especially to finish the construction of SP and to validate SP in comparison to SC. The “new” SC and SP yeast construction had been finalized and the characterized, resulting in a backup or complementary system for the already established SC system by the SP. Overall the “new” systems are a bit less sensitive and little higher operating expense make them less efficient compared to the “old” SC system.

The next aim was the use and validation of the SC system in further excretion studies with problematic steroids in comparison to MS. A number of different substances have been analysed and in parts results of this of the project part have already been published. Summarizing this, the obtained results are encouraging, but seem to depend on the substance and their specific metabolism. This information is very helpful to further characterise the advantages but also limitations of SC.

Another aim of this founding period was to validate whether the SC system is capable to detect SARM abusing, which was carried out as an animal experiment. Animal experiments with SARMs were performed and post administration urines analysed with

the SC. The results indicate that SC is also able to detect such substances in the urines of excretion studies.

A very important aim was the demonstration of the capability of the SC to identify longterm metabolites. This highlight of our so far conducted experiments was the general test whether the SC is a suitable bio detector to identify stable metabolites in the urine for further structure analysis. The presented results demonstrate that that we succeed in this proof of the principle. We are optimistic that we will be able to succeed in identifying a number of long-term metabolites structures in the future using a larger preparative experimental scale.

Last but not least our aim was to develop a standard operation procedure (SOP) to use SC system in routine anti-doping analysis. In our focused parallel analysis in Cologne and Dresden we have been able to acquire a SOP working in both laboratories with the identical efficiency. The simultaneously analyzed blinded urine samples impressively demonstrated that the results do not vary in quality even if performed in different laboratories. These results confirm the stability as well as the reliability of the SC assay and demonstrate its value as a screening assay in routine anti-doping analysis.

Publications

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