

PROJECT REVIEW

"Advancement of the yeast transactivation system as a screening test for the structure independent identification of anabolic steroid misuse"

P. Diel (German Sports University, Cologne, Germany), **O. Zierau** (University of Dresden, Germany), **W. Schänzer** (German Sports University, Cologne, Germany)

The classical methodology to detect anabolic steroids or other anabolic substances in 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. ***Last year the Wada has founded a pilot study of our groups aiming to develop in vitro test systems for the structure independent identification of anabolic substances.***

Our successfully established test system is a stable transfected yeast transactivation system for the identification of substances with affinity to the androgen receptor. Using this test system we could identify several compounds, including 1-Testosterone, THG, Androstendione, Norandrostendione, Norboletone and Propyltrenbolone as potent binders to the AR and determine their relative potency (Friedel et al. 2006 a and b) The results were in part verified by the Herschberger Assay. ***Based on these results our test system is ready to be used routinely for the characterisation of the anabolic potency of substances and nutrition supplementation products.***

Reaching this milestone, ***our future aim is now to end up with a yeast system which can also detect anabolic steroids in the human urine with high sensitivity and can be used in routine doping analytics.*** Such a test system would be a powerful supplementation of the established doping tests. It is easy to perform, without high tech equipment, cheap and the results are clear. Therefore it is most suitable to be used as a pre-screening system to identify the misuse of anabolic steroids, independent of the chemical structure, especially in training controls. Similar systems are successfully in use to identify anabolic misuse for agriculture applications. ***First experiments have shown that our yeast assay also works in unpurified urine and is able to detect doping abuse also in the urine of athletes being tested positive by GCMS.*** Nevertheless we believe that we can still enhance the sensitivity of the system, by using new yeast strains, new

"Advancement of the yeast transactivation system as a screening test for the structure independent identification of anabolic steroid misuse"

P. Diel (German Sports University, Cologne, Germany), **O. Zierau** (University of Dresden, Germany),

W. Schänzer (German Sports University, Cologne, Germany)

Results and Conclusions

In the funding period we further characterized and improve our *Saccharomyces cerevisiae* reporter gene system (SC) and started with the construction of a new reporter gene system in *Schizosaccharomyces pombe* (SP).

SC was able to detect a variety of metabolites of anabolic steroids with high sensitivity. We also tested the specificity of SC for androgens. Our results demonstrate that SC responds with highest sensitivity and selectivity to AR binding ligands. To test the background activity of SC, the response to male and female unspiked urine samples was compared. No differences could be observed; demonstrating that natural steroid background in males and females does not activate the system. In contrast in urine of abusers of anabolic steroids, SC responds with high sensitivity.

These results have been published in a paper (Zierau et al. 2008). SC was also able to detect non-steroidal androgen receptor binding substances (SARMS). Treatment of the urine (concentration, purification) further increases the sensitivity of the yeast system. Very convincing were excretion studies with Methyltestosterone in close cooperation with the doping control laboratory in Cologne. Urine samples were analyzed time dependent by GCMS and our yeast system. The yeast system responds in a time dependent manner and detects Methyltestosterone abuse up to 307 hours (GCMS detection limit was 118 hours).

To generate a new reporter gene system in the yeast *Schizosaccharomyces pombe* new reporter gene constructs and AR expression vectors were cloned. Using one of our new GFP and β -Gal reporter gene plasmids we could successfully reduce the time span of incubation in SC from 2 days down to 18 h. This makes our assay significantly faster, with similar or even higher sensitivity. In the second year of funding we tried to introduce these new constructs together with a new constructed AR expression vector in *S. pombe* (SP). First transgenic SP yeasts have been now successfully generated.

In conclusion SC is now ready to supplement routine doping analytics. Therefore our next aims are to use and validate the system in further excretion studies with

problematic steroids in comparison to GCMS. Here is the possibility to use the system for the identification of long-term stable metabolites. In addition we want to develop a standard operation procedure for doping testing under routine conditions together with the doping control lab cologne and finish and validate the SP system.

Publications

-Detection of anabolic steroid abuse using a yeast transactivation system. Zierau O, Lehmann S, Vollmer G, Schänzer W, Diel P. Steroids. 2008 Oct;73(11):1143-7. Epub 2008 May 4.

reporter genes and an optimized protocol for sample preparation. In our current assay design, performing the assay includes a reaction time of the reporter gene of 2 days. Using new, GFP- based reporter genes can reduce this time span down to 12 h. Therefore we are planning in our new project to create specific recombinant yeast with more sensitive reporter genes optimised for our purpose based on the yeast strain *Sacharomyces pombe*, which has in addition a much more permeable cell wall. The sensitivity and reliability of the yeast test system will be characterised and standardized using different anabolic steroids and metabolites, ***including SARMS***. For this purpose it will be tested with characterised, standard urine samples provided by the Department of Biochemistry of the German Sports University and in anonymous urine samples of athletes abusing anabolic steroids in comparison to the results of GCMS analytics