Project Review

“Pharmacological characterization of nucleosides as potential modulators of erythropoietin production and effects”

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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 Conclusion:

The results of this research Project present biologic and pharmacological "in vitro" and "in vivo" evidence that increases of EPO secretion during limited oxygen availability may be affected by extracellular adenosine generation and signaling. Therefore, we suggest that adenosine may be a doping agent "in vivo" since bone marrow is an hypoxic tissue. The A3 receptor does not appear to be involved in these direct effects of adenosine in HeL023 differentiation. On the contrary, the A3 receptor appears to be the mediator for the increase in EPO production induced by Adenosine.

The molecular signaling induced by Adenosine to increase EPO release by A3 receptors promotes HIF-1 expression. In particular, Adenosine can affect hematopoiesis at three levels: 1) increasing EPO concentration; 2) increasing ET-differentiation and 3) impairing MK-commitment. Adenosine, through A1 and A3 receptors, improves ET differentiation, accelerating the early commitment signaling. Adenosine may impair MK differentiation tuning PM fate toward the ET commitment. The mechanism appeared to involve the distal molecular effectors of EPO signaling pathway. Moreover, Adenocard increases EPO blood levels. Even if the increase was of modest entity, it is possible that adenosine, given as a unique bolo, has been rapidly degraded in few hours "in vivo". We suggest that a chronic Adenocard treatment may generate a greater EPO increase with relevant physiological effects on PM cells.