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Androgenic Activity Measurement-based Bioanalytical Detection of Steroid Abuse in Sports

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Introduction

Sport has a vital part in society because it instills values such as hard work, perseverance and excellence. However, it is frequently polluted by the illicit use of doping drugs to improve athletic performance and perform under pressure. This undermines fair play and poses a major health danger to athletes. Steroids are now the most often discovered drugs by WADA-accredited laboratories. AAS was first used in athletics in 1950. The World Anti-Doping Agency is now standardizing ways for detecting steroid usage in the Anti-Doping industry through technical publications and recommendations.

Urine analysis processes commonly comprise deconjugation using the glucuronidase enzyme, followed by extraction and concentration. Finally, the target analytes are subjected to mass spectrometry analysis, often tandem MS–MS. These target testing approaches, however, will not discover steroids of unknown composition. The detection of testosterone administration in the battle against doping in sports is based on the measurement of a series of testosterone metabolites and their integration into the athlete's biological passport to carry out a longitudinal evaluation of the fluctuations. Among professional and amateur athletes, steroids are the most often utilized doping drugs. Their popularity has skyrocketed in recent years. However, the usage of steroids is risky and can result in major health problems for both professional and amateur athletes.

Description

In doping control laboratories, detection has mostly relied on the testosterone epitestosterone ratio in urine and the presence of other metabolites. In recent years, it has been demonstrated that persons with a common genetic deletion of the *UGT2B17* gene, which alters glucuronidation, may be able to utilise microdose steroids without being detected. In contrast to the standard anti-doping laboratory approach, E. coli ß glucuronidase hydrolyzed with weak HCl was used to deconjugate the sulphate metabolites with glucuronide forms [1].

At the moment, more techniques and indicators are needed to identify exogenous testosterone administration, particularly in athletes with *UGT2B17* mutations, where detection at low levels is more challenging. With the goal of enhancing ABP's steroidal module, the idea was offered that longitudinal monitoring of androgenic activity in urine might provide complementary and extra information. Based on our findings, there is a strong case to be made for utilizing the androgen receptor as an indirect method of identifying steroid usage in urine. Conducting bioluminescence experiments on modified mammalian cell lines with receptors promises to be a key component in identifying chemicals in urine. This method of detection might be used for any steroid, dose, or route of administration [2].

In comparison to mass spectrometry, bioassays have the benefit of detecting any component, including novel chemicals that interact with AR, regardless of chemical structure, route of administration, or dispensation. These findings support the notion that androgen receptor (AR) assays can be used as a supplement to doping detection as an indirect marker of any substance with an androgenic character and in this case, they can improve the detection of T administration based on the measurement of the T/E ratio and steroid profile, which is heavily influenced by the *UGT2B17* gene polymorphism [3].

We envision determining normal values within a certain population or subpopulation, followed by screening for deviations from these normal values, because BDS' CALUX® bioassays also respond to endogenous hormones. A system that combines screening for unknown compounds with effect-based CALUX bioassays and more specific sensitive and identifying chemical-analytical methods such as GC-MS is expected to provide a robust protocol for detecting the use of almost any chemical compound that interferes with normal steroid hormone action [4].

The comparison of AR CALUX® bioassay activity with the principal endogenous steroids discovered by GC-MS in human urine samples revealed a high correlation, giving proof of principle of the compatibility of both methods, as found in earlier research. These findings support the notion that, in addition to chemical–analytical approaches, the AR CALUX® bioassay can be a useful tool for the analysis of steroids for doping control reasons [5].

Conclusion

The findings allow us to conclude that the AR CALUX® bioassay analysis was capable of detecting exogenous testosterone injection regardless of genotype for the three forms of polymorphism. T intramuscular injection caused a statistically significant change in bioluminescence generated by anabolic androgenic drugs (p 0.001). The analysis method based on the AR CALUX® bioassay allows for the measurement of variations in the androgenic signal in a reproducible and consistent manner without the need for hydrolysis, though the signal without hydrolysis is always significantly weaker, which can facilitate the development of a simple and fast screening method in the future.

While it is not possible to determine an universal cut-off value based on the data obtained, the procedure is successful with a longitudinal follow-up of each individual. Unlike routine methods of analysis in anti-doping that are based on the structure of the analytes, the developed method measures an effect, making it capable of detecting the possible administration of compounds with unknown structures. This can be used for the development of rapid screening methods applicable not only to physiological samples, but also to the possible analysis of nutritional supplements.

Conflict of Interest

None.

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