

PROJECT REVIEW

“Bioassay-based screening and detection of novel designer androgens.”

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The recent identification of two designer androgens, norbolethone and tetrahydrogestrinone (THG), has raised new concerns regarding risks from novel designer androgens custom-produced as undetectable sports doping agents. These two synthetic androgens were produced by modification of existing steroids to produce previously unknown (THG) or non-marketed (norbolethone) potent androgens. THG was produced from gestrinone, a progestin known to have androgenic activity and banned by WADA. Yet, among 36 commercially marketed progestins many have similar or greater androgenic potential than gestrinone and the capability to be chemically modified, potentially forming potent and currently undetectable designer androgens. Banning all synthetic progestins is not feasible given their importance in female reproductive health. Therefore it is essential to characterize the available progestins according to their risk profile and to develop detection methods for those with most androgenic activity and most capable of being developed into so-far undetectable doping agents.

This project involves 3 stages, the first involves an in vitro yeast-based androgen bioassay that we used to provide the first proof that THG was a potent androgen. This in viro androgen bioassay is a sensitive and efficient screening method that can be applied to all available progestins and their analogs or metabolites as compared with a reference panel of synthetic androgens. The second stage involves confirming the androgenic bioactivity identified as positive in the in vitro screening bioassay by an in vivo mouse-based androgen bioassay. The third stage involves developing urinary Mass Spectrometric detection methods for progestins with significant in vitro and in vivo androgenic bioactivity.

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Results and Conclusions

A comprehensive review of commercially marketed synthetic progestins was completed using the yeast-based in vitro androgen bioassay. This led to a detailed description of steroid androgen receptor (SAR) for androgenic activity of synthetic progestins.

The practical implications of this analysis for the WADA antidoping program were that the risk from newer synthetic progestins was relatively low as they lacked significant intrinsic androgenic potency. The major risk was from the older, first generation synthetic progestins, especially those derived from 19-nortestosterone, which had significant intrinsic androgenic potency that could be enhanced by simple chemical modifications. Nevertheless existing GC/MS profiling was already available for these compounds. These conclusions are supported by the rarity of novel progestin-derived illicit designer androgens subsequent to the discovery of THG.

The simplified and modified mouse in vivo androgen bioassays gave quite consistent findings with rank order of potency of the in vitro androgen bioassays. Specific class-based comparisons confirmed that the newer third generation synthetic progestin, as exemplified by nesterone, had negligible androgenic bioactivity whereas the older early generation progestin, norgestrel, had significant intrinsic androgenic potency in vivo. These findings confirmed that no further detailed metabolic analysis of excretory products of modern progestins in humans is warranted as the significant risks are already covered by the known commercially marketed progestins.

Publications

L. McRobba, D.J. Handelsman, R. Kazlauskas, S. Wilkinson, M.D. McLeode, A.K. Heather. Structure–activity relationships of synthetic progestins in a yeast-based in vitro androgen bioassay. *Journal of Steroid Biochemistry & Molecular Biology*, 110 (2008) 39–47.