_PROJECT REVIEW

"The use of Cytochrome P450 inhibitors in sport. A new generation of doping masking agents"

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Cytochrome P450 (CYP450) enzymes are essential for the metabolism of many drugs. Although this class has more than 50 enzymes, six of them metabolize 90% of known drugs, with the two most significant enzymes being CYP3A4 and CYP2D6. Genetic variability in these enzymes may influence the individual response to commonly prescribed drug classes. Moreover, CYP450 enzymes can be inhibited by several drugs, resulting in clinically significant drug-drug interactions that can cause alteration in metabolic routes of absorption and elimination with consequent pharmacological effect or toxic reactions. The extent to which a CYP450 inhibitor affects the metabolism of a drug depends on different factors such as the dose and the ability of the inhibitor to bind to the enzyme.

In anti-doping testing, the knowledge of the pharmacokinetics of a drug/class of drugs is a key component of the analytical strategies for the detection of banned drug administration. Athletes may intentionally take advantage of CYP450 inhibition by co-administrating different drugs to obtain an alteration of the metabolism of the banned drug(s), making more complicated the detection by the anti-doping laboratories. This project is designed to provide information on the role that inhibitors of the most abundant CYP450 enzymes may have in doping scenarios. Specifically, the metabolic profile of selected banned compounds, with special emphasis on threshold compounds and to compounds that are excreted in urine mainly as CYP450 metabolites will be assessed individually and in the presence of selected CYP inhibition agents in vitro, in order to establish if the co-administration of CYP inhibitors with doping agents could be used by athletes as masking strategy.

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Result and Conclusion

This project was designed to investigate the role that inhibitors of the most abundant CYP450 enzymes may play in doping scenarios. The *in vitro* metabolic profile of some representative banned compounds was investigated, with special emphasis to compounds that are excreted in urine mainly as CYP450 metabolites, specifically evaluating the effect of selected, non-banned drugs that are also CYP450 inhibitors, with the aim of verifying whether the co-administration of CYP inhibitors with doping agents could be used by athletes as a masking strategy.

In the first part of this project the *in vitro* metabolism protocol using either pooled human liver microsomes or recombinant human CYP450 isoenzymes (CYP3A4, CYP2D6, CYP2C9 and CYP2C19) was optimized and validated with five representative banned compounds (the selective oestrogen receptor modulator toremifene, the anabolic agents stanozolol, methandienone and the glucocorticoids ciclesonide and deflazacort) in order to obtain a good correlation with the metabolism reported in humans. The optimized *in vitro* model was subsequently utilized in presence of non banned medicaments commonly used by athletes (primarily among them non antifungals, antiacid and antidepressant agents) to investigate their effects on the *in vitro* metabolic profile and on the activity of the CYP450 isoforms involved in the phase I metabolism of the selected banned agents.

The *in vitro* model set up in this study showed good correlation with the previously described metabolism in humans. The CYP450 isoforms involved in the phase I metabolism of the selected banned compounds are the CYP2C9, the CYP2C19, the CYP2D6 and the CYP3A4 isoforms.

On the basis of our results i) the co-administration of banned compounds with antifungals or antidepressants could lead to an incorrect interpretation of the analytical results, producing a masking effect based on the alteration of the phase I metabolic pathways; ii) the number of the markers of drug abuse currently selected during routine analyses should be expanded as much as possible to include also the parent compounds and the possible additional metabolites produced by alternative routes; iii) being the inclusion in our normal routine screening method by LC-MS/MS of the CYPs inhibitors considered in this study very straightforward, if so allowed by the WADA rules, a monitoring study on the real occurrence of CYP inhibitors in the urine samples analyzed by the WADA laboratories would markedly enhance the statistical and epidemiological relevance of our in vitro observations.