

## ***"Masking strategies in sport doping: modulation of phase II metabolism"***

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### **Aims:**

The metabolic steps that lead to drug clearance in the human body are divided into two distinct parts: chemical modifications of the parent compound (phase I); and conjugation of either the parent drug and/or the phase I metabolite(s) with endogenous molecules (phase II). Generally, cytochrome P450 systems are most important for phase I, while UDP glycosyltransferases (UGTs) for phase II reactions. UGTs catalyze the conjugation of the glucuronic acid moiety from UDP-glucuronic acid to many xenobiotics, including drugs/metabolites banned by the WADA. Conjugation facilitates the excretion of the compounds into urine. The kinetics of elimination of a compound could be influenced by genetic variability and/or by the co-administration of drugs that interact with UGTs. In this context, previous studies demonstrated that non steroidal anti-inflammatory drugs, like diclofenac and ibuprofen, decrease the enzymatic activity of the most important UGTs involved in the phase II metabolism of testosterone, whereas benzodiazepines and antifungals alter the glucuronidation kinetics of morphine and codeine.

The preliminary results of an ongoing project, also funded by the WADA, focused on the study of CYP450s inhibitors as potential "masking agents", proved that in the presence of CYP450s inhibitors the in vitro phase I metabolic profiles of several classes of banned substances were extensively modified. In this project we plan to follow an analogous approach to preliminarily investigate whether the co-administration of doping agents with other xenobiotics that are substrate of the UGTs could be used by athletes as a strategy to evade anti-doping testing. Specifically, the in vitro phase II metabolic profile of selected banned compounds, with especially emphasis on threshold substances (i.e 19-norandrosterone, morphine and tetrahydrocannabinol) will be assessed individually and in the presence of selected medicaments commonly used by athletes (non steroidal anti-inflammatory agents, antifungal agents and benzodiazepines).

### **Result and Conclusions:**

This research project focused on the evaluation of, the inhibitory effect of on-prohibited drugs on the phase II metabolism of prohibited drugs. More specifically, we have considered the effect, on the kinetics of the glucuronation reaction catalyzed by uridinediphosphateglucuronosyltransferases (UGT) of 19-norandrosterone, testosterone, epitestosterone and morphine, of non-prohibited drugs

belonging to the classes of antifungals (fluconazole, itraconazole, ketoconazole and miconazole), benzodiazepines (alprazolam, bromazepam, diazepam and triazolam) and non-steroidal anti-inflammatory drugs (diclofenac, ibuprofen, ketoprofen and nimesulide).

In the first part of this project, the in vitro metabolism protocol using either pooled human liver microsomes or recombinant human UGT isoenzymes (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A10, UGT2B4, UGT2B7, UGT2B10, UGT2B15 and UGT2B17) was optimized and validated in order to obtain a good correlation with the metabolism reported in humans. The optimized in vitro protocol was subsequently used to perform the inhibition studies in the presence of non banned medicaments commonly used by athletes (primarily among them antifungals, benzodiazepines and non-steroidal anti-inflammatory drugs).

19-norandrosterone, testosterone, epitestosterone and morphine undergo extensive glucuronidation confirming that the in vitro model developed and optimized in this study provides a good representation of the metabolic glucuronidation reactions in humans, being these data in accordance with information reported by other investigators. The UGT isoforms principally involved in the phase II metabolism are the UGT2B7 and UGT2B17 for 19-norandrosterone, the UGT2B15 and UGT2B17 for testosterone, the UGT2B4 and UGT2B7 for epitestosterone and UGT2B7 for morphine.

Concerning the inhibition study, the results obtained showed that the enzymatic activity of the UGT isoforms involved in the glucuronidation of 19-norandrosterone, testosterone, epitestosterone and morphine are extensively altered in the presence of the antifungals ketoconazole and miconazole. Moderate variations were, instead, registered in the presence of itraconazole, triazolam, diclofenac and ibuprofen and no significant modifications were measured in the presence of the others agents studies. The inhibitory effect of diclofenac, ketoconazole, ibuprofen, itraconazole, miconazole and triazolam on the 19-norandrosterone, epitestosterone, testosterone and morphine glucuronide formation is not an irreversible process, but involves competitive and mixed type mechanisms.

These evidences impose that drug-drug interaction has to be taken into account in doping control analysis, to reduce the risk of reporting uncorrected results.