"An in vitro study on the biotransformation pathways of vaptans. Selection of the most appropriate markers of misuse"

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Project Overview

Vaptans are a relatively new class of compounds with diuretic suitable for publication on WADA's website effects. They increase water excretion by inhibiting its reabsorption in the renal collecting ducts. For these reasons, vaptans have been included by the World Anti-Doping Agency in the section S5 "Diuretics and Masking agents" of the list of substances and methods that are prohibited in sport. Previous investigators have reported that this class of compounds is extensively cleared by hepatic metabolism via oxidative enzymes. Consequently, the most efficacy strategy to detect them in urine samples could not be achieved by simply targeting the drug itself: the selection of one or more diagnostic metabolites is necessary. Very few data are reported in literature on the metabolic profile of these compounds in humans; at the same time, the influence of physiological and environmental factors on their excretion profile is also almost completely unexplored yet.

This project aims to define the phase I and phase II metabolic reactions and to characterize the enzymatic isoforms involved in the biotransformation pathways of vaptans. Alterations of the metabolic profile of vaptans provoked by physiological (i.e. sex, genetic polymorphism) and environmental (drug-drug interactions) factors, will be evaluated to obtain information on the impact of these factors on the biotransformation pathways detected. Finally, the possibility of obtaining sufficient amounts of vaptans metabolites by enzyme-assisted synthesis will also be explored. Human liver microsomes will be the source of the isoenzymes involved in the phase I metabolism of vaptans; the metabolites once formed will be isolated by HPLC, characterized by MS and used as reference materials to set up and validate efficacy analytical procedures to detect vaptans in urine

Results and Conclusions:

This research project focused on the characterization of the main biotransformation pathways of vaptans with the aim to identify class-specific metabolic pathways of vaptans and select the most appropriate marker(s) of intake.

In the first part of this project, the *in vitro* metabolism protocols using either pooled human liver microsomes or recombinant human CYP and UGT isoenzymes were optimized and validated in order to obtain a good correlation with the metabolism reported in humans. The optimized *in vitro* protocols were subsequently used to evaluate the effects of physiological

(gender and genetic polymorphism) and environmental (drug-drug interactions) factors on the metabolic profile of vaptans.

Lxivaptan, mozavaptan and tolvaptan were extensively biotransformed (tolvaptan>lixivaptan>mozavaptan) mainly by CYP3A subfamily and to a lesser degree by CYP2C19 and 2D6 enzymatic isoforms to 13, 4 and 20 metabolic products respectively. Conivaptan was moderately biotransformed by CYP3A subfamily to 2 hydroxylated metabolites due to its well-known inhibitory activity against the CYP3A4 enzymatic isoform. The phase-I biotransformation pathways include hydroxylation in different positions, carboxylation, dehydrogenation, hydrogenation, N-dealkylation, isomerization and combinations of them. Most of the above-mentioned phase I metabolites once formed undergo an extensive glucuronidation by UGT2B isoforms.

Concerning the effects of physiological an environmental factors, the results obtained showed that (i) no appreciable gender differences were registered in our experimental conditions for all the compounds under investigation; (ii) significant differences were instead registered depending on the specific allelic variant used. More in details, in the presence of the allelic variant CYP3A5 *1*1, the formation of the phase I metabolites of second step appeared to be faster if compared to the other CYP3A5 allelic variants (CYP3A5 *3*3 and CYP3A5 *1*3) and finally (iii) the levels of all the metabolites were significantly reduced in the presence of the imidazole antifungals tested. On the contrary, in the presence of the steroidal progestins the reduction was less evident, and observed only at the highest concentrations studied.

On the basis of the above observations the most appropriate merkers of intake for conivaptan is the parent compound; the parent compound and the hydroxylated metabolites for lixivaptan; the parent compound and the de-methylated metabolite for mozavaptan; and finally the parent compound and the hydroylated and N-dealkilated hydroxylated metabolites for tolvaptan. These analytes were easily added to the LC-MS/MS analytical procedure currently used in our laboratory to detect more than 150 prohibited compounds.