

“Salbutamol metabolism how to differentiate oral vs. inhaled administrations: Looking outside the box”

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Project overview

Salbutamol belongs to the class of beta-2-agonists, and its use is prohibited by the WADA if administered for reasons different than the pharmacological treatment of respiratory diseases (i.e. asthma, bronchospasm). At present, discrimination between the therapeutic and illicit use of salbutamol is based on concentration thresholds, being the urinary concentration of non-sulfated drug mainly, but not exclusively, related to the administered dose and to the route of administration. Small differences in metabolism can be observed due to the difficulty of avoiding a partial oral administration during inhalation administration where a fraction of the dose is swallowed. It appears that investigation of the location of the majority of salbutamol metabolism is of great interest and may add additional knowledge for better discrimination of salbutamol route of administration.

It seems adequate in terms of analytical and metabolic reasons to develop a LC/MS/MS method for the simultaneous detection of the free, glucuronated and sulphated forms of salbutamol. In addition, an evaluation of the spontaneous urinary salbutamol hydrolysis could be estimated (stability test). The combination of chiral liquid chromatography coupled to mass spectrometry will add a dimension to salbutamol metabolism assessment. The determination of the well accepted S/R ratio of the free + glucuronated salbutamol to differentiate oral vs. inhaled administration would be complemented by the determination of the S/R ratio of the sulphated fraction. The determination of the sulphated fraction will offer the possibility to better understand and discriminate between different routes of administration.

The genotypic characterization of selected sulfotransferases (SULT's) of the individuals participating to the study (coming from two different ethnic groups) will also be performed, in order to correlate the genotypic expression of the sulfotransferases (SULT), that are the enzymes responsible for salbutamol metabolism with the phenotypic data obtained from the experiments described in the previous step.

Results and Conclusions

Salbutamol is one of the most used β 2-adrenoceptor agonists by athletes for relieving bronchospasm and for prevention of exercise-induced-asthma. Inhalation is the preferred route of administration, but also the oral route is recommended, in particular for populations where inhalation presents practical problems (i.e. young children). Since salbutamol, similar to other β 2-adrenoceptor agonists, produces an anabolic effect when sufficiently high doses are administered orally, its use is approved by the World Anti-Doping Agency (WADA) only after inhaled administration. Currently, sport authorities monitor salbutamol use in and out of competition: urine concentrations greater than the Decision Limit of 1200 ng/mL (free + glucuronated salbutamol) are considered adverse analytical finding unless the non- inhaled administration could be excluded by other means. Previous studies have demonstrated that after oral administration, the majority of salbutamol is found in the urine either as the parent compound (24-33%) or as conjugated sulfate metabolite (48%). On the contrary, no significant biotransformation occurs in the lungs, thus the percentage of salbutamol metabolite depends mainly on the percentage of the dose that is swallowed and absorbed from the gastrointestinal tract.

Salbutamol is administered as a mixture of two enantiomers: S(+)-and R(-)-salbutamol and enantioselective disposition studies have demonstrated that after oral administration the enantiomers are conjugated at a different rate by the body tissues. The active R(-) enantiomer undergoes a higher rate of sulfation, and therefore, after oral intake the non metabolized S(+) enantiomer is excreted in a greater level than the non metabolized R(-) enantiomer. For the above reasons the ratio between S(+) and R(-) of the unchanged salbutamol was proposed as marker of oral administration.

Based on the above evidence, an analytical method involving a solid-phase clean-up procedure followed by a chiral HPLC separation and a mass spectrometric detection to quantify separately the enantiomers of salbutamol and its sulphate conjugates has been developed and validated according to ISO17025 and WADA requirements.

The urinary results confirmed that after inhalation the enantiomeric ratio between S(+) and R(-) of the non metabolized and of the metabolized salbutamol strongly depends on the percentage of the dose that is swallowed. The sulphotransferases (SULT's) responsible for the conjugation of salbutamol in different tissues, and for the different excretion of S(+) and R(-) enantiomers depending on the route of administration, are polymorphic and then large differences between individuals may be expected. In some specific cases, this may be the reason for reaching elevated concentrations of salbutamol after inhaled therapeutic administrations (permitted).

To access to the information of the genotyping of this SULT's may be crucial for the correct interpretation of the phenotypic data. To obtain genomic DNA from blood or saliva samples is a common practice in forensic investigations. In doping analysis urine still being the unique biological specimen available in more than 80-90% of the cases. Then it demonstrated the feasibility of obtaining genomic DNA from urine samples of the adequate quality for the genotyping of the sulfotranferases of interest. A method for the extraction and amplification of genomic DNA form blood, urine and saliva samples was developed and validated, permitting to obtain genomic DNA of adequate quality starting from 2-5 μ L of serum, 1-10 mL of urine or 2-5 μ L of saliva respectively.