“Enantioselective pharmacokinetics of formoterol and application to doping control”

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Project overview

Formoterol is a long acting beta2-agonist allowed for use in athletes via inhalation at doses up to a predetermined limit, and is not allowed to be administered orally. The drug is usually administered and measured as a racemic mixture consisting of an active R/R- and inactive S/S- enantiomer, each with a different time course in the body which also differs by route of administration. The inactive S/S-formoterol is preferentially eliminated in urine relative to the active R/R-formoterol. Recent work has demonstrated that the Adverse Analytical Finding (AAF) limit, currently 40 ng/ml in urine, is difficult to reach and some authors have even questioned whether it is worth having an AAF limit for formoterol at all. To date, anti-doping strategies have not capitalised on this difference between formoterol enantiomer elimination from the body. We will use our advanced analytical technique (enantioselective LC-MS/MS) to characterise the urinary levels and ratio of both R/R- and S/S-formoterol enantiomers, as well as the glucuronide metabolite in athletes dosed with formoterol in prohibited regimens; an “acute” treatment regimen that exceeds the current allowable dose via inhalation and an oral regimen consisting of 160 µg/day for 7 days. The secondary objective is to characterise the urinary levels and ratio of formoterol enantiomers and glucuronide metabolite in athletes using repeated “chronic” inhaled rac-formoterol under permitted regimens. Study design will be double-blinded placebo controlled with two “chronic” 7-day treatment regimens; formoterol delivered by inhalation at just below the maximum allowable dose, and approximately half of the maximum allowable dose. This project will allow doping agencies to better discriminate between permitted and prohibited formoterol dosing.

Results and conclusions:

Oral dosing of beta2-agonists is known to lead to beneficial performance effects in athletes, but some drugs of this class are allowed to be delivered by inhalation for use in athletes with asthma. Formoterol is a long acting beta2-agonist permitted for use in athletes at inhaled doses up to 54 µg over 24 hours, however, a threshold limit of 40 ng/ml in urine is used to control for supratherapeutic dosing. The drug is usually administered as the racemic (*rac*-) mixture consisting of the active (R,R)- and inactive (S,S)- enantiomers with different metabolic profiles. The primary objective of the study was to examine whether the urinary levels and ratio of formoterol enantiomers in individuals dosed with formoterol could be used to improve the sensitivity and specificity of a urine threshold approach to ascertain whether individuals were using formoterol in a permitted manner. Urine levels were measured

following prohibited regimens; an “acute” treatment regimen that exceeds the current allowable dose (54 μg in 24 hours) by administering a once-off 72 μg dose via inhalation at baseline, and an oral regimen consisting of 156 μg/day for 7 days. These levels were compared with repeated “chronic” inhaled *rac*-formoterol under permitted regimens (12 μg twice daily for 7 days, 24 μg twice daily for 7 days). Comparisons were made with current urine dosing threshold and decision limits (TD2019DL) to assess the utility of enantioselective ratios. Using the current TD2019DL method, none of the samples from the chronic permitted regimens exceeded the current threshold for total formoterol (40 ng/ml) supporting the existing approach. The acute inhaled and oral prohibited regimens resulted in 7/127 and 21/124 samples exceeding the threshold (TD2019DL), and 5/127 and 13/124 exceeding the decision limit respectively. There were an increased number of samples exceeding threshold when urine was normalised for hydration by specific gravity. Females demonstrated higher urine levels than males. As predicted, there were differences in enantiomer ratio between oral and inhaled administration, but receiver operating characteristic (ROC) analysis for log(R,R:S,S) formoterol enantiomer ratio showed poor diagnostic performance (ROC area=0.716 for oral treatment) and was inferior to the current TD2019DL approach, possibly due to considerable pharmacogenetic enantioselective differences in glucuronidation metabolism. In conclusion, the current urine method (TD2019DL) based on a threshold and decision limit of 40 ng/ml and 50 ng/ml respectively appears a valid approach to discriminate between oral (prohibited) and permitted inhaled dosing regimens in urine spot samples but requires further validation with regard to intra-day spot sampling variability and dosing regimens. Further work is required to understand the basis of the significant differences in enantioselective glucuronidation between subjects and genders, and improve the sensitivity of the current threshold. Formoterol elimination seems to be particularly sensitive to urine hydration and a greater understanding of exercise on hydration and excretion is also warranted with regard to application of TD2019DL threshold approach.