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UDP-Glucuronosyltransferases in conjugation of 5α- and 5β-androstane steroids

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Abstract

This study is an extension of our earlier work on the glucuronidation of testosterone and epitestosterone and carried out to reach a better understanding of the substrate specificity, particularly stereo- and regioselectivity of human UDP-glucuronosyltransferases (UGTs) in the steroid glucuronidation.

The substrates of the study were androsterone, etiocholanolone, 5α-androstane-3α,17β-diol (5α-diol), and 5β-androstane-3α,17β-diol (5β-diol), allowing the examination of the effects of a flat versus bent A-B-ring steroid scaffold and the presence or absence of 17-OH group. Nineteen recombinant human UGTs were members of subfamilies UGT1A, UGT2A and UGT2B, and expressed in baculovirus-infected insect cells. The screening of UGT activities of each analyte and glucuronidation kinetics of androsterone and etiocholanolone were determined in an *in vivo* assay, and the quantitative analyses of intact glucuronide-conjugated analytes were performed by UPLC-ESI-qTOF instrument in negative ion mode.

Among the nine UGTs of subfamily UGT1A, only UGT1A3 and UGT1A4 exhibited any detectable, although low glucuronidation activity and the enzymes of this subfamily do not contribute much to androgen glucuronidation. UGT2A1 and UGT2A2 glucuronidated most compounds of this study with unique stereo- and regioselectivity, both having clear preference toward 17-OH of 5α-diol but strictly toward 3-OH of 5β-diol. Glucuronidation took place mostly at low rates, but by UGT2A1 for etiocholanolone the reaction was unusual with low affinity but extremely high rate.

The most important UGTs with respect to androgen metabolism belong to subfamily UGT2B and large differences were revealed in specificity of UGT2B7, UGT2B15 and UGT2B17. UGT2B7 conjugated all four substrates, but only at 3-OH and preferring the flat 5α-backbone. UGT2B17 readily conjugated 3-OH, but exhibited high activity towards 17-OH whenever it was available in the structure. UGT2B15 did not glucuronidate any of the studied substrates at the 3-OH, but did conjugate both diols at the 17-OH, with a clear preference for 5α-diol.

In routine doping control the key role of UGT2B17 and its deletion polymorphism has been recognized in testosterone glucuronidation and therefore, it might be useful to identify urinary androgen metabolites, such as 5α-diol-3-*O*-glucuronide, the level of which is not directly affected by the presence or absence of UGT2B17. The overlap in substrate selectivity of the UGTs, especially when focusing on an individual metabolite, is not as broad as often assumed.

For the complete paper, please, see the following reference:

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