Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (11)

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Sport und Buch Strauß, Köln, 2003

T. KUURANNE, M. KURKELA, M. THEVIS, W. SCHÄNZER, M. FINEL, R. KOSTIAINEN:

Structure-Function Relationships in the Glucuronidation of Anabolic Androgenic Steroids by Recombinant Human UDP-Glucuronosyltransferases

In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (11). Sport und Buch Strauß, Köln, (2003) 163-165

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Structure-function relationships in the glucuronidation of anabolic androgenic steroids by recombinant human UDP-glucuronosyltransferases

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UDP-glucuronosyltransferases (UGTs; E.C. 2.4.1.17) are a family of membrane-bound enzymes of the endoplasmic reticulum that catalyze the glucuronidation of endogenous and exogenous compounds. Via glucuronidation the parent compounds are generally transformed into less toxic metabolites. Conjugation of the aglycone substrate with glucuronic acid moiety increases the polarity of the steroid aglycone, leading to enhanced excretion in the urine. The human genome encodes at least 16 different UGTs, and most of them are expressed in the liver that is considered to be the major site of glucuronidation. However, some UGTs are extra-hepatic enzymes, and many of the liver UGTs are also found in other tissues.

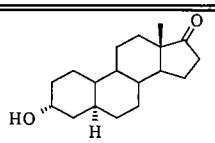
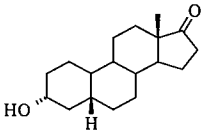
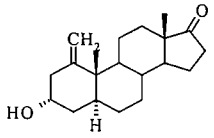
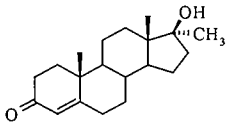
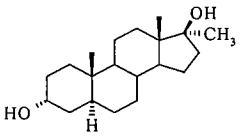
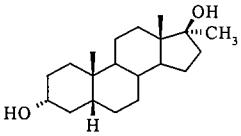
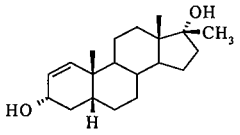
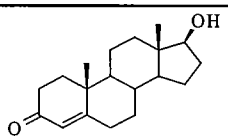
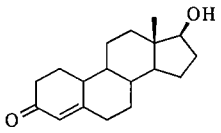
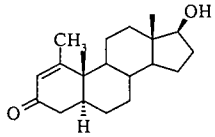
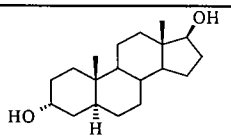
In the present multi-dimensional study we examined the activity of individual recombinant human UGTs in glucuronidation of a set of 11 exogenous anabolic steroids and their phase-I metabolites, in order to gain insight into the structural factors that affect the enzyme-aglycone interactions. The analyses were carried out using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with electrospray ionization (ESI), which allowed direct determination of steroid glucuronides. Large differences were detected between the enzymes with respect to the conjugation profiles of the 11 tested aglycones. Two UGTs, 1A6 and 1A7, did not exhibit measurable activity towards any of the aglycones that were examined in this

study. Regio-selectivity was demonstrated by UGTs 1A8, 1A9, and 2B15 that preferentially catalyzed hydroxyl glucuronidation at the 17β-position, whereas most of the other enzymes glucuronidated hydroxyl groups at both the 3α- and the 17β-positions. Stereo-selectivity was observed in glucuronidation of diastereomeric nandrolone metabolites (5α-estran-3α-ol-17-one and 5β-estran-3α-ol-17-one), while such specificity was not seen when analogous methyltestosterone metabolites were assayed. 5α-androstane-3α,17β-diol was readily glucuronidated by UGTs 1A1, 1A3, 1A4, 1A8, 1A9, 1A10, 2B4, 2B7 and 2B15, but none of them exhibited methyltestosterone glucuronidation activity. Methyltestosterone glucuronidation activity of human liver microsomes was extremely low, whereas in induced rat liver microsomes it was significantly higher. It was also surprising to find large differences in the activity of highly homologous UGTs 1A7-1A10 towards this set of aglycones.

This presentation was based on scientific paper published completely as:

Kuuranne, T., Kurkela, M., Thevis, M., Schänzer, W., Finel, M., and Kostiainen, R. Glucuronidation of anabolic androgenic steroids by recombinant human UDP-glucuronosyltransferases. Drug Metab. Dispos. 31:1117-1124 (2003).

Table 1. Structures and nomenclature of steroid glucuronides (parent compounds in capital letters).

Compound	Structure	Sites of glucuronidation	Precursor
5α-estran-3α-ol-17-one		3α-OH	nandrolone
5β-estran-3α-ol-17-one		3α-OH	nandrolone
1-methylen-5α-androstane 3α-ol-17-one		3α-OH	metenolone
17α-methylandrost-4-en-17β-ol-3-one		17β-OH	METHYL-TESTOSTERONE
17α-methyl-5α-androstane-3α,17β-diol		3α-OH	mestanolone, methyltestosterone, oxymetholone
17α-methyl-5β-androstane-3α,17β-diol		3α-OH	metandienone, methandriol, methyltestosterone,
17β-methyl-5β-androst-4-ene-3α,17α-diol		3α-OH, 17α-OH	metandienone
4-androsten-17β-ol-3-one		17β-OH	TESTOSTERONE
estr-4-en-17β-ol-3-one		17β-OH	NANDROLONE
1-methyl-5α-androst-1-en-17β-ol-3-one		17β-OH	METENOLONE
5α-androstane-3α,17β-diol		3α-OH, 17β-OH	testosterone