Journal of Steroid Biochemistry & Molecular Biology 127 (2011) 374–381

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Metabolic studies with promagnon, methylclostebol and methasterone in the uPA$^{+/+}$-SCID chimeric mice

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ARTICLE INFO

Article history:
Received 26 January 2011
Received in revised form 6 June 2011
Accepted 25 June 2011

Keywords:
Doping
Steroids
Chimeric mouse
GC–MS
Urine

ABSTRACT

The chimeric uPA$^{+/+}$-SCID mouse model, transplanted with human hepatocytes, was previously validated as an alternative tool to study *in vivo* the human steroid metabolism. This humanized mouse model was now applied, in the framework of anti-doping research, to test different nutritional supplements containing steroids. These steroids, intentionally or accidentally added to a nutritional supplement, usually are derivatives of testosterone. Information about the metabolism of these derivatives, which is important to assure their detection, is quite limited. However, due to ethical constraints, human volunteers cannot be used to perform experimental excretion studies.

Therefore the chimeric mice were selected to perform three separated excretion studies with superdrol (methasterone), promagnon and also methylclostebol. The urine of the humanized mice was collected 24 h after a single dose administration and analyzed by gas chromatography–mass spectrometry (GC–MS). The results indicated the presence of several metabolites including a 3-keto reduced metabolite and numerous hydroxylated metabolites. Also phase 2 metabolism was investigated to update the complete picture of their metabolism.

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1. Introduction

Anabolic steroids are a popular class of doping substances due to their performance-enhancing properties, like stimulating muscle growth [1]. Steroids are mentioned on the prohibited list of the World Anti-Doping Agency (WADA) and hence their use in sports is forbidden [2]. More and more newly designed steroids and prohormones become freely available via the worldwide web without essential information on their toxicological and pharmacological characteristics. This, in combination with the known side effects and health risks of steroid use, raise ethical concerns towards human excretion studies. Use of supplements, containing steroids, can lead to an adverse analytical finding in doping control screening, which implements suspension for the athlete or even the end of his/her sports career [3]. The continuous production of new synthetic steroids is challenging the anti-doping research laboratories in order to assure proper detection after intake. Open screening methods can aid in the detection of unknown substances [4,5], but for the more sensitive target screening methods the specific metabolites need to be identified via *in vivo* or *in vitro* experiments.

In the human body the parent steroid compound is often metabolised to enhance its bioavailability and/or excretion. In general, the liver is the main organ responsible for these drug transformations. Therefore several *in vitro* models have been developed using liver (cell) fractions, e.g. hepatocyte cell cultures and intact liver-slices. They offer a good practical approach, but these cell cultures have a limited viability and are difficult to sustain, requiring complex culture media and methods [6]. Although these *in vitro* based technologies have proven to be extremely valuable in the assessment of drug safety, *in vivo* experiments show a better correlation with the human situation. However, the use of animals for *in vivo* studies also has two major drawbacks: (1) ethical considerations towards animal experiments (especially primates) and (2) high inter-species differences in metabolism which make extrapolation of the results to humans difficult.

To overcome these concerns, transgenic mice were generated which carry a liver specific homozygous over-expression of the mouse urokinase-type plasminogen activator (uPA$^{+/+}$) gene, inducing liver disease and deficiency. The combination of the high regenerative capacity of the liver and the severe combined immune deficiency (SCID) background renders these transgenic mice susceptible for transplantation of human hepatocytes without the risk

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doi:10.1016/j.jsbmb.2011.06.010

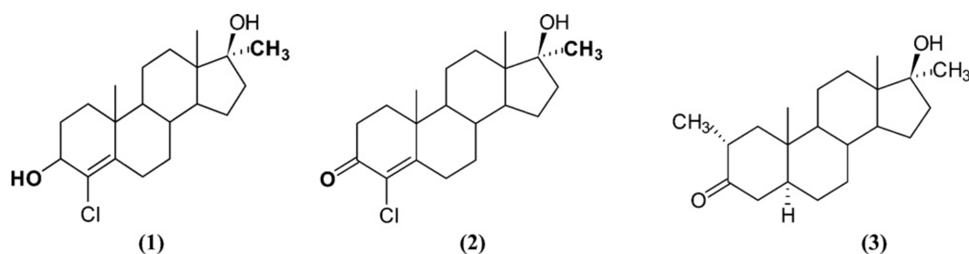

Fig. 1. Chemical structures of (1) promagnon, (2) methylclostebol and (3) methasterone.

of transplant rejection [7]. This model has superior qualities compared to other *in vivo* or *in vitro* models to perform metabolic studies due to its excellent correlation with the human liver [8]. Previous studies have demonstrated that the steroids administered to these chimeric mice underwent approximately the same metabolic transformations as in human subjects [8–11]. In this way the *in vivo* human metabolism of illicit substances can be studied without involving human volunteers.

For the study described herein, this small humanized animal model was applied to investigate drug metabolism of steroids present in supplements for the discovery of urinary markers, which will assist in identifying steroid abuse. Therefore some selected 'nutritional supplements', containing designer steroids or prohormones, were administered to chimeric mice to obtain data about their metabolism and thus making their detection possible.

Most 'nutritional supplements' are not marketed as pharmaceutical preparations and in many cases even a proper labeling of the contents is missing or they can contain steroids that have never been approved as medicinal drugs [3,12]. Prohormones are steroid precursors which will be converted to the active steroid in the body, while designer steroids are modified steroid compounds with similar or enhanced effects but synthesized in such a way (1) to avoid detection in routine doping analyses, and (2) to circumvent legal regulations that preclude the over-the-counter sale of defined steroidal products [3,12].

Three selected compounds were administered to the chimeric mice: (1) 4-chloro-17α-methyl-andro-4-ene-3,17β-diol, available on the supplement market as promagnon; (2) methylclostebol; and (3) methasterone, sold under the name of superdrol (Fig. 1). These products were used to perform *in vivo* excretion studies with the chimeric mouse model in order to evaluate their phase I and phase II metabolism via GC–MS analyses in the framework of doping control.

2. Materials and methods

2.1. Reagents and references

Phosphate buffered saline (PBS) was bought from Invitrogen (Merelbeke, Belgium). Diethyl ether and ethanol were obtained from Biosolve (Valkenswaard, The Netherlands). Na_2SO_4, K_2CO_3 and NH_4I were purchased from Merck (Darmstadt, Germany). $NaHCO_3$ was from Fisher Scientific (Loughborough, UK). 17α-Methyltestosterone (Internal Standard, ISTD) was a gift from Organon (Oss, The Netherlands). N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Karl Bucher (Waldstetten, Germany), ethanethiol (97%) was from Acros (Geel, Belgium), β-glucuronidase from *Escherichia coli* K12 was from Roche Diagnostic (Mannheim, Germany) and β-glucuronidase from *Helix pomatia* from Sigma–Aldrich (Steinheim, Germany). Phosphate buffer (pH 7) and acetic buffer (pH 5.2) were in-house prepared by dissolving respectively $Na_2HPO_4{\cdot}2H_2O/NaH_2PO_4{\cdot}H_2O$ from Merck (Darmstadt, Germany) and sodium acetate from VWR (Leuven, Belgium) in aqua bidest.

The following 'supplements' were ordered via the internet: Promagnon was bought from Peak Performance Laboratories and Superdrol from Anabolic Xtreme/Designer Supplements. The reference material of methylclostebol and methasterone were bought from Toronto Research Chemicals (TRC, Toronto, Canada). The reference material of the metabolites 6β-hydroxymethylclostebol and 15α-hydroxymethylclostebol were a kind gift from the Department of Chemistry, Wroclaw University (Wroclaw, Poland). Other reference standards of possible metabolites were not commercially available at the time that this study was carried out.

2.2. Excretion studies with mice

The project was approved by the Animal Ethics Committee of the Faculty of Medicine of the Ghent University (ECD 06/09). The chimeric $uPA^{+/+}$-SCID mice were produced and maintained as described before [7]. The administration studies were performed in special metabolic cages (Tecniplast, Italy). In this way the mice had unlimited access to water and powdered food, while the design of the cages allows for easy and accurate urine collection without disturbing the mice. Moreover, the mouse urine is perfectly separated from the faeces.

The excretion studies were performed by oral gavation of 100 μL of a PBS suspension containing the supplement or the reference standard of the steroids. Urine was collected prior to (=blank) and 24 h after a single dose administration. All samples were stored at −20 °C until analysis. In addition, non-transplanted $uPA^{+/-}$-SCID mice, without human hepatocytes, were included in these administration studies as a control group for the interspecies differences. In a pilot study the detection window was set by selecting a correct dose which is no longer detectable 24 h after its oral administration, which implements reuse of the mice after respecting a sufficient washout period.

2.3. Sample preparation

A general sample preparation method, used in routine doping control screening, was followed to extract the steroids from the mouse urine [8]. Since the mice produce a limited amount of urine every day (±1.5 mL/24 h), the extraction method was started from only 100 μL of mouse urine and 17α-methyltestosterone was used as internal standard. The first analytical step was hydrolysis of glucuronides by adding 1 mL of phosphate buffer (pH 7) and 50 μL β-glucuronidase from *E. coli*. The mixture was placed in the oven for 2.5 h at ± 56 °C. After cooling to room temperature 300 mg $NaHCO_3/K_2CO_3$ (2/1; w/w) solid buffer and 5 mL of diethyl ether were added. Extraction of the combined free and released glucuronides conjugates was performed by rolling for 20 min followed by 5 min centrifugation. Afterwards the organic layer was separated and dried over anhydrous Na_2SO_4 and evaporated under oxygen-free nitrogen. The residue was derivatised with 100 μL of

the MSTFA/NH_4I/ethanethiol mixture for 1 h at ± 80 °C. Afterwards, 50 μL was transferred in a vial to be analyzed on GC–MS.

Besides this regular extraction procedure, also the separated fractions were investigated (without hydrolysis = free, or with hydrolysis: glucuro-conjugated or sulpho-conjugated). For the hydrolysis of sulphates 1 mL of acetic buffer (pH 5.2) and 50 μL β-glucuronidase from *H. pomatia* were added and followed by the similar liquid-liquid extraction with diethyl ether [8].

2.4. GC–MS parameters

The analysis of the samples was conducted in scan mode on an Agilent 6890 gas chromatograph (GC) directly coupled to an Agilent 5973 mass selective detector (MS) (Palo Alto, USA) with a mass scan range from 50 to 800 mass to charge ratio (*m*/*z*). The GC-column was an HP-Ultra 1 (J&W, Folsom, CA, USA), 100% methyl silicone column with a length of 17 m, an internal diameter of 0.2 mm and a film thickness of 0.11 μm. The electron energy was set at 70 eV and the ion source temperature at 250 °C. Injection volume was 0.5 μL, splitless. A general temperature program was used: 120 °C, 70 °C/min → 180 °C (0.1 min), 4 °C/min → 234 °C (0.1 min), 30 °C/min → 300 °C (2 min).

3. Results and discussion

Evaluation of the different excretion urines was done by comparing pre- and post-administration mouse urine samples from chimeric and non-chimeric mice. The results of the promagnon, methylclostebol and methasterone excretion studies are described. The presented GC–MS chromatograms are all obtained after the regular extraction procedure with hydrolysis, unless otherwise stated.

3.1. Promagnon

Promagnon was the first 'supplement' administered to the mice to investigate its urinary detection and the presence of species-specific metabolites. According to the label it contains the following steroid compound 4-chloro-17α-methyl-andro-4-ene-3,17β-diol as active substance and this structure is presented as promagnon in Fig. 1.

Promagnon is provisionally not commercially available as a reference standard. Therefore the pink pentagonal shaped supplement pills were analyzed for their content by using GC–MS. The presence of two isomers (e.g. C3α and C3β), compatible with the claimed structure of promagnon (4-chloro-17α-methyl-andro-4-ene-3,17β-diol), was confirmed (Fig. 2). The TMS-derivatized promagnon (M1) has a molecular weight of 482/484 *m*/*z*. However the mass spectrum has 447 *m*/*z* and 357 *m*/*z* as most abundant and typical fragment ions (Fig. 2). These fragment ions resulted from the cleavage of the chlorine group (loss of −35/37) and loss of the –HOTMS group (loss of −90) respectively. The fragment at *m*/*z* 143 is a typical D-ring fragment for 17-hydroxylated and 17-methylated compounds [13,14]. The analysis also indicated that, at first sight, no major pill impurities could be observed (Fig. 2).

These mass spectrometric findings are in agreement with previously reported results based on the content of the pills, however no details on metabolic or excretion data were described in former publications of promagnon [15,16].

However, during the excretion studies with the promagnon supplement, it became apparent that the pill was contaminated with a minor impurity of another chlorinated substance M2 (Fig. 2). Identification with the reference standard revealed the presence of methylclostebol in the supplement (Fig. 2). Although there is only a difference in the substituent at the C_3 position between

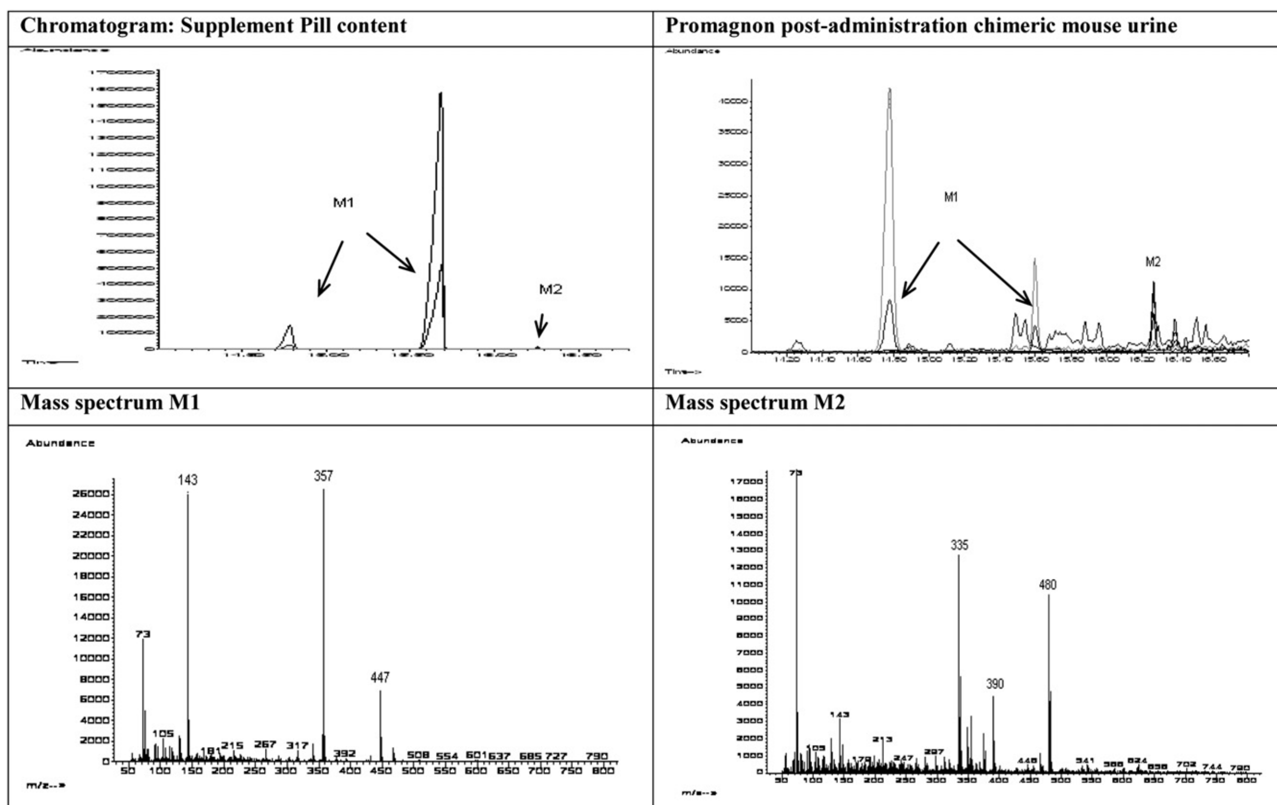

Fig. 2. Results from promagnon: content of the supplement pills and post-administration chimeric mouse urine. M1 (promagnon, two isomers) and M2 (methylclostebol) are indicated. Results obtained after hydrolysis of glucuronides. The corresponding mass spectra of M1 and M2 are presented. M2 was only in a minor amounts present in the supplement pills.

promagnon (C3-hydroxy, MW 482/484) and methylclostebol (C3-keto, MW 480/482) (Fig. 1), the fragmentation pattern of their TMS derivatives is completely different (Fig. 2). For methylclostebol the chlorine clusters are still present in the derivatized spectrum. Apparently the fragmentation of the chlorine atom-containing part of the molecule is delayed due to the structural configuration of the A-ring in methylclostebol (Fig. 2). Also a loss of −145 resulting in m/z 335, the most abundant ion, was observed. Since this compound was available as a pure reference standard it was preferred to perform an excretion study with methylclostebol in the chimeric mice.

Other metabolites could not be identified in the promagnon post-administration urines with sufficient certainty due to high background. This is probably more due to the bad administration and supplement interferences than by concluding that promagnon is not metabolised. The metabolism of promagnon still remains unclear, however for further experiments pill purifications are necessary prior to administration or the use of the reference standard (if available) is recommended to obtain good metabolic data.

3.2. Methylclostebol

Since methylclostebol was detected in the promagnon supplement pills and this compound was available as a pure reference standard it seemed appropriate to administer this compound to the chimeric mice. Methylclostebol is a substance structurally related to clostebol (4-chlorotestosterone) and numerous results about the metabolism of clostebol in human [13,17,18] and cattle [19,20] have been published. But there is only limited data available concerning the metabolism of methylclostebol itself. In literature only a few studies with *in vitro* cultures are found [21,22] and in humans it seemed that methylclostebol was already abused in Germany during the time period 1966–1974 as a substance named STS482 [23]. However, methylclostebol and promagnon are not explicitly mentioned on the WADA Prohibited List, while clostebol is [2]. In fact, all these compounds are 4-chlorinated substances introducing more anabolic activities and less aromatisation [19]. Based on the 17-methylated structure, which influence D-ring metabolism methylclostebol and promagnon can also be related to 4-chloro-1,2-dehydro-17α-methyltestosterone (oral turinabol).

Methylclostebol is available as a supplement and as a pure reference standard. In general, a reference standard is preferred for the administration studies, because the purity of these standards usually is of higher quality than for the supplements. One hundred μL of a reference standard/PBS suspension (10 mg/mL) was prepared and administered to the chimeric and non-chimeric mice. The urine was collected on the day before and on 24 h after the single dose administration.

Methylclostebol, itself, was detected in all mouse urine samples, providing evidence of a successful administration at a sufficiently high dose. The results of only one chimeric mouse are presented here to describe the detected compounds after methylclostebol administration in detail (Fig. 3). Besides the parent drug (M2), the 3-hydroxylated metabolite (=promagnon) was found in all samples, indicated as M1 (Fig. 3A).

Several hydroxylated compounds were detected in the post-administration chimeric and non-chimeric mouse urine (Fig. 3B). The fragment ions at m/z 218 and 231 indicated that these substances are 17-methyl-16,17-dihydroxylated steroid compounds (Fig. 3B, Table 1) [13,14]. The mass spectrum of compound M3 has a molecular weight of 570/572 m/z (with presence of chlorine cluster). Based upon its fragmentation pattern and the presence of the 218 and 231 m/z typical ions, 16-hydroxylated-promagnon could be proposed as possible structure for M3. This structure identification is only tentative and confirmation with a reference standard is needed.

The mass spectrum of the major peak M4a has m/z at 658 and 568 (loss of HOTMS −90) as typical ions, with the chlorine cluster present (Fig. 3). Three other peaks with the same mass spectra but at different retention times were also detected as M4b, M4c and M4d (Fig. 3). As the mass spectra of these substances (M4a–b–c–d) showed an m/z of 218 and 231, typical for 16,17-dihydroxylated steroids, the structure was tentatively identified as *x*,16-dihydroxylated-promagnon (MW 658), where the position of the first x-hydroxyl group varies for the different metabolites.

M5a and M5b indicated a closely related spectrum (Table 1 and Fig. 3) but with a molecular ion at 660/662. For M5a–b, the presumably observed reduced double bond in the A-ring, was already described for norclostebol, a structurally related compound [13]. However, synthesis of the reference standards of these compounds is necessary to unequivocally identify the structures of these compounds.

Table 1
Overview of phase I and II metabolism of the detected metabolites after methylclostebol administration to the chimeric mice.

	Compound	Characteristic TMS-ions m/z	RT	Fraction
M1	Promagnon	447 357	14.89	Glucuronidated
M2	Methylclostebol	480–482 390 335	16.35	Free
M3	16-Hydroxylated-promagnon	570–572 480 218 231	16.42	Glucuronidated
M4a M4b M4c M4d	*x*,16-Dihydroxylated promagnon	658–660 568 218 231	16.45 16.60 16.71 16.82	Free + glucuronidated
M5a M5b	*x*,16-Dihydroxylated promagnon, with a tentative reduced double bond	660–662 218 231	16.73 16.96	Free + glucuronidated
M6a[a] M6b[a]	12-Hydroxylated compound	143 170	16.22 16.68	Free

[a] Typical chimeric humanized mouse metabolite (=not-detected in the non-chimeric mouse).

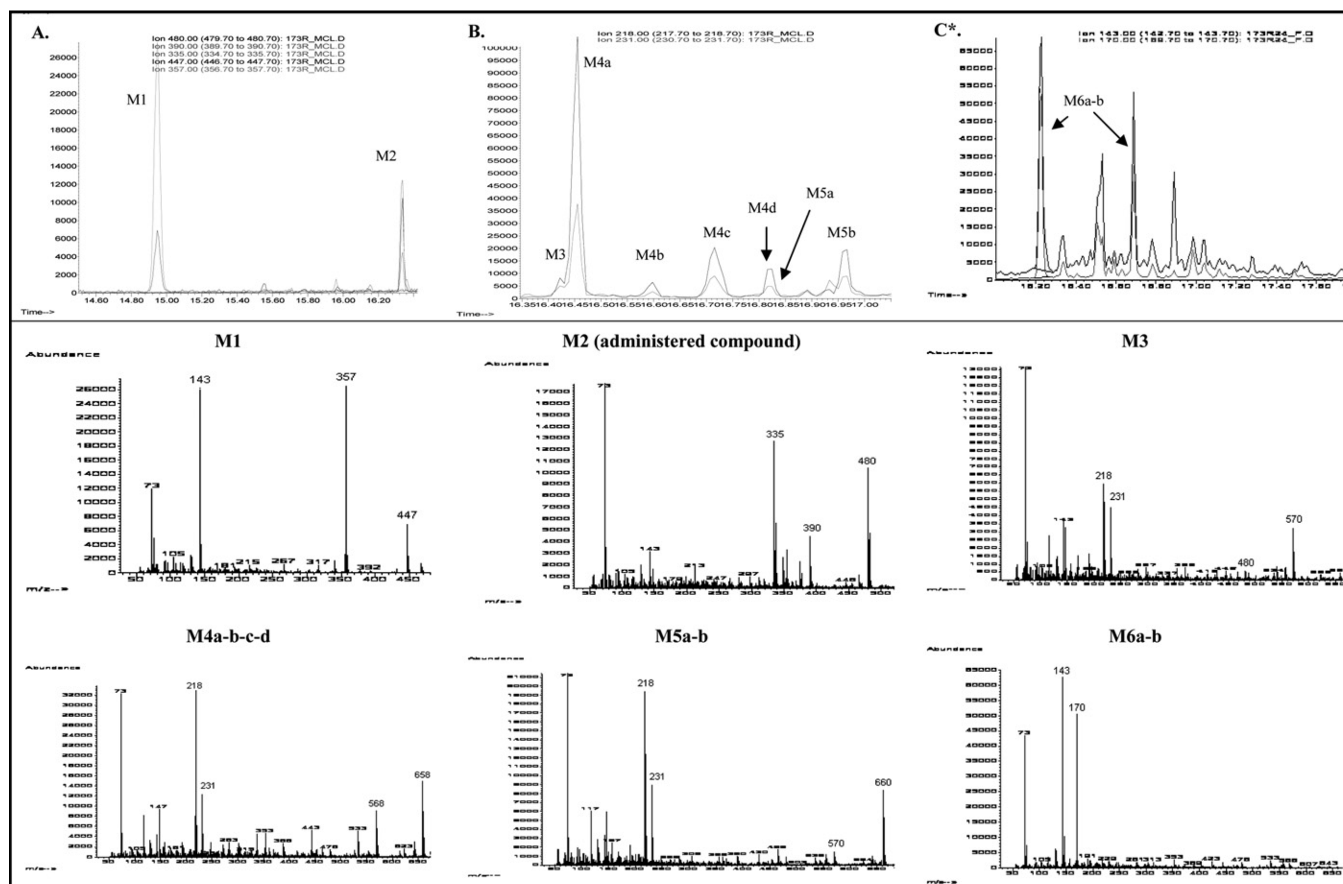

Fig. 3. Results of the methylclostebol post-administration chimeric mouse urine: GC–MS chromatograms with the corresponding mass spectra. *Legend*: Promagnon (M1), methylclostebol (M2) and several hydroxylated metabolites with a tentative structure and name: 16-hydroxylated-promagnon (M3), 4 isomers of *x*,16-dihydroxylated promagnon (M4a–b–c–d), two isomers of reduced *x*,16-dihydroxylated promagnon (M5a–b) and two isomers of a 12-hydroxylated metabolite (M6a-b). * Chromatogram C obtained without hydrolysis (free fraction).

Direct comparison with a reference standard showed, remarkably, the absence of 6β-hydroxymethylclostebol in the mouse urine sample [21], while in literature 6-hydroxylation is one of the major metabolic pathways for 17-hydroxylated,17-methylated compounds [13].

As a conclusion could be stated that in contrast to clostebol, but similar as for 4-chloro-1,2-dehydro-17α-methyltestosterone (oral turinabol), several polyhydroxylated metabolites were detected after methylclostebol administration [24,25].

In addition, the conjugation of the metabolites was also tested in the chimeric mouse urine samples after methylclostebol administration. Methylclostebol itself is predominantly excreted in an unconjugated form, while promagnon was excreted as a glucuroconjugated compound (Table 1), which could be explained by the available hydroxyl group in the C_3 position of promagnon. Phase II conjugation with sulphates was examined by using β-glucuronidase from *H. pomatia*. This resulted in dirty extracts which hampered the analysis. However no additional compounds, in sufficient high abundances, were detected, although the sulphatase activity was tested to be sufficient.

By evaluating the excretion distribution of the detected compounds in the different fractions, an additional compound M6 (two isomeric peaks M6a–b) was detected in the chimeric mouse urine obtained without hydrolysis (unconjugated fraction). This compound M6 has *m*/*z* 143 and 170 as most characteristic ions (Table 1 and Fig. 3C), which are typical for 12-hydroxylated steroids [13]. It could not be detected in the non-chimeric mice after methylclostebol administration. The 12-hydroxylated compound is the major metabolite excreted in the free fraction after methylclostebol administration to the chimeric mice (Fig. 3).

3.3. Superdrol – methasterone

The second nutritional supplement tested in the chimeric mice, was superdrol. According to the label it contains 10 mg of the anabolic steroid 2α,17α-dimethyl-5α-androst-3-one (Fig. 1). Superdrol, also known as methasterone or 17α-methyldrostanolone, is on the WADA prohibited list since 2006. In the mean time some serious health disorders (incidents) have been reported after the use of superdrol supplements [26].

One of the metabolites reported *in vivo* is the reduction of the keto-function in the C_3 position to form 2α,17α-dimethyl-5α-androstane-3α,17β-diol [27,28]. Using *in vitro* cultures, a few additional 16-hydroxylated compounds have also been detected [29].

GC–MS analysis of the superdrol pill confirmed the presence of methasterone, the parent drug, in high amounts. However, the commercially available reference standard was chosen to perform the excretion studies with the chimeric mice. As shown in Fig. 4, the mass spectrum of methasterone after TMS-derivatization (MW 462) correlates with previous reported results [27,28]. An excretion study with the reference standard, even with a high dose (8 mg/mL), resulted in the recovery of only a minor amount of the parent drug from the mouse urine (Fig. 4). Other attempts were made to formu-

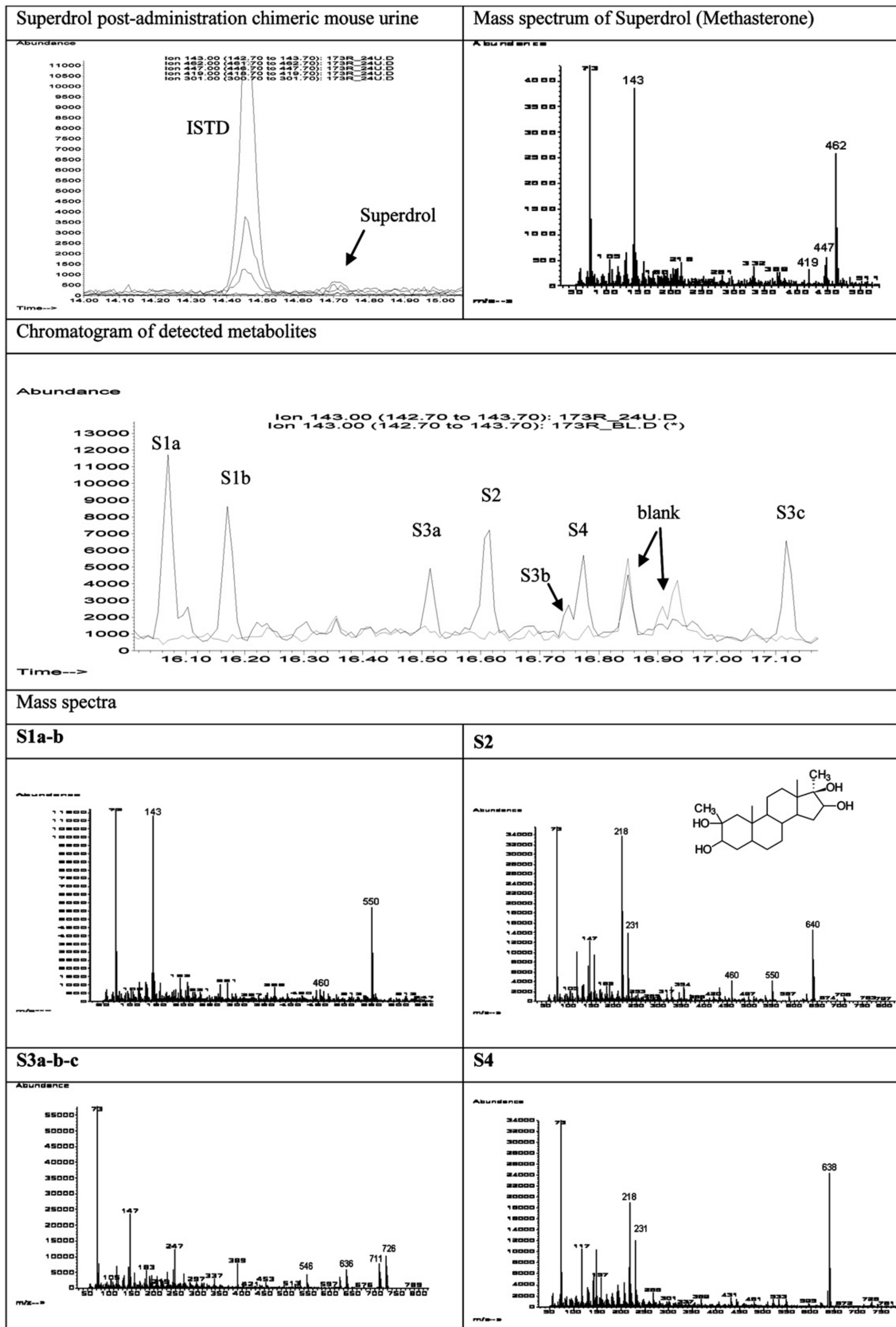

Fig. 4. Results of the superdrol (methasterone) post-administration chimeric mouse urine: GC–MS chromatograms with the corresponding mass spectra. *Legend*: 17a-methyltestosterone (ISTD, internal standard), and several metabolites with tentative structure and name: 2 isomers of x-hydroxy-superdrol (S1a–b), confirmed [29] 2,16-dihydroxy-2a,17a-dimethyl-5a-androstane-3a,17b-diol (S2) with chemical structure presentation, 3 isomers of trihydroxy-superdrol (S3a–b–c) and *x*,16-dihydroxy-superdrol metabolite (S4).

Table 2
Overview of phase I and II metabolism of the detected metabolites after superdrol (methasterone) administration to the chimeric mice.

	Compound	Characteristic TMS-ions *m/z*	RT	Fraction
	Superdrol	462	14.70	Glucuronidated
		447		
		419		
S1a	x-Hydroxy-superdrol	550	16.08	Glucuronidated
S1b		460	16.18	
		143		
S2[a]	2,16-Dihydroxy-DM-AD	640	16.60	Free
		550		
		460		
		218		
		231		
S3a	Trihydroxy-superdrol	726	16.52	Free + glucuronidated
S3b		711	16.75	
S3c		636	17.11	
		546		
S4[a]	*x*,16-Dihydroxy-superdrol	638	16.77	Free + glucuronidated
		218		
		231		

DM-AD = 2α,17α-dimethyl-5α-androstane-3α,17β-diol (3-hydroxy-superdrol).
[a] Typical chimeric humanized mouse metabolite (=not-detected in the non-chimeric mouse).

late the suspension differently, e.g. with PEG400. The solubility of the reference standard of methasterone with PEG was slightly better but no differences in detected compounds could be observed (data not shown). Therefore it is assumed that methasterone is intensively metabolised, since only a minor amount of the parent drug could be recovered (Fig. 4).

As shown in Fig. 4 several metabolites were detected by comparing the post-administration with the pre-administration urines. Comparison of the mass spectra of these metabolites with previous reported results from *in vivo* human studies with methasterone indicated that the chimeric mice revealed other metabolic pathways [27,28]. The only reported metabolite *in vivo*, namely the 3-keto-reduced metabolite of methasterone: 2α,17α-dimethyl-androstanediol (DM-AD, MW 464) was not detected in the mouse urine. However, one of the compounds mentioned in the *in vitro* cultures of Gauthier et al. [29] could be detected in the chimeric mouse urine, namely S2 (Fig. 4). This compound was described as 2,3,16,17-tetrol metabolite [24] and is here indicated as 2,16-dihydroxy-DM-AD (Table 2).

In vitro hydroxylations at the C12 or C16 position of superdrol (methasterone) itself were already described [29]. In the chimeric mice a closely related spectrum as those previously described [29] was found for metabolites S1a-b (MW 550), so a tentative structure of x-hydroxy-superdrol was proposed (Fig. 4 and Table 2). The position of the hydroxyl-group is unknown, however when comparing with the metabolites detected by Gauthier et al. it is clear that it is not at the C16 position (because lack of *m/z* 218/231) and not at C12 position (lack of *m/z* 170), but rather on the B or C ring [29,30]. The structures/metabolites proposed are based on molecular weight and GC–MS fragmentation pattern only. Structure elucidation is still ongoing for the identification of these compounds.

Evaluation and comparison with the excretion urine of the non-chimeric mice revealed that S2 and S4 could not be detected in the non-chimeric mice. This indicates that these compounds are exclusively produced in the chimeric mice and could therefore be considered as typical human metabolites.

Phase II metabolism was also investigated for methasterone in the chimeric mice (Table 2). Via this investigation it could be established that the parent compound, methasterone (whenever it is excreted in minor amounts), it is excreted as a glucuronidated compound (Table 2). No sulphated metabolites could be detected.

4. Conclusion

The market of new and more enhanced doping products is continuously growing, which necessitates the need of developing new detection methodologies and updated screening methods. Research in this respect resulted in the application of the mouse model to investigate how anti-doping control laboratories can screen for prohormones or designer steroids in order to maintain the integrity of a healthy and ethical sport.

The ultimate goal of this study was to investigate whether the $uPA^{+/+}$-SCID mice transplanted with human hepatocytes can be useful to discover additional information about the metabolism of promagnon, methylclostebol and superdrol (methasterone). In previous validation studies, good similarity between the chimeric mice and humans was demonstrated, unlike the non-chimeric mice which were used as a control group for interspecies characteristics [7].

This study, involving the detection of chlorinated and 2,17-dimethylated substances in the framework of doping analysis, showed that the results of the previous reported *in vitro* cultures could not completely be confirmed via the chimeric mouse model. For superdrol one previously reported metabolite and, in addition, several closely related compounds were found. For methylclostebol the 3-keto-reduced promagnon was detected together with some mono- and dihydroxylated compounds. The observed metabolic transformations include reduction and hydroxylation of the parent compound. Compared to *in vitro* cultures the 6β-hydroxymethylclostebol was not detected. Structural characterisation of the metabolites produced, could not solely be obtained from the GC–MS data, so further structural confirmations are necessary. Nevertheless this *in vivo* chimeric mouse model, with transplanted human hepatocytes, revealed essential data on the metabolism of these steroids. A remark must be included here to place these results in a good perspective. The initial goal of directly administering supplements to the mice is not completely achieved, due to high impurity interferences. For promagnon no good results were obtained, since no reference standard was available. But for methylclostebol and superdrol the pure reference standard was available to administer to the mice and more metabolic results were obtained. So for future supplement experiments prior pill purifications are

mandatory, especially when the pure reference standard is not available.

Acknowledgements

This study was financially supported by WADA, the Special Research Fund (BOF) of the Ghent University (LL) and a Concerted Action Grant from the Ghent University (01G00507). The authors want to thank Lieven Verhoye for technical assistance and the Wroclaw University, Department of Chemistry, Poland for their generous gift of the methylclostebol metabolites.

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