PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 85 (2003) 235–245

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

The cynomolgus monkey (*Macaca fascicularis*) is the best animal model for the study of steroid glucuronidation☆

Olivier Barbier*, Alain Bélanger

Oncology and Molecular Endocrinology Research Center, Laval University Medical Center (CHUL), Laval University, 2705, Boulevard Laurier, Sainte-Foy, Québec, Canada G1V 4G2

Abstract

Intense research efforts performed during the past decade clearly established the major role of glucuronidation and uridine-diphospho-glucuronosyltransferase (UGT) enzymes for steroid metabolism in humans. However, a clear understanding of the physiological importance of this metabolic process requires in vivo studies. Numerous evidences ascertain that simians are the most appropriate animal models for such studies. Indeed human and monkey have a similar pattern of steroidogenesis, unlike common laboratory mammals such as rat or mouse. Furthermore, human and monkey are unique in having high levels of circulating androsterone glucuronide and androstane-3α-diol glucuronide (3α-Diol-G). In addition, characterization of eight monkey UGT proteins demonstrated the similarity of their conjugation activity toward steroid hormones. Like human ones, monkey enzymes are expressed in steroid target tissues, where they preferentially glucuronidate androgen and estrogen metabolites. In monkey tissues, immunohistochemical studies demonstrated that UGT2B proteins are expressed in a cell-type specific manner in ovary and kidney, where they control androgens and aldosterone inactivation. These results identify the cynomolgus monkey as an appropriate animal model for the determination of cellular localization of UGT enzymes in steroid target tissues and for the identification of endogenous or exogenous stimuli affecting steroid glucuronidation.

Keywords: Steroidogenesis; Cynomolgus monkey; Metabolites

1. Introduction

Conjugation of compounds by glucuronidation is a pathway found in all vertebrates [1]. This reaction is catalyzed by uridine-diphosphate-glucuronosyltransferase (UGT) enzymes and corresponds to the transfer of the glucuronosyl group from uridine-5′-diphosphoglucuronic acid (UDPGA) to acceptor molecules [1]. Substrates for UGT enzymes are generally hydrophobic compounds with oxygen, nitrogen and sulfur functional groups. The glucuronide products are more polar, generally water soluble and more easily excreted in bile or urine [1]. Glucuronidation is a major metabolic pathway for more than 40% of all the xenobiotics to which the human body is exposed, and for numerous endogenous molecules such as bilirubin, bile acids and steroids [2,3].

2. UDP-glucuronosyltransferase (UGT) enzymes

To date, numerous UGT cDNAs have been isolated from several mammalian species. In humans, 16 proteins were characterized and categorized into two major families, UGT1 and UGT2, according to their primary amino acid sequence homology [4]. The entire UGT1 family is derived from a single gene locus (*UGT1A*), located on chromosome 2 (2q37), coding for nine functional proteins (UGT1A1, UGT1A3–1A10) and three pseudogenes [5,6]. The physiological importance of UGT1 enzymes is demonstrated by mutations in *UGT1A* genes, which cause several diseases with varying degrees of hyperbilirubinemia (Crigler–Najjar Types I and II, and Gilbert's disease) [7,8]. These mutations provoke lowered or absent levels of bilirubin glucuronidation that may lead to toxic levels of substrate accumulation in the body. UGT1A enzymes were first associated to specific bilirubin and xenobiotics conjugation; however, recent studies have shown their role in C18-steroids inactivation [9–12].

Enzymes of the UGT2 family are further divided into two sub-families: UGT2A and UGT2B. UGT2A mRNAs are expressed in rodent, bovine and human olfactory epithelium, where the corresponding enzymes control the

☆ Presented at the 11th International Congress on Hormonal Steroids and Hormones and Cancer, ICHS & ICHC, Fukuoka, Japan, 21–25 October 2002.

* Corresponding author. Tel.: +1-418-654-2296; fax: +1-418-654-2279.
E-mail address: olivier.barbier@crchul.ulaval.ca (O. Barbier).

0960-0760/$ – see front matter © 2003 Published by Elsevier Ltd.
doi:10.1016/S0960-0760(03)00235-8

inactivation of odorant molecules [13–15]. UGT2B enzymes catalyze the glucuronidation of bile acids, steroids, fatty acids, carboxylic acids, phenols and carcinogens. Seven human UGT2B proteins have been characterized: UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17 and UGT2B28 [16–22]. These enzymes are encoded by different genes clustered on chromosome 4 (4q13-4q21.1) [23–26]. UGT2B4, UGT2B10 and UGT2B11 are more specific for bile acids and fatty acids glucuronidation [27,28]; whereas UGT2B7, UGT2B15, UGT2B17 and UGT2B28 conjugates sex steroids (namely androgens) and a variety of other endogenous and exogenous molecules [16–21].

All mammalian UGT proteins share an identical organization with two major domains. The amino-terminal part, encoded by the first exon of both *UGT1A* and *UGT2B* genes, contains the substrate-binding domain and is responsible for the substrate specificity of each isoform [3,4]. By contrast, the UDPGA-binding domain is found in the carboxyl terminal part of the proteins, which is highly conserved among mammalian UGT enzymes [3,4].

3. Glucuronidation of steroid hormones in humans

Numerous studies on steroid metabolism demonstrated that androsterone glucuronide (ADT-G) and 5α-androstane-3α, 17β-diol glucuronide (3α-Diol-G) are the major circulating C19-steroid metabolites in humans [29]. Furthermore, the plasma levels of these glucuronide derivatives reflect the peripheral tissue conversion of adrenal and gonadal precursor C19-steroids to active androgens in various pathophysiological conditions [30]. More recently, identification and characterization of human UGT2Bs demonstrated the major role that these enzymes play in androgen conjugation [16,19–21,31]. A recent study clearly established the relative contribution of each human UGT2B in the glucuronidation of various C19-steroid metabolites [32]. Indeed, UGT2B7 plays a major role in the glucuronidation of 3α-Diol, whereas UGT2B15 and UGT2B17 are major ADT, dihydrotestosterone (DHT) and testosterone-conjugating isoforms [32]. In addition to androgens, UGT2B7 also conjugates bile acids, estrogen, mineralocorticoid and glucocorticoid hormones [17,33,34].

Estrogens and catecholestrogens are also important endogenous substrates for UGT enzymes. As such, all of the UGT1A tested so far, with the exception of UGT1A6, glucuronidate estrogens. While UGT1A3, UGT1A4, UGT1A9 and UGT1A10 enzymes conjugate some C19-steroids, their activity is apparently much higher for estrogens and catecholestrogens [3,10]. The glucuronidation of catecholestrogens, such as 4-hydroxyestrone, is a potentially important catabolic pathway for the elimination of these genotoxic steroid metabolites from a given tissue, and the prevention of the cell damages they can induce [35,36].

These results clearly established the role of UGT enzymes in sex steroids inactivation and elimination.

4. Human UGT enzymes are expressed in steroid target tissues

It is widely accepted that the liver is a major site of glucuronidation. However, there is accumulating evidences that extrahepatic tissues are also involved in the conjugation of steroids hormones. High levels of 5α-reduced C19-steroid glucuronides were measured in the human prostate, breast cyst fluid and ovarian follicular fluid, indicating that glucuronidation of these androgen metabolites occurs in these tissues [37–39]. In the skin, where local androgens synthesis occurs, 5α-reduced C19-steroids are also glucuronidated [40]. Furthermore, Northern-, Western-blot and reverse transcription (RT)-PCR analyses demonstrated the expression of UGT2B and UGT1A transcripts and proteins in a large variety of human steroid target tissues, including skin, testis, ovary, breast and prostate [3,16,19–21,32,41,42]. These observations strongly demonstrate the local inactivation of androgens inside their target tissues.

5. Characterization of the cynomolgus monkey as an animal model for studying steroid glucuronidation in extrahepatic tissues

Identification and characterization of human UGT enzymes during the 1990s evidenced the importance of these enzymes in the metabolism of steroid hormones. However, a clear understanding of the physiological role of UGTs in sex hormones inactivation and elimination required in vivo analyses. To obtain a relevant system in which to study the role of steroid glucuronidation, it was suggested that non-human primates may be the most appropriate animal model. Indeed, the crab-eating (or cynomolgus) monkey (*Macaca fascicularis*) is a commonly used animal model for the development of drugs, and particularly for the characterization of pharmacokinetic and toxicological properties of novel molecules. Using this model, it was demonstrated that glucuronidation is a major metabolic pathway for numerous molecules [43–46]. In addition, humans and other primates are unique in having adrenals that secrete large amounts of dehydroepiandrosterone (DHEA) and its sulfated derivative DHEA-sulfate. These steroids act as precursors of active androgens and estrogens formed in peripheral target tissues, where the same enzymes involved in active steroid synthesis are expressed. As observed in humans, these precursors are converted into androgens and estrogens in a large series of monkey peripheral tissues. In addition, oral administration of DHEA to monkeys resulted in a rapid increase of serum DHEA, testosterone, androstenedione, 3α-Diol-G and ADT-G concentrations, as observed in humans [47]. These observations indicate that in cynomolgus monkey, and

like in humans, the measurement of androgen glucuronides reflects the peripheral conversion of adrenal precursors [47].

5.1. Steroid glucuronides are formed in monkey target tissues

A comparison of the circulating levels of 5α-reduced C19-steroid glucuronides among mammalian species, revealed that human and monkey are unique in having high levels of circulating ADT-G and 3α-Diol-G (Fig. 1a). Using polyclonal antibodies raised against human UGT1A and UGT2B enzymes, Western-blot experiments indicated the presence of UGT proteins in various monkey steroids target tissues, including uterus, mammary gland, testis and prostate (Fig. 1b) [48]. In addition, a comparison of the glucuronidation activity in 24 monkey tissues, demonstrated that xenobiotics, as well as estrogens or androgens are significantly conjugated in a wide variety of tissues [48]. However, the steroid glucuronidation versus xenobiotics glucuronidation ratio was higher in steroid target tissues, suggesting a preferential expression of steroid-conjugating enzymes in these tissues. By contrast, tissues of the gastrointestinal tract, which

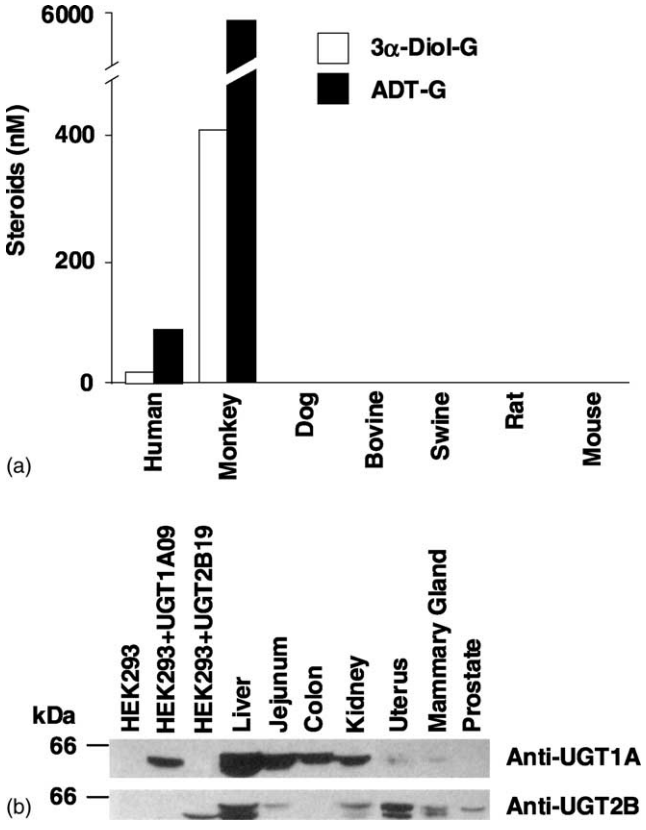

Fig. 1. Human and monkey have high concentrations of circulating of 5α-reduced C19-steroid glucuronides, which are formed by UGT enzymes expressed in extrahepatic tissues. (a) The concentration of androgen glucuronides was determined by radioimmuno assays in plasma from 20 men, and 4 monkey, dog, bovine, swine, rat and mouse adult males; (b) expression of UGT1A and UGT2B proteins in microsome preparations from various monkey tissues, as measured by Western-blot analyses using anti-UGT1A or anti-UGT2B antibodies.

are first exposed to exogenous compounds, preferentially express UGT enzymes conjugating xenobiotics [48–51]. These observations revealed the presence of steroid glucuronidation enzymes in monkey extrahepatic target tissues and provided the first evidence that steroid conjugation occurs in a specific manner in target tissues. Furthermore, these data suggested that glucuronidation is an important pathway for the termination of the steroid signaling in these tissues.

To compare the molecular and biochemical mechanisms of steroid glucuronidation in human and monkey, 10 simian UGT enzymes have been cloned and characterized.

5.2. Cloning and characterization of monkey UGT2B enzymes

To isolate UGT2B clones, monkey liver and prostate cDNA libraries were made with mRNA isolated from the cynomolgus monkey. Screening of these libraries with a combination of human probes resulted in the identification of six monkey enzymes: UGT2B9, UGT2B18, UGT2B19, UGT2B20, UGT2B23 and UGT2B30 [52–57]. The primary structures of these enzymes, deduced from the nucleotide sequence of the corresponding cDNAs were compared to those of human, rat and rabbit enzymes (Fig. 2). Clearly, simian UGT2B enzymes are closer to the human ones than rat and rabbit proteins. The primary structure of the human and simian UGT2B proteins share 62–92% of identity [3,57]. The amino-terminal sections of the protein are less homologous than the carboxyl-terminal portions [52–57]. The human and monkey UGTs thus share similar primary structures. Moreover, they also present identical post-translational modifications. Indeed, we demonstrated that human UGT2B7, UGT2B15 and UGT2B17 and monkey UGT2B20 are glycosylated proteins, and that this post-translational modification is essential for their glucuronidation activity [58]. However, *N*-glycosylation is also an important modification for rat UGT2B1 and UGT2B2 proteins [59], which indicates that this post-translational modification affects both higher mammals and rodents UGT enzymes. The recent determination of the intron–exon structure of the *UGT2B30* gene demonstrated that this gene, which spans approximately 15 kb, is composed of six exons and five introns as human *UGT2B* genes (Fig. 3) [57]. This observation further demonstrates that the structure of *UGT2B* genes is conserved among rat, monkey and humans (Fig. 3).

Simian UGT2B cDNAs have been stably transfected in human embryonic kidney (HEK) 293 cells and the substrate specificity of each enzyme was analyzed and compared to human isoforms. The monkey enzymes can conjugate C19-steroids, such as testosterone, DHT, 3α-Diol or ADT, and other steroid substrates as seen with the human proteins (Fig. 4). However, the overall substrate specificity of a given simian enzyme is slightly different from that of the human proteins, which make it difficult to classify these enzymes as true orthologues. Based on their stereospecificity of androgen glucuronidation, monkey UGT2B enzymes

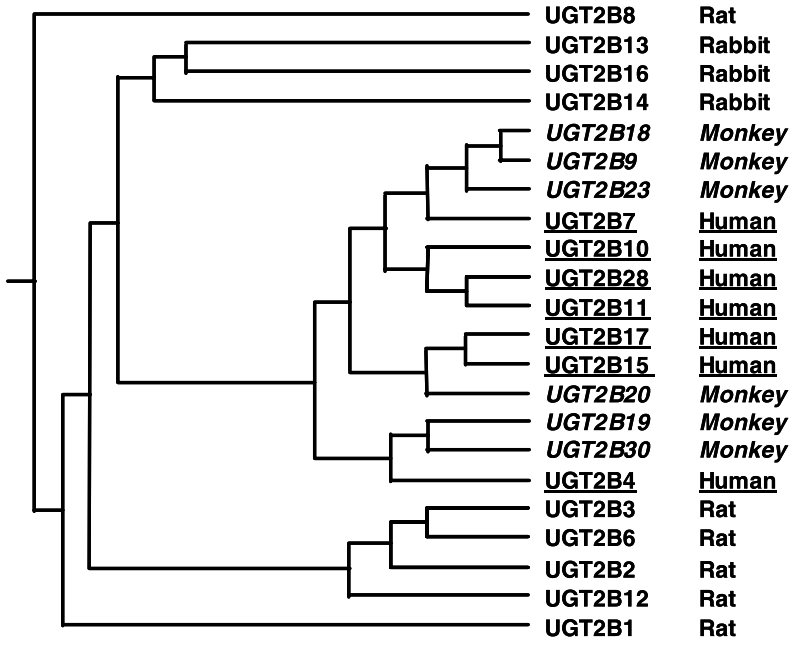

Fig. 2. Dendrogram of homology between the UGT2B enzymes from rats, rabbit, human and monkey. Amino acid sequences were from the GenBank data base.

can be divided into two groups: UGT2B19 and UGT2B20 conjugate specifically the 17β-hydroxy position of testosterone, DHT and 3α-Diol, whereas other enzymes can conjugate androgen at both 3α-hydroxy position (ADT) and 17β-hydroxy position [52,54–57,60] (Fig. 4). It is important to note that despite 3α-Diol presents hydroxyl groups at both 3α and 17β positions, only 17β-glucuronides of this metabolite are detected in monkey plasma. Interestingly, the stereospecificity of monkey UGT2B enzymes for androgen glucuronidation, can be extended to other substrates, such as C18-steroids, which are conjugated at a lower rate than androgens. As such, UGT2B9 and UGT2B30 conjugate both estrogens (estradiol and estriol) and catecholestrogens, while UGT2B19 and UGT2B20 only glucuronidate estrogens, and UGT2B9 and UGT2B18 are unable to catalyze C18-steroid glucuronidation [52–57]. Interestingly, similar results could be observed with the human isoforms, since UGT2B7, UGT2B17 and UGT2B28 glucuronidate 3α- and 17β-hydroxyandrogens, whereas UGT2B15 only conjugates 17β-hydroxyandrogens (testosterone and DHT) [16,19,21,31,32,34].

Kinetic analyses of the human and monkey UGT2B enzymes illustrate the conjugation of steroids with K_m values in the low micromolar range which is consistent with a

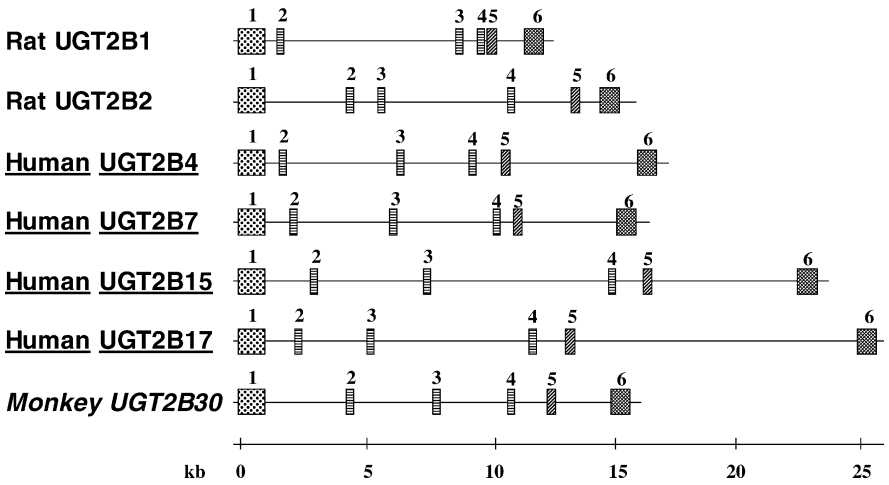

Fig. 3. Intron–exon structure of rat, human and monkey *UGT2B* genes. The organization of all *UGT2B* genes characterized to date in different species is represented in a schematic view.

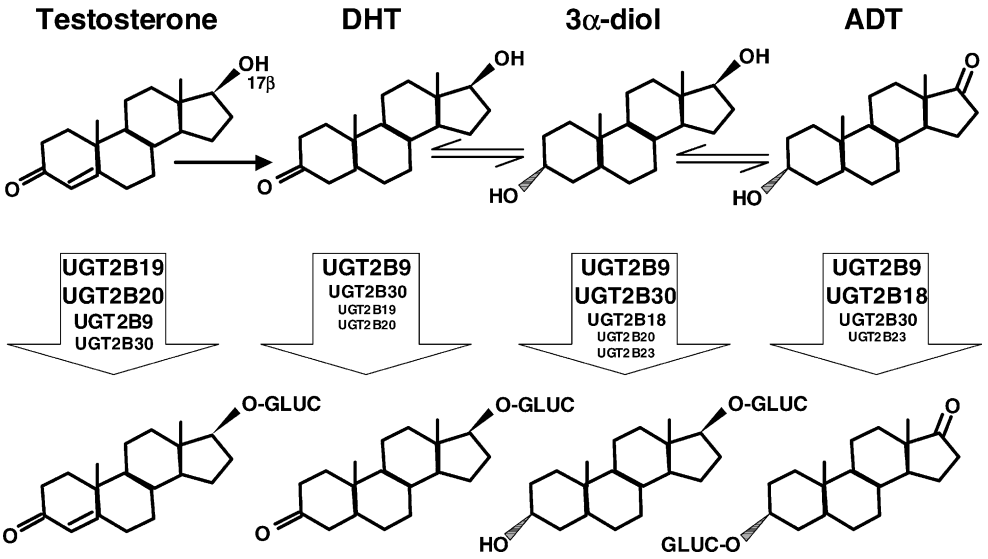

Fig. 4. Relative activity of monkey UGT2B enzymes toward androgenic substrates. The levels of testosterone, DHT, 3α-Diol and ADT glucuronidation by monkey UGT2B enzymes stably expressed in HEK293 cells were normalized by the level of protein expression in the corresponding transfected cell lines.

physiological role of these proteins in steroid glucuronidation [16,19–21,52–57]. The rate of substrate glucuronidation of a given isoform, as determined using stable transfectant, depends on the level of enzyme expression in HEK293 cells, it is therefore difficult to compare the specific roles of different enzymes in androgen glucuronidation. To address this specific role, Girard et al. [57] have compared the glucuronidation activity toward testosterone, DHT, ADT and 3α-Diol of the six monkey UGT2B enzymes. The glucuronidation activity values were subsequently corrected with the amount of proteins contained in the corresponding cell lines, as determined by Western-blot. This study evidenced that testosterone is mainly glucuronidated by UGT2B19 and UGT2B20, while UGT2B9 plays an important role for DHT, 3α-Diol and ADT glucuronide conjugation (Fig. 4) [57]. UGT2B30 and UGT2B18 are also important enzymes for 3α-Diol and ADT glucuronidation, respectively (Fig. 4). These data demonstrate that despite a relative overlap of substrate specificity, each UGT2B enzyme plays a specific role in androgens inactivation.

Interestingly, the rhesus monkey (*Macaca mulatta*) homologue of UGT2B9 (UGT2B9*2, GenBank number: AF294901) has been cloned, but its steroid glucuronidation activity was not investigated. However, this demonstrate that monkey UGT2B enzymes are conserved among cynomolgus and rhesus species.

Overall, these data demonstrate that monkey UGT2B enzymes are close to human proteins and conjugate steroids with similar sterospecificity and enzymatic properties.

5.3. Cloning and characterization of monkey UGT1A enzymes

In order to clone and characterize simian UGT1A isoforms, a monkey liver cDNA library was screened, and 100 positive recombinants were isolated. Among these recombinants, cDNA clones homologous to the human UGT1A6, UGT1A8 and UGT1A9 were named UGT1A06, UGT1A08 and UGT1A09 [42]. Alignment of the deduced amino acid sequences of these proteins demonstrated a region of total identity from residues 286 to 530, whereas the amino-terminal parts of the proteins were variable. From these results it is apparent that the monkey UGT1A proteins contain a variable domain at the amino-half that is probably encoded by exon 1, and is followed by a conserved carboxyl-region encoded by exons 2–5 as found in the human [6], rat [61], and mouse [62] *UGT1A* genes. Based on this observation, the monkey UGT1A01 was cloned from liver by PCR using a sense primer specific for human UGT1A1 and an antisense oligonucleotide hybridizing the common 3′-region of monkey UGT1As [42].

Comparison of the primary structure of the amino-part of monkey UGT1A proteins with the corresponding human enzymes revealed a more than 90% sequence identity (Table 1). In addition, comparison of the conserved carboxyl-region demonstrates 97% sequence identity between human and monkey proteins. Overall, these data illustrate the elevated homology of UGT1A isoforms and suggest a similar *UGT1A* gene organization between human and monkey.

As performed for UGT2Bs, human and monkey UGT1A1 and UGT1A9 enzymes were stably expressed into HEK293 cells for subsequent comparison of their enzymatic properties [41,42]. Interestingly, human and monkey homologues of both enzymes present an identical endogenous substrate specificity (Table 2). Furthermore, human and monkey UGT1A1 and UGT1A9 fail to conjugate androgens, whereas these enzymes catalyze estrogen and catecholestrogen glucuronidation [41,42]. Although, UGT1A1 glucuronidates only estradiol, UGT1A9 is able to convert estradiol, estrone and estriol to their glucuronide derivatives

Table 1
Homology between monkey and human UGT1A proteins

Human enzymes	GenBank numbers	Monkey homologues	GenBank numbers	Percentage of homology
UGT1A1	AAA63195	UGT1A01	AAF15549	94
UGT1A6	AAG30420	UGT1A06	AAF15547	94
UGT1A8	AAA86625	UGT1A08	AAF15548	94
UGT1A9	AAC31425	UGT1A09	AAF15546	90

Homology of amino acid sequence of cynomolgus monkey UGT1A01, UGT1A06, UGT1A08 and UGT1A09 putative exon 1 and the first exon of human homologous UGT1A1, UGT1A6, UGT1A8 and UGT1A9.

(Table 2). Furthermore, human and monkey UGT1A1 enzymes are more active on the 2-hydroxyestrogens [41]. By contrast, UGT1A9 and UGT1A09 conjugate preferentially catecholestrogens with a hydroxyl group at position 4 [42], thus indicating a clear stereospecificity for C18-steroid glucuronidation by human and monkey UGT1A enzymes, as observed for UGT2Bs and androgens. Kinetic analyses also demonstrated that human and monkey UGT1A enzymes conjugate estrogens and catecholestrogens with low K_m values in the micromolar range, suggesting that steroid glucuronidation by UGT1A enzymes occurs with a high affinity.

The glucuronidation activity of human and monkey homologues of UGT1A1 and UGT1A9 was also identical with other substrates, such as bilirubin (for UGT1A1) or planar phenols (for UGT1A9) [41,42], thus indicating that human and monkey UGT1A enzymes share an identical substrate specificity for a variety of endogenous or exogenous molecules. Moreover, as observed for UGT2B9, the rhesus monkey UGT1A01 isoform (GenBank number: AF360121) was also isolated, and presents a greater than 99% amino acid sequence identity to the cynomolgus monkey UGT1A01, and is also capable to convert estradiol into its glucuronide derivative [63].

Overall, these results demonstrate that UGT1A enzymes are conserved between humans and monkey, and establish the relevance of the cynomolgus monkey as a model for studying the role of UGT1A enzymes in the metabolism of C18-steroids.

Table 2
Reactivity of estrogens and catecholestrogens with monkey and human UGT1A1 and UGT1A9 expressed in HEK293 cells

	UGT1A1		UGT1A9	
	Human	Monkey	Human	Monkey
Glucuronide formation				
Estrogens				
Estradiol	+	+	+	+
Estriol	−	−	+	+
Estrone	−	−	+	+
Catecholestrogens				
2-Hydroxyestradiol	+	+	+	+
4-Hydroxyestradiol	+	+	+	+
2-Hydroxyestriol	+	+	+	+
2-Hydroxyestrone	+	+	+	+
4-Hydroxyestrone	+	+	+	+

(+) indicates a reactive substrate and (−) indicates no reactivity.

5.4. Tissue distribution of monkey UGT transcripts

Characterization of monkey UGT enzymes demonstrated that, although a given isoform may play a specific role in steroid inactivation, a certain degree of redundancy in their substrate specificities is observed. The physiological role of a UGT isoform in the metabolism of sex steroids depends not only on its substrate specificity, but also on the steroid target tissues where the enzyme is expressed. Using specific oligonucleotides for RT-PCR, the presence of UGT2B and UGT1A mRNAs was analyzed in various monkey tissues (Table 3). UGT1A09 transcripts were observed in all studied tissues, including estrogen-sensitive ones [42], which is consistent with this enzyme having a role in estrogen metabolism. Similar analyses demonstrated the presence of UGT1A01 mRNAs in various tissues such as liver, kidney and ovary, where it may catalyze bilirubin glucuronidation and contribute to estrogen elimination [41]. Interestingly, all enzymes analyzed are expressed in liver, prostate and ovary, whereas at least five isoforms are found in other tissues (Table 3). Furthermore, each UGT2B enzyme displays a specific expression pattern, thus suggesting that UGT enzymes with similar substrates specificities may play complementary roles in the tissue specific steroid inactivation. Similarly to the catabolism of xenobiotics in liver and intestine [9,49], the expression of all UGT isoforms in tissues such as the prostate or ovary, reveal the importance of glucuronidation as a steroid inactivating pathway.

6. Cellular localization of UGT2B enzymes in monkey tissues

The physiological importance of UGT enzymes depends not only on their ability to conjugate steroid hormones, but also on their tissue- and cell-specific expression. As example, in human prostate the expression of UGT2B15 and UGT2B17, two major androgen-conjugating enzymes is cell-type specific in the two cell layers epithelium. Indeed, UGT2B17 is expressed only in androgen producing basal cells, whereas UGT2B15 expression is detected only in androgen responsive luminal cells [60]. These observations illustrate a complementary role of these two en-

Table 3
Tissue distribution of monkey UGT2B and UGT1A transcripts

	UGT2B9	UGT2B18	UGT2B19	UGT2B20	UGT2B23	UGT2B30	UGT1A01	UGT1A09
Liver	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+
Epididymis	+	+	+	+	+	−	+	+
Ovary	+	+	+	+	+	+	+	+
Mammary gland	−	−	+	+	+	+	−	+
Adrenal	−	+	+	+	−	+	−	+
Intestine	+	+	−	+	+	+	+	+
Kidney	−	+	+	−	+	+	+	+

Total RNA isolated from the tissues were analyzed by RT-PCR using specific oligonucleotides for each UGT transcript. The specificity of each RT-PCR was confirmed by direct sequencing. + or − indicate the presence or absence of transcript.

zymes: UGT2B17 may control the formation of ADT-G and 3α-Diol-G in basal cells, whereas UGT2B15 inactivates DHT in luminal cells. It is therefore tempting to speculate that monkey enzymes expressed in a given tissue also display complementary roles. To date, two studies report that UGT2B proteins are expressed in a cell-type specific manner in monkey tissues: ovary and kidney [33,64].

6.1. Localization of UGT2B in ovary

In the ovary, androgens produced by theca cells, serve not only as substrates for the P450 aromatase-catalyzed estrogen synthesis in the granulosa cells, but also have a fundamental trophic role in follicular development [65]. Measurement of unconjugated and glucuronidated C19-steroids in human follicular fluid demonstrated that the levels of 3α-Diol-G, ADT-G and DHT-G are two- to six-fold higher than their unconjugated forms [39]. In addition, ovarian UGT enzymes conjugate ADT and 3α-Diol with five-fold higher rates than the xenobiotic eugenol [48], indicating that UGT enzymes expressed in this tissue have specific activity for androgens. Immunohistochemistry analyses showed that UGT2B proteins are equally expressed in theca and granulosa cells of growing follicles, whereas theca cells represent the exclusive

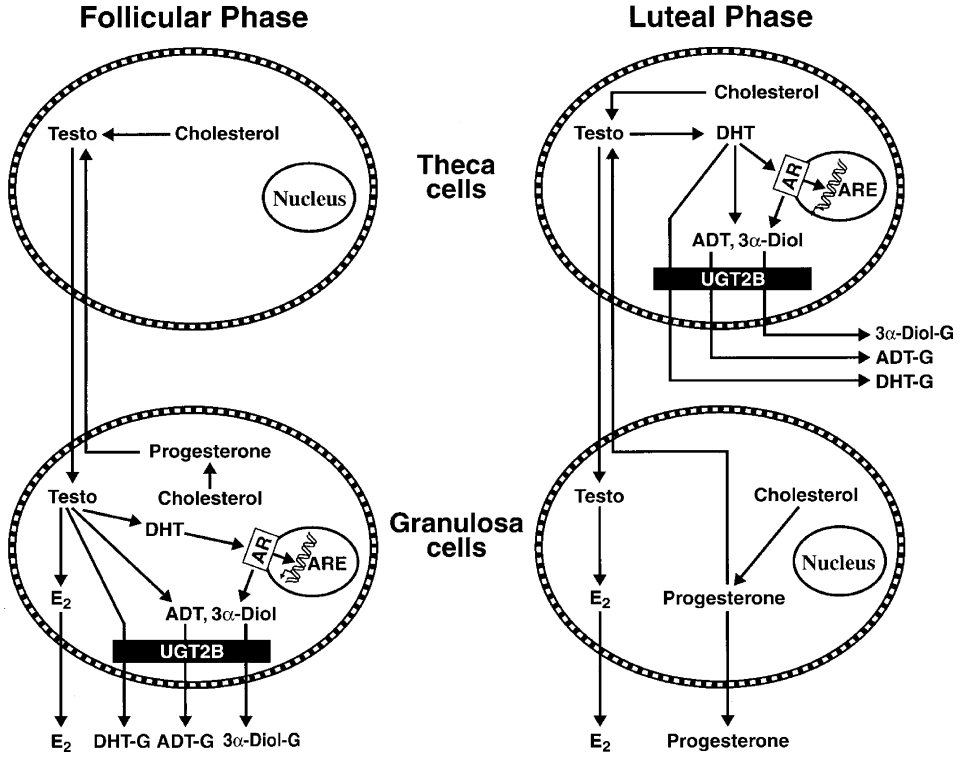

Fig. 5. The androgen-conjugating UGT2B enzymes are differentially expressed temporally and spatially in the monkey follicle throughout the menstrual cycle. As revealed by immunohistochemistry, UGT2B enzymes are expressed in granulosa cells of growing follicles, whereas in preovulatory follicles and in corpus luteum their expression is restricted to theca cells.

site of UGT2B expression in secondary follicles and corpus luteum (Fig. 5) [64]. This differential cell-type expression of UGT2B enzymes during the maturation and degradation of follicles suggests that these enzymes have important physiological roles throughout this process. The fact that UGT2B and the androgen receptor co-localize in the granulosa cells of growing follicles suggests that these enzymes may increase the inactivation of androgen diffusing from theca cells, and may thus influence the androgen response (Fig. 5). By contrast, the presence of UGT2B in theca cells of preovulatory follicles and of corpus luteum demonstrates that androgens may be conjugated in the cells where their formation occurs. This process may limit the concentration of DHT diffusing from theca to granulosa cells, and thus may concur to the control of granulosa cell growth and proliferation.

6.2. Cellular expression of UGT2B proteins in monkey kidney

Aldosterone, the major mineralocorticoid that regulates plasmatic electrolyte homeostasis, is also metabolized as a glucuronide derivative [66], and in a recent study we have identified human UGT2B7 and monkey UGT2B19 as aldosterone-conjugating enzymes [33]. Furthermore, using cynomolgus monkey tissues, it was observed that aldosterone glucuronidation occurs in liver and kidney. In the later, aldosterone regulates electrolytes absorption and secretion through binding to the mineralocorticoid receptor (MR), which is expressed in the epithelial cells of distal tubules [67]. To determine whether aldosterone glucuronidation could interfere with its biological activity, the cellular localization of UGT2B enzymes in the cortex of monkey kidney was investigated by immunohistochemistry (Fig. 6). The expression of UGT2B enzymes was found exclusively in epithelial cells of proximal tubules (Fig. 6a and b). When the antibody was replaced by the preimmune serum a total absence of labelling confirmed the immunostaining specificity. This specific cellular expression is consistent with the detoxifying role of UGTs, because the proximal tubule cells are exposed to the initial filtrate through the glomerulus, so that it is reasonable to suggest that they play the primary role in xenobiotic conjugation [68]. However, the expression of MR in distal tubule epithelial cells [67] indicates that aldosterone activity and catabolism occurs in different cell types. Such a two-cell mechanism of hormone action and metabolism has been previously described for androgens in human prostate [60].

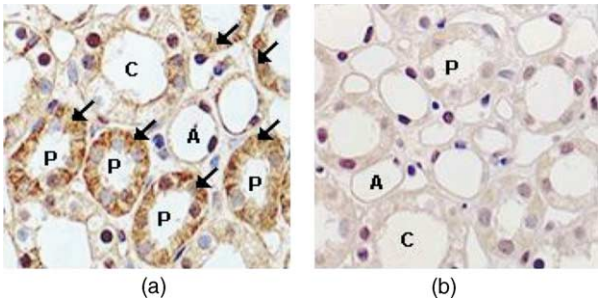

Fig. 6. Cell-type specific expression of UGT2B proteins in the monkey kidney cortex determined by immunohistochemistry using the anti-UGT2B antibody. (a) An intense immunostaining reaction is observed in the epithelial cells of the proximal tubules (P), while some epithelial cells of the collecting ducts (C) and of capillaries (A) were significantly stained (250×); (b) Similar sections as in (a), where the antibody was replaced by the preimmune serum. No immunostaining reaction could be detected (250×).

7. Conclusion

The present data identify the cynomolgus monkey as a relevant animal model for studying steroid glucuronidation. This model constitutes a unique tool for determining whether UGT enzymes conjugate active steroids or their reduced metabolites inside target cells. Emerging evidences from in vitro systems indicate that UGT enzymes expression is regulated by a wide variety of endogenous or exogenous stimuli [23,26,69–73]. However, complexities of glucuronidation renders difficult in vitro to in vivo extrapolation, especially for steroids. By contrast, a model such as the cynomolgus monkey will allow physiologically relevant future studies on the stimuli affecting UGT enzymes expression in the whole body.

Acknowledgements

The authors thank Dr. V. Bocher for critical reading of the manuscript.

References

[1] G.J. Dutton, Glucuronidation of Drugs and Other Compounds, CRC Press, Boca Raton, FL, 1980.
[2] W.E. Evans, M.V. Relling, Pharmacogenomics: translating functional genomics into rational therapeutics, Science 286 (5439) (1999) 487–491.
[3] D.W. Hum, A. Bélanger, É. Lévesque, O. Barbier, M. Beaulieu, C. Albert, M. Vallée, C. Guillemette, A. Tchernoff, D. Turgeon, S. Dubois, Characterization of UDP-glucuronosyltransferases active on steroid hormones, J. Steroid Biochem. Mol. Biol. 69 (1999) 413–423.
[4] P.I. Mackenzie, I.S. Owens, B. Burchell, K.W. Bock, A. Bairoch, A. Bélanger, S. Fournel-Gigleux, M. Green, D.W. Hum, T. Iyanagi, D. Lancet, P. Louisot, J. Magdalou, J.R. Chowdhury, J.K. Ritter, H. Schachter, T.R. Tephly, K.F. Tipton, D.W. Nebert, The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence, Pharmacogenetics 7 (4) (1997) 255–269.
[5] I.S. Owens, J.K. Ritter, Gene structure at the human UGT1 locus creates diversity in isozyme structure, substrate specificity, and regulation, Prog. Nucleic Acid. Res. Mol. Biol. 51 (1995) 305–338.

[6] I.S. Owens, J.K. Ritter, M.T. Yeatman, F. Chen, The novel *UGT1* gene complex links bilirubin, xenobiotics, and therapeutic drug metabolism by encoding UDP-glucuronosyltransferase isozymes with a common carboxyl terminus, J. Pharmacokinet. Biopharm. 24 (5) (1996) 491–508.
[7] I.S. Owens, J.K. Ritter, The novel bilirubin/phenol UDP-glucuronosyltransferase *UGT1* gene locus: implications for multiple nonhemolytic familial hyperbilirubinemia phenotypes, Pharmacogenetics 2 (3) (1992) 93–108.
[8] J.K. Ritter, M.T. Yeatman, P. Ferreira, I.S. Owens, Identification of a genetic alteration in the code for bilirubin UDP-glucuronosyltransferase in the *UGT1* gene complex of a Crigler–Najjar type I patient, J. Clin. Invest. 90 (1) (1992) 150–155.
[9] C.P. Strassburg, M.P. Manns, R.H. Tukey, Expression of the UDP-glucuronosyltransferase 1A locus in human colon: identification and characterization of the novel extrahepatic UGT1A8, J. Biol. Chem. 273 (15) (1998) 8719–8726.
[10] C.D. King, M.D. Green, G.R. Rios, B.L. Coffman, I.S. Owens, W.P. Bishop, T.R. Tephly, The glucuronidation of exogenous and endogenous compounds by stably expressed rat and human UDP-glucuronosyltransferase 1.1, Arch. Biochem. Biophys. 332 (1) (1996) 92–100.
[11] S.B. Senafi, D.J. Clarke, B. Burchell, Investigation of the substrate specificity of a cloned expressed human bilirubin UDP-glucuronosyltransferase: UDP-sugar specificity and involvement in steroid and xenobiotic glucuronidation, Biochem. J. 303 (Pt 1) (1994) 233–240.
[12] T. Ebner, B. Burchell, Substrate specificities of two stably expressed human liver UDP-glucuronosyltransferases of the *UGT1* gene family, Drug Metab. Dispos. 21 (1) (1993) 50–55.
[13] J. Heydel, S. Leclerc, P. Bernard, H. Pelczar, D. Gradinaru, J. Magdalou, A. Minn, Y. Artur, H. Goudonnet, Rat olfactory bulb and epithelium UDP-glucuronosyltransferase 2A1 (UGT2A1) expression: in situ mRNA localization and quantitative analysis, Brain. Res. Mol. Brain Res. 90 (1) (2001) 83–92.
[14] D. Lazard, K. Zupko, Y. Poria, P. Nef, J. Lazarovits, S. Horn, M. Khen, D. Lancet, Odorant signal termination by olfactory UDP glucuronosyl transferase, Nature 349 (6312) (1991) 790–793.
[15] S. Leclerc, J.M. Heydel, V. Amosse, D. Gradinaru, M. Cattarelli, Y. Artur, H. Goudonnet, J. Magdalou, P. Netter, H. Pelczar, A. Minn, Glucuronidation of odorant molecules in the rat olfactory system: activity, expression and age-linked modifications of UDP-glucuronosyltransferase isoforms, UGT1A6 and UGT2A1, and relation to mitral cell activity, Brain Res. Mol. Brain Res. 107 (2) (2002) 201–213.
[16] M. Beaulieu, É. Lévesque, D.W. Hum, A. Bélanger, Isolation and characterization of a novel cDNA encoding a human UDP-glucuronosyltransferase active on C19 steroids, J. Biol. Chem. 271 (37) (1996) 22855–22862.
[17] B.L. Coffman, C.D. King, G.R. Rios, T.R. Tephly, The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y (268) and UGT2B7H (268), Drug Metab. Dispos. 26 (1) (1998) 73–77.
[18] C.J. Jin, J.O. Miners, K.J. Lillywhite, P.I. Mackenzie, cDNA cloning and expression of two new members of the human liver UDP-glucuronosyltransferase 2B subfamily, Biochem. Biophys. Res. Commun. 194 (1) (1993) 496–503.
[19] É. Lévesque, M. Beaulieu, M.D. Green, T.R. Tephly, A. Bélanger, D.W. Hum, Isolation and characterization of UGT2B15(Y85): a UDP-glucuronosyltransferase encoded by a polymorphic gene, Pharmacogenetics 7 (1997) 317–325.
[20] É. Lévesque, M. Beaulieu, D.W. Hum, A. Bélanger, Characterization and substrate specificity of UGT2B4 (E458): a UDP-glucuronosyltransferase encoded by a polymorphic gene, Pharmacogenetics 9 (2) (1999) 207–216.
[21] É. Lévesque, D. Turgeon, J.S. Carrier, V. Montminy, M. Beaulieu, A. Bélanger, Isolation and characterization of the UGT2B28 cDNA encoding a novel human steroid conjugating UDP-glucuronosyltransferase, Biochemistry 40 (13) (2001) 3869–3881.
[22] M. Beaulieu, É. Lévesque, D.W. Hum, A. Bélanger, Isolation and characterization of a human orphan UDP-glucuronosyltransferase, UGT2B11, Biochem. Biophys. Res. Commun. 248 (1) (1998) 44–50.
[23] D. Turgeon, J.S. Carrier, É. Lévesque, B.G. Beatty, A. Bélanger, D.W. Hum, Isolation and characterization of the human *UGT2B15* gene, localized within a cluster of *UGT2B* genes and pseudogenes on chromosome 4, J. Mol. Biol. 295 (3) (2000) 489–504.
[24] G. Monaghan, B. Burchell, M. Boxer, Structure of the human *UGT2B4* gene encoding a bile acid UDP-glucuronosyltransferase, Mamm. Genome 8 (9) (1997) 692–694.
[25] J.S. Carrier, D. Turgeon, K. Journault, D.W. Hum, A. Bélanger, Isolation and characterization of the human *UGT2B7* gene, Biochem. Biophys. Res. Commun. 272 (2) (2000) 616–621.
[26] M. Beaulieu, É. Lévesque, A. Tchernof, B.G. Beatty, A. Bélanger, D.W. Hum, Chromosomal localization, structure, and regulation of the *UGT2B17* gene, encoding a C19 steroid metabolizing enzyme, DNA Cell Biol. 16 (10) (1997) 1143–1154.
[27] D. Turgeon, S. Chouinard, P. Bélanger, S. Picard, J.F. Labbe, P. Borgeat, A. Bélanger, Glucuronidation of arachidonic and linoleic acid metabolites by human UDP-glucuronosyltransferases, J. Lipid. Res. 44 (6) (2003) 1182–1191.
[28] T. Pillot, M. Ouzzine, S. Fournel-Gigleux, C. Lafaurie, A. Radominska, B. Burchell, G. Siest, J. Magdalou, Glucuronidation of hydrodeoxycholic acid in human liver: evidence for a selective role of UDP-glucuronosyltransferase 2B4, J. Biol. Chem. 268 (34) (1993) 25636–25642.
[29] F. Labrie, A. Bélanger, L. Cusan, B. Candas, Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology, J. Clin. Endocrinol. Metab. 82 (8) (1997) 2403–2409.
[30] M. Brochu, A. Bélanger, R.R. Tremblay, Plasma levels of C-19 steroids and 5 alpha-reduced steroid glucuronides in hyperandrogenic and idiopathic hirsute women, Fertil. Steril. 48 (6) (1987) 948–953.
[31] F. Chen, J.K. Ritter, M.G. Wang, O.W. McBride, R.A. Lubet, I.S. Owens, Characterization of a cloned human dihydrotestosterone/androstanediol UDP-glucuronosyltransferase and its comparison to other steroid isoforms, Biochemistry 32 (40) (1993) 10648–10657.
[32] D. Turgeon, J. Carrier, É. Lévesque, D.W. Hum, A. Bélanger, Relative enzymatic activity, protein stability, and tissue distribution of human steroid-metabolizing UGT2B subfamily members, Endocrinology 142 (2) (2001) 778–787.
[33] C. Girard, O. Barbier, G. Veilleux, M. El Alfy, A. Bélanger, Human UDP-glucuronosyltransferase UGT2B7 conjugates mineralocorticoid and glucocorticoid metabolites, Endocrinology, in press.
[34] W.E. Gall, G. Zawada, B. Mojarrabi, T.R. Tephly, M.D. Green, B.L. Coffman, P.I. Mackenzie, A. Radominska-Pandya, Differential glucuronidation of bile acids, androgens and estrogens by human UGT1A3 and 2B7, J. Steroid Biochem. Mol. Biol. 70 (1–3) (1999) 101–108.
[35] J.A. Lavigne, K.J. Helzlsouer, H.Y. Huang, P.T. Strickland, D.A. Bell, O. Selmin, M.A. Watson, S. Hoffman, G.W. Comstock, J.D. Yager, An association between the allele coding for a low activity variant of catechol-*O*-methyltransferase and the risk for breast cancer, Cancer Res. 57 (24) (1997) 5493–5497.
[36] B.T. Zhu, A.H. Conney, Functional role of estrogen metabolism in target cells: review and perspectives, Carcinogenesis 19 (1) (1998) 1–27.
[37] A. Bélanger, S. Caron, F. Labrie, C. Naldoni, L. Dogliotti, A. Angeli, Levels of 18 non-conjugated and conjugated steroids in human breast cyst fluid: relationships with cyst type, Eur. J. Cancer 26 (3) (1990) 277–281.

[38] A. Bélanger, J. Couture, S. Caron, R. Roy, Determination of nonconjugated and conjugated steroid levels in plasma and prostate after separation on C-18 columns, Ann. N.Y. Acad. Sci. 595 (1990) 251–259.
[39] J. Prévost, M. Brochu, A. Bélanger, R. Lambert, Conjugated and unconjugated C-21, C-19 and C-18 steroid concentrations in human follicular fluid from hyperstimulated follicles, Gynecol. Endocrinol. 1 (4) (1987) 331–338.
[40] W. Chen, D. Thiboutot, C.C. Zouboulis, Cutaneous androgen metabolism: basic research and clinical perspectives, J. Invest. Dermatol. 119 (5) (2002) 992–1007.
[41] M. Vallée, C. Albert, G. Beaudry, D.W. Hum, A. Bélanger, Isolation and characterization of the monkey UDP-glucuronosyltransferase cDNA clone monUGT1A01 active on bilirubin and estrogens, J. Steroid Biochem. Mol. Biol. 77 (4-5) (2001) 239–249.
[42] C. Albert, M. Vallée, G. Beaudry, A. Bélanger, D.W. Hum, The monkey and human uridine diphosphate-glucuronosyltransferase UGT1A9, expressed in steroid target tissues, are estrogen-conjugating enzymes, Endocrinology 140 (7) (1999) 3292–3302.
[43] C. Prakash, D. Cui, Metabolism and excretion of a new antianxiety drug candidate, CP-93,393, in cynomolgus monkeys: identification of the novel pyrimidine ring cleaved metabolites, Drug Metab. Dispos. 25 (12) (1997) 1395–1406.
[44] F.M. Moran, R. Tarara, J. Chen, S. Santos, A. Cheney, J.W. Overstreet, B.L. Lasley, Effect of dioxin on ovarian function in the cynomolgus macaque (*M. fascicularis*), Reprod. Toxicol. 15 (4) (2001) 377–383.
[45] H. Kurebayashi, R. Harada, R.K. Stewart, H. Numata, Y. Ohno, Disposition of a low dose of bisphenol a in male and female cynomolgus monkeys, Toxicol. Sci. 68 (1) (2002) 32–42.
[46] D.K. Dalvie, J.P. O'Donnell, Metabolism of CP-195,543, a leukotriene B4 receptor antagonist, in the Long-Evans rat and cynomolgus monkey, Xenobiotica 29 (11) (1999) 1123–1140.
[47] M. Leblanc, C. Labrie, A. Bélanger, B. Candas, F. Labrie, Pharmacokinetics of oral dehydroepiandrosterone (DHEA) in the ovariectomised cynomolgus monkey, J. Steroid Biochem. Mol. Biol. 81 (2) (2002) 159–164.
[48] C. Albert, O. Barbier, M. Vallée, G. Beaudry, A. Bélanger, D.W. Hum, Distribution of UDP-glucuronosyltransferase (UGT) expression and activity in the cynomolgus monkey tissues: evidence for a differential expression of steroid conjugating UGT enzymes in steroid target tissues, Endocrinology 141 (7) (2000) 1472–1480.
[49] C.P. Strassburg, N. Nguyen, M.P. Manns, R.H. Tukey, UDP-glucuronosyltransferase activity in human liver and colon, Gastroenterology 116 (1) (1999) 149–160.
[50] C.P. Strassburg, A. Strassburg, N. Nguyen, Q. Li, M.P. Manns, R.H. Tukey, Regulation and function of family 1 and family 2 UDP-glucuronosyltransferase genes (*UGT1A*, *UGT2B*) in human oesophagus, Biochem. J. 338 (Pt 2) (1999) 489–498.
[51] R.H. Tukey, C.P. Strassburg, Genetic multiplicity of the human UDP-glucuronosyltransferases and regulation in the gastrointestinal tract, Mol. Pharmacol. 59 (3) (2001) 405–414.
[52] O. Barbier, A. Bélanger, D.W. Hum, Cloning and characterization of a simian UDP-glucuronosyltransferase enzyme UGT2B20, a novel C19 steroid conjugating protein, Biochem. J. 337 (1999) 567–574.
[53] O. Barbier, É. Lévesque, A. Bélanger, D.W. Hum, UGT2B23, a novel UDP-glucuronosyltransferase enzyme expressed in steroid target tissues that conjugates androgen and estrogen metabolites, Endocrinology 140 (12) (1999) 5538–5548.
[54] M. Beaulieu, E. Lévesque, O. Barbier, D. Turgeon, G. Bélanger, D.W. Hum, A. Bélanger, Isolation and characterization of a simian UDP-glucuronosyltransferase UGT2B18 active on 3-hydroxyandrogens, J. Mol. Biol. 275 (5) (1998) 785–794.
[55] G. Bélanger, M. Beaulieu, E. Lévesque, D.W. Hum, A. Bélanger, Expression and characterization of a novel UDP-glucuronosyltransferase, UGT2B9, from cynomolgus monkey, DNA Cell Biol. 16 (10) (1997) 1195–1205.
[56] G. Bélanger, O. Barbier, D.W. Hum, A. Bélanger, Molecular cloning, expression and characterization of a monkey steroid UDP-glucuronosyltransferase, UGT2B19, that conjugates testosterone, Eur. J. Biochem. 260 (3) (1999) 701–708.
[57] C. Girard, O. Barbier, D. Turgeon, A. Bélanger, Isolation and characterization of the monkey *UGT2B30* gene that encodes a uridine diphosphate-glucuronosyltransferase enzyme active on mineralocorticoid, glucocorticoid, androgen and oestrogen hormones, Biochem. J. 365 (Pt 1) (2002) 213–222.
[58] O. Barbier, C. Girard, R. Breton, A. Bélanger, D.W. Hum, *N*-Glycosylation and residue 96 are involved in the functional properties of UDP-glucuronosyltranferase enzymes, Biochemistry 39 (38) (2000) 11540–11552.
[59] P.I. Mackenzie, The effect of N-linked glycosylation on the substrate preferences of UDP glucuronosyltransferases, Biochem. Biophys. Res. Commun. 166 (3) (1990) 1293–1299.
[60] O. Barbier, M. El Alfy, H. Lapointe, A. Bélanger, D.W. Hum, Cellular localization of UDP-glucuronosyltransferase 2B (UGT2B) enzymes in human prostate epithelium by in situ hybridization and immunohistochemistry, J. Clin. Endocrinol. Metab. 85 (12) (2000) 4819–4826.
[61] Y. Emi, S. Ikushiro, T. Iyanagi, Drug-responsive and tissue-specific alternative expression of multiple first exons in rat UDP-glucuronosyltransferase family 1 (*UGT1*) gene complex, J. Biochem. (Tokyo) 117 (2) (1995) 392–399.
[62] A.N. Kong, M. Ma, D. Tao, L. Yang, Molecular cloning of two cDNAs encoding the mouse bilirubin/phenol family of UDP-glucuronosyltransferases (mUGTBr/p), Pharm. Res. 10 (3) (1993) 461–465.
[63] B. Dean, S. Chang, J. Stevens, P.E. Thomas, C. King, Isolation and characterization of a UDP-glucuronosyltransferase (UGT1A01) cloned from female rhesus monkey, Arch. Biochem. Biophys. 402 (2) (2002) 289–295.
[64] O. Barbier, C. Girard, L. Berger, M. El Alfy, A. Bélanger, D.W. Hum, The androgen conjugating UDP-glucuronosyl-transferase 2B (UGT2B) enzymes are differentially expressed temporally and spatially in the monkey follicle throughout the menstrual cycle, Endocrinology 142 (6) (2001) 2499–2507.
[65] S.G. Hillier, P.F. Whitelaw, C.D. Smyth, Follicular oestrogen synthesis: the two-cell, two-gonadotrophin model revisited, Mol. Cell. Endocrinol. 100 (1–2) (1994) 51–54.
[66] C.E. Gomez-Sanchez, O.B. Holland, Urinary tetrahydroaldosterone and aldosterone-18-glucuronide excretion in white and black normal subjects and hypertensive patients, J. Clin. Endocrinol. Metab. 52 (2) (1981) 214–219.
[67] S. Bachmann, M. Bostanjoglo, R. Schmitt, D.H. Ellison, Sodium transport-related proteins in the mammalian distal nephron: distribution, ontogeny and functional aspects, Anat. Embryol. 200 (5) (1999) 447–468.
[68] J.W. Lohr, G.R. Willsky, M.A. Acara, Renal drug metabolism, Pharmacol. Rev. 50 (1) (1998) 107–141.
[69] C. Guillemette, É. Lévesque, M. Beaulieu, D. Turgeon, D.W. Hum, A. Bélanger, Differential regulation of two uridine diphospho-glucuronosyltransferases, UGT2B15 and UGT2B17, in human prostate LNCaP cells, Endocrinology 138 (7) (1997) 2998–3005.
[70] C. Guillemette, D.W. Hum, A. Bélanger, Regulation of steroid glucuronosyltransferase activities and transcripts by androgen in the human prostatic cancer LNCaP cell line, Endocrinology 137 (7) (1996) 2872–2879.
[71] O. Barbier, I. Pineda Torra, A. Sirvent, T. Claudel, C. Blanquart, D. Duran-Sandoval, F. Kuipers, V. Kosykh, J.C. Fruchart, B. Staels, FXR

induces the UGT2B4 enzyme in hepatocytes: a potential mechanism of negative feedback control of FXR activity, Gastroenterology 124 (7) (2003) 1926–1940.
[72] O. Barbier, L. Villeneuve, V. Bocher, C. Fontaine, I. Pineda Torra, C. Duhem, V. Kosykh, J.C. Fruchart, C. Guillemette, B. Staels, The UDP-glucuronosyltransferase 1A9 enzyme is a peroxisome proliferator-activated receptor alpha and gamma target gene, J. Biol. Chem. 278 (16) (2003) 13975–13983.
[73] J.H. Lin, Complexities of glucuronidation affecting in vitro in vivo extrapolation, Curr. Drug Metab. 3 (6) (2002) 623–646.