Cytochrome P450 3As Gene Expression and Testosterone 6β-Hydroxylase Activity in Human Fetal Membranes and Placenta at Full Term

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Received October 16, 2009; accepted November 24, 2009; published online November 27, 2009

Expression levels of cytochrome P450 (CYP) 3A4, CYP3A5 and CYP3A7 mRNAs in placentas and fetal membranes, which were split into amnion and chorion leave attached decidua (chorion/decidua), obtained from pregnant women with normal delivery (5 subjects) and Caesarean section (15 subjects) were determined. These CYP3A mRNAs were also expressed in amnion and chorion/decidua together with placenta, although the expression level of these mRNAs was strikingly different between subjects. The expression level of the CYP3A4 mRNA in the placenta was about 2-fold higher than those in amnion and chorion/decidua. On the other hand, the expression levels of CYP3A5 and CYP3A7 mRNAs were highest in chorion/decidua. The immunologically related protein(s) with CYP3A7 was detected in all tissues examined. Testosterone 6β-hydroxylase activity in homogenate of human placenta, amnion and chorion/decidua were 26.6, 3.7 and 4.6 pmol/h/mg protein, respectively. These results suggest that CYP3As in fetal membranes have the metabolic function to protect the fetus from exposure to drugs.

Key words cytochrome P450 3A; fetal membrane; amnion; chorion; decidua; testosterone 6β-hydroxylation

Cytochrome P450 represent a superfamily of heme proteins that play a critical role in the oxidative metabolism of xenobiotics and endogenous substances, and there are several isoforms. The human CYP3A subfamily contains mainly three isoforms, CYP3A4, CYP3A5, and CYP3A7.[3)] CYP3A4 is a major isoform that accounts for about 30% of the amount of total CYP in human adult livers and is responsible for approximately 50% of the metabolism by CYPs of known drugs in humans.[4,5)] CYP3A5 is expressed polymorphically throughout development in the liver.[6,7)] CYP3A7 is a major CYP isoform in the human fetal and newborn liver.[8)]

It is known that the human placenta contains multiple CYP isoforms in the mitochondria and endoplasmic reticulum of trophoblastic cells.[9)] The type and amount of expressed CYPs varies depending on the period of gestation and maternal health status.[10,11)] In general, it appears that the placenta expresses a wider variety of CYP isoforms in the first trimester than at term.[12,13)] Thus it has been suggested that xenobiotic metabolism in the placenta may be feto-protective during the critical embryogenic and organogenesis stage prior to the second trimester, when it is most susceptible to the effects of teratogens, the expression of CYP mRNAs is maximal.[14)] However, the central nervous system develops throughout the pregnancy and may also be affected in the later stages of pregnancy.

Even though, as described above, various studies of enzymes related to pharmacokinetics in human placenta have been reported, there is only limited research in fetal membranes. The fetal membranes, which extend from the placenta, are an important barrier between the fetus and mother. The membranous structure that surrounds the developing fetus and forms the amniotic cavity is derived from fetal tissue except for decidua and is composed of three layers: amnion (inner layer), chorion leave (middle layer) and decidua (outer layer). The amnion is a translucent structure adjacent to the amniotic fluid. The chorion leave is a more opaque membrane that is attached to the decidua (*i.e.*, maternal tissue that lines the uterus during pregnancy). The amnion and chorion leave are separated by the exocelomic cavity until approximately three months gestation, when they become fused. These membranes regulate the access of substances including drugs and other harmful chemicals to and from the amniotic fluid and, hence, the fetus.[15)] In recent years, it has been reported that the multidrug resistance/P-glycoprotein gene (MDR1/P-gp) and breast cancer resistance protein (BCRP), belong to the ATP-binding cassette transporter family are expressed in human fetal membranes including decidua.[16,17)] It is considered that the role of these transporters is to discharge drugs in amniotic fluid and to keep out transfer of drugs from the maternal side *via* the uterus. It is well established that P-glycoprotein transports many drugs that are metabolized by CYP3A4 and many modulators of P-glycoprotein also modulate the CYP3A gene family.[18)] To the best of our knowledge, the expression of CYP3A isoforms in the fetal membranes, however, has not been investigated, even at the mRNA level. Furthermore, the CYP3A mRNAs and proteins have been detected from placenta,[10,19,20)] but thus far no study describing steroid 6β-hydroxylation, erythromycin or dextromethorphan *N*-demethylation or any other relevant marker activities for CYP3A isoforms in human placenta has been published.[13,21)]

In the present study, we investigated the expression of CYP3As in human fetal membranes and placenta at term, by use of quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR), Western blot analysis and testosterone 6β-hydroxylation.

MATERIALS AND METHODS

Materials Anti-P-450 HFLa (CYP3A7) immunoglobu-

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Table 1. Sequences of Primers and Real-Time PCR Conditions

Primer name	Forward primer sequence (5′- -3′)	Reverse primer sequence (5′- -3′)	Product size (bp)	Annealing temp.	Number of cycles	Gene Bank accession number
CYP3A4	CTGTGTGTTTCCAAGAGAAGTTAC	TGCATCAATTTCCTCCTGCAG	298	60	45	AF182273
CYP3A5	CTCTCTGTTTCCAAAAGATACC	TGAAGATTATTGACTGGGCTG	194	60	45	NM_000777
CYP3A7	AGATTTAATCCATTAGATCCATTCG	AGGCGACCTTCTTTTATCTG	148	60	45	AF315325
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG	185	60	45	BC013310

lin G (IgG) was a generous gift from Professor M. Kitada (Division of Pharmacy, University Hospital, Chiba University School of Medicine, Chiba, Japan).[22] RNA*later* was obtained from Ambion (Austin, TX, U.S.A.). Complete protease inhibitor cocktail tablets was obtained from Roche Diagnostics (Mannheim, Germany). SuperScript III First-Strand Synthesis System for reverse transcription-polymerase chain reaction (RT-PCR) was from Invitrogen Life Technologies (Carlsbad, CA, U.S.A.). TaKaRa SYBR Premix Ex Taq was obtained from Takara Bio Inc. (Otsu, Japan). Amersham ECL Advance Western blotting Detection Kit, illustra RNAspin Mini kit and Amersham Hyperfilm ECL were obtained from GE Healthcare U.K. Ltd. (Little Chalfont, Buckinghamshire, England). Histofine simple-stain MAX-PO (R) (horseradish peroxidase-conjugated goat anti-rabbit IgG polyclonal antibody) was obtained from Nichirei Bioscience (Tokyo, Japan). Clear Blot Membrane-p was purchased from ATTO Co. (Tokyo, Japan). DC protein assay kit was obtained from Bio-Rad Lab. Inc. (Hercules, CA, U.S.A.). 6β-Hydroxytestosterone and ethoxyresorufin were obtained from Sigma (St. Louis, MO, U.S.A.). ($\pm$) 4-Hydroxymephenytoin was obtained from Toronto Research Chemicals Inc. (North York, Ontario, Canada). LC/MS-grade distilled water was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Ammonium acetate was obtained from Nacalai Tesque Co., Inc. (Kyoto, Japan). HPLC-grade formic acid was obtained from Wako Pure Chemicals (Osaka, Japan). All other chemical reagents used were of the highest quality available.

Tissue Preparation Fetal membrane and placental tissues were obtained under a protocol approved by the Ethics Committee of Shinshu University School of Medicine after term normal delivery or Caesarean section (33—42 weeks) from Shinshu University Hospital. Smoking status was assessed by patient interview. Fetal membranes were split into amnion and chorion leave attached decidua (chorion/decidua) in the mesenchymal layer, which is located between the amnion epithelium and the chorion leave trophoblast. The amnion and chorion/decidua contained a part of the mesenchymal layer. Tissues for analyses of testosterone 6β-hydroxylation and Western blotting were stored at $-150\,^{\circ}C$ until the homogenate preparation. A portion of tissue was dipped in RNA*later* and stored at –150°C until extraction of total RNA.

RNA Extraction and Reverse Transcription Reaction Total RNA was extracted from tissue (30—50 mg) dipped in RNA*later* using illustra RNAspin Mini kit according to the manufacturer's instructions. First-strand cDNA was generated from 4 μg of total RNA. Reverse transcription reaction was performed using a SuperScript III First-Strand Synthesis System for RT-PCR in accordance with the manufacturer's instruction.

Real-Time RT-PCR Analysis For detection of expression levels, CYP mRNAs were analyzed by SYBR Green real-time quantitative RT-PCR. The levels of these mRNAs were normalized relative to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. All PCR procedures were performed using the ABI 7500 Fast System SDS software version 1.3.1 (Applied Biosystems, Foster City, CA, U.S.A.) according to each manufacturer's instructions. PCR was performed using diluted cDNA template in a 25 μl reaction mixture containing 0.30 μM of each primer and 12.5 μl SYBR Green real-time PCR Master Mix. The primers used are summarized in Table 1.

Western Blotting Tissues (0.3—0.4 g) were homogenized in 1 ml of lysis buffer, according to the methods of Yeboah *et al.*[17] The protein concentration was measured using a DC protein assay kit using bovine serum albumin as a standard. Samples containing 1 mg protein were diluted in an equal volume of sample buffer and separated on 10% polyacrylamide gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously reported.[23,24] SUPERSOMES expressed CYP3A7 (BD Genetest, Franklin Lakes, NJ, U.S.A.) was used as the reference standard. Western blotting analysis was carried out according to the method reported previously.[25] The proteins were transferred to Clear Blot Membrane-p by electrophoresis at 1 mA/cm^2 for 60 min. Non-specific binding was blocked overnight at 4 °C with 2% ECL Advance Blocking Reagent in 20 mM Tris buffered saline (pH 7.6) containing 0.1% Tween-20. The membrane was incubated for 1 h at room temperature with an anti-CYP3A7 antibody. This was followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG polyclonal antibody. Proteins were visualized by chemiluminescence detection with an ECL system and Hyperfilm for ECL in accordance with the manufacturer's instruction.

Assay for Testosterone 6β-Hydroxylase Activity Tissues, about 1 g, were homogenized in 3 ml of 10 mM Tris–HCl buffer (pH 7.5) containing 250 mM sucrose, 0.1 mM ethylenediaminetetraacetic acid (EDTA) and protease inhibitor. Tissue homogenates were added into 0.26 mM testosterone, 1 mM NADPH and 100 mM potassium phosphate buffer (pH 7.4) to a final volume of 0.5 ml. The mixture was incubated at 37 °C for 3 h after preincubation at 37 °C for 2 min. After incubation, 10 μl of 1 μM ethoxyresorufin was added to the reaction mixture as an internal standard and was vortex-mixed with 1.25 ml of ethyl acetate and centrifuged. Aliquots (1 ml) of organic phase were transferred to microcentrifuge tubes. Ethyl acetate was evaporated under nitrogen gas and the samples were dissolved with 100 μl of mixture of 10 mM ammonium acetate (98%) and 0.1% formic acid in

methanol (2%). Metabolites were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

Instrument An Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) consisting of a binary pump, a degasser linked to a CTC HTS PAL new wash system autosampler (AMR Inc., Tokyo, Japan) was used. Mass spectrometric detection was performed on an API 4000 triple quadruple instrument (Applied Biosystems-Sciex, CA, U.S.A.) equipped with a Turbo Ion Spray ionization (ESI) interface. Data processing was performed with Analyst 1.4.2 software package (Applied Biosystems-Sciex, CA, U.S.A.).

Chromatographic Conditions The chromatographic separation was achieved on a reversed-phase CAPCELL PAK C18 MG III column (50×4.6 mm i.d., 5 μm, Shiseido Co., Inc., Tokyo, Japan). The column temperature was kept constant at 40 °C. The mobile phase consisted of a mixture of 10 mM ammonium acetate in water (A) with 0.1% formic acid in methanol (B) and was delivered at a flow-rate of 0.5 ml/min. A step-wise gradient of B into A was run as shown in Table 2.

Mass Spectrometric Conditions The mass spectrometer was operated using ESI source in the positive ion detection. In order to optimize all the MS parameters, standard solutions (100 ng/ml) and internal standard were infused into the mass spectrometer at a flow-rate of 250 μl/min. The ion spray voltage (IS) was set at 4500 V. The turbo ion spray probe temperature was maintained at 600 °C. The instrument parameters *viz.*, nebulizer gas, curtain gas, auxillary gas and collision gas were set at 60, 15, 80 and 5, respectively. Compounds parameters *viz.*, declustering potential, collision energy, entrance potential and collision exit potential were 80, 25, 10 and 15, respectively, for 6β-hydroxytestsosterone and ethoxyresorufin. Zero air was used as source gas while nitrogen was used as both curtain and collision gas. The mass spectrometer was operated at ESI positive ion mode and detection of the ions was performed in the multiple reaction monitoring (MRM) mode, monitoring the transition of *m*/*z* 305 precursor ion $[M+H]^+$ to the *m*/*z* 270 product ion for 6β-hydroxytestosterone (retention time; 6.8 min) and *m*/*z* 242 precursor ion $[M+H]^+$ to the *m*/*z* 158 product ion for ethoxyresorufin (7.4 min). Quadrupoles Q1 and Q3 were set on unit resolution. Data acquisition and quantitation were performed using analyst software version 1.4.2 (Applied Biosystems, MDSSciex, Toronto, Canada).

Calibration Standards Calibration standards to cover the assay range of 1—5000 nM of 6β-hydroxytestosterone were prepared by adding 50 μl of 0.01, 0.05, 0.1, 0.5, 1, 5, 10 and 50 μM working standards to 0.5 ml aliquots of control reaction mixture.

Statistical Analysis Statistical differences were determined by two-sided Student's *t*-test. Difference with $p<0.05$ was considered significant.

Table 2. HPLC Timetable

Time (min)	Solvent A (%)	Solvent B (%)
0.0	98	2
3.0	98	2
4.0	10	90
9.0	10	90
9.1	98	2
16.0	98	2

Solvents A and B are 10 mM ammonium acetate in water and 0.1% formic acid in methanol, respectively.

RESULTS

Demographic Data Demographic data, including medication and smoking status of 20 women before term normal delivery or Caesarean section, are shown in Table 3. Normal delivery and Caesarean section were 5 and 15 subjects, re-

Table 3. Characteristics of Pregnant Women Who Donate Placenta and Fetal Membrane in This Study

	Gestational period (weeks)	Labor	Medication (daily dosage)	Present illness
No. 1	38	C-section	None	None
No. 2	42	C-section	Levothyroxine sodium hydrate (150 mg)	Hypothyroidism (due to thyroid carcinoma)
No. 3	37	C-section	None	None
No. 4	38	Normal	Ritodrine hydrochloride (15 mg)	Threatened premature delivery
No. 5	38	C-section	None	None
No. 6	41	Normal	Dexamethasone (0.25 mg), hydrocortisone (20 mg)	Adrenal insufficiency
No. 7	37	C-section	None	None
No. 8	40	Normal	None	None
No. 9	33	C-section	Ritodrine hydrochloride (*ca.* 200 mg)	Threatened premature delivery
No. 10	34	C-section	Carbamazepine (dosage unspecified), zonisamide (dosage unspecified), ritodrine hydrochloride (*ca.* 288 mg)	Epilepsy, Threatened premature delivery
No. 11	39	Normal	None	None
No. 12	38	C-section	None	None
No. 13	39	C-section	None	None
No. 14	—	Normal	—	—
No. 15	42	C-section	Methylprednisolone (4 mg)	Nephrosis
No. 16	38	C-section	None	None
No. 17	37	C-section	None	None
No. 18	40	C-section	None	None
Bo. 19	37	C-section	None	None
No. 20	38	C-section	None	None

All women have not smoked at least last one month before labor. C-section: Caesarean section, —: missing information, **bold**; these numbered tissues (five women's) were used in Western blot analysis and testosterone 6β-hydroxylase activity.

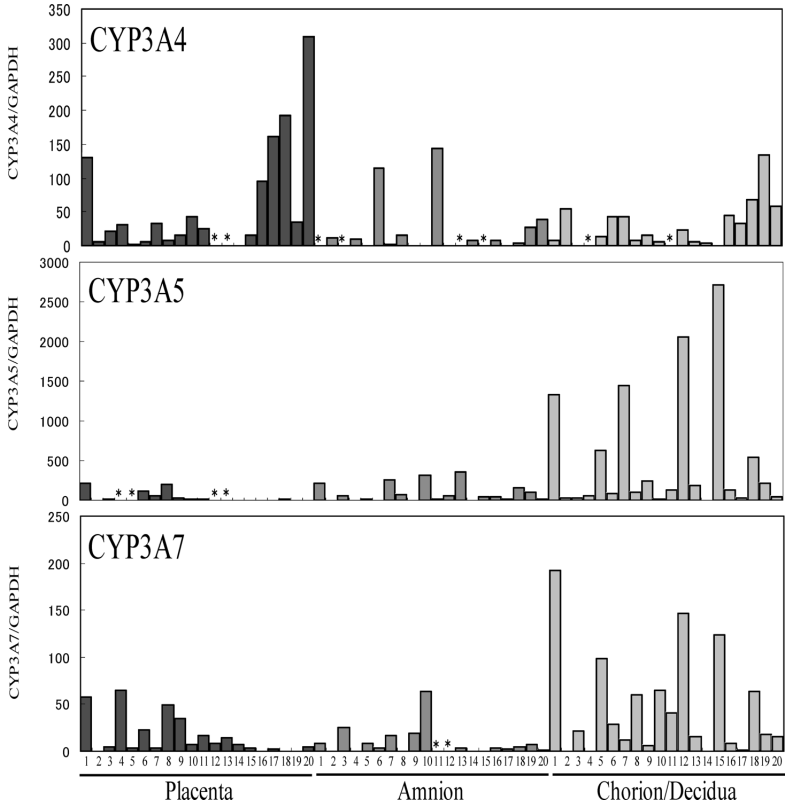

Fig. 1. Expression of CYP3A4, CYP3A5 and CYP3A7 mRNAs in Placenta, Amnion and Chorion/Decidua of Human

Expression of these mRNAs was analyzed by SYBR Green real-time quantitative RT-PCR. Data presented are the ratio of CYP3A4, CYP3A5, or CYP3A7 to GAPDH. * No data available.

spectively. Thirteen women were not treated with any drugs at least last one month before labor. All women have not smoked at least last one month before labor.

Real-Time RT-PCR Analysis of CYP3A4, CYP3A5 and CYP3A7 Gene Expression Expression levels of CYP3A4, CYP3A5 and CYP3A7 mRNAs in placenta, amnion and chorion/decidua were determined by quantitative real-time RT-PCR. These CYP3A mRNAs were expressed in amnion and chorion/decidua together with placenta, although the expression level of these mRNAs was strikingly different between subjects (Fig. 1). The means of expression levels of CYP3A4, CYP3A5 and CYP3A7 mRNAs in these tissues are shown in Fig. 2. The expression level of CYP3A4 mRNA in the placenta was about 2-fold higher than that in amnion and chorion/decidua. On the other hand, the expression levels of CYP3A5 mRNA in amnion and chorion/decidua were about 2- and 10-fold, respectively, higher than that in placenta. The expression level of CYP3A7 mRNA was highest in chorion/decidua and the level was about 3-fold of placenta and amnion.

Western Blot Analysis of CYP3A To determine whether CYP3As protein was expressed, we used the homogenates of human placenta, amnion and chorion/decidua for Western blot analysis. The immunologically related protein(s) with CYP3A7 was detected at the same mobility to CYP3A7 in all tissues examined (Fig. 3). The content of this protein(s) in amnion was slightly lower than those in placenta and chorion/decidua.

Testosterone 6β-Hydroxylase Activity Testosterone 6β-hydroxylase activity as a marker of CYP3A, especially

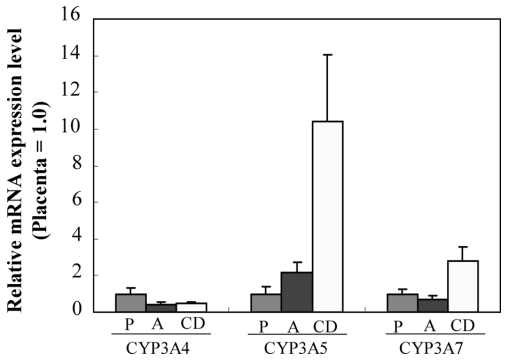

Fig. 2. Average of Ratio of CYP3A mRNA Expression in Placenta (P) as Standard Value of 1, Amnion (A) and Chorion/Decidua (CD)

The results are presented as the means±S.E. of 16—20 samples.

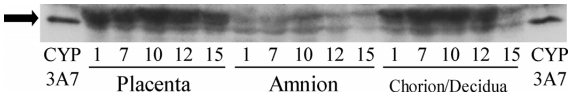

Fig. 3. Western Blot Analysis of CYP3A in Placenta, Amnion and Chorion/Decidua of Human

Tissue homogenates prepared from placenta, amnion and chorion/decidua obtained by Caesarean section before labor and following labor at term were subjected to SDS-PAGE, and electrophoretically transferred to membrane. The proteins were reacted with anti-CYP3A7 IgG. The positive control (C) contains microsomes of insect cells expressed CYP3A7 (0.2 pmol). Each lane of tissue samples contained 40 mg protein.

CYP3A4, was determined (Fig. 4). The activities in the homogenates of human placenta, amnion and chorion/decidua were 26.6, 3.7 and 4.6 pmol/h/mg protein, respectively. Testosterone 6β-hydroxylase activity of amnion or chorion/

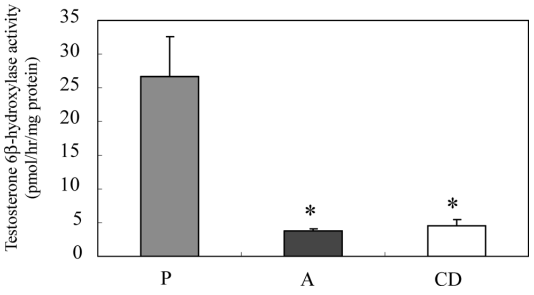

Fig. 4. Testosterone 6β-Hydroxylase Activity in Homogenates of Human Placenta (P), Amnion (A) and Chorion/Decidua (CD)

The results are presented as the means±S.E. of 5 samples (number: #1, 7, 10, 12 and 15). Statistical significance of differences between testosterone 6β-hydroxylase activity in homogenate of placenta and that of amnion or chorion/decidua ($*p<0.05$).

decidua was significantly lower than that of placenta.

DISCUSSION

Pregnant women are exposed to a wide variety of foreign chemicals. This exposure is most commonly due to maternal medication, lifestyle factors, such as smoking and alcohol consumption, or food sources. A number of CYP isoforms related to drug metabolism are expressed in human placenta.[9] It was thought that the fetus was dependent on the maternal capacity for drug metabolism or on the placenta to act as a "shield" against potentially harmful compounds.[26] Subsequent works have demonstrated that most xenobiotics freely cross the placenta into the fetal circulation and that the human fetal liver has a significant capacity for drug metabolism.[26] There is the possibility of recycling of drugs from amniotic fluid to the fetal circulation and their persistence in the fetus.[27] In this study, we clarified that CYP3A4, CYP3A5 and CYP3A7 mRNAs were also expressed in human amnion and chorion/decidua, not only in the placenta, although the expression levels of these CYP3A mRNAs were markedly different among individuals (Fig. 1).

Seven out of 20 pregnant women were treated with medicines such as synthetic and natural glucocorticoids, some antiepileptic drugs and ritodrine hydrochloride used for suppression of preterm labor (Table 3). We identified that ritodrine hydrochloride did not affect the expression of CYP3A mRNAs in human fetal liver cells (Maezawa K., Matsunaga T. and Ohmori S., unpublished data). On the other hand, it is well known that the expression level of CYP3A4 in liver is enhanced by treatment with various agents, such as glucocorticoids and some antiepileptic drugs.[28—30] In this study, the expression levels of CYP3A mRNAs in placenta of mother treated with glucocorticoids or antiepileptic drugs were not especially high as compared with those in placentas of mothers who were not taking medicines (Fig. 1). Our results are consistent with previous reports, because several drug therapies including phenobarbital and phenobarbital-like inducers such as carbamazepine have failed to affect placental CYP-associated activities.[31,32] Paakki *et al.* indicated that the formation of androstenedione from testosterone as the substrate, and *O*-deethylase activities of 7-ethoxycoumarin and 7-ethoxyresorufin were slightly decreased in the glucocorticoid-treated patients compared to control patients' values.[33] The expression of CYP3A4 mRNA is regulated by a number of nuclear receptors, including pregnane X receptor (PXR), constitutive androstane receptor (CAR), vitamin D receptor (VDR), glucocorticoid receptor (GR), hepatocytes nuclear factor 4α (HNF4α) and farnesoid X receptor.[34,35] CYP3A5 and CYP3A7 are transactivated by PXR and CAR.[36,37] PXR and HNF4α determine basal hepatic expression of CYP3A4 and CYP3A5.[38,39] The xenobiotic-induced transcriptional regulation of CYPs in human extrahepatic tissues is different from liver.[40] GR and VDR, but not PXR, are expressed in both placental syncytiotrophoblast and cytotrophoblast.[41,42] Pavek *et al.* reported that expression of CYP3A4 mRNA in placenta trophoblast cell line JEG3 cells was not changed significantly after treatment with dexamethasone.[42] They also indicated that non-responsiveness of CYP3A4 promoter to glucocorticoids is responsible for low expression of HNF4α because CYP3A4 promoter was activated in placenta cells only after co-transfection with HNF4α.[42] The expression of CYP3A mRNA in placenta might be hard to be induced by various chemicals including drugs.

Western blot analysis was also performed to assess CYP3A expression in amnion, chorion/decidua and placenta at the protein level. Immunological related proteins to antibody raised against human CYP3A7 were detected in all tissues examined (Fig. 3). The content in amnions was slightly lower than that of placenta or chorion/decidua. CYP3A4, CYP3A5 and CYP3A7 cannot be distinguished from each other by the polyclonal antibody used in this experiment. Furthermore, these CYP3A isoforms were difficult to electrophoretically separate using SDS-PAGE. These findings indicate that the bands of Western blot indicate the total amount of CYP3A isoforms. The antibody also reacted with proteins that have higher molecular weight as compared with the cDNA-expressed CYP3A7. We do not know what the immunologically related proteins are.

In the present study, 6β-hydroxytestosterone was detected as a major metabolite of testosterone in reaction mixture of amnion, chorion/decidua and placenta homogenates when the metabolites were analyzed by LC-MS/MS, as described in Materials and Methods. According to these findings, it was clarified that CYP3A isoforms in these tissues have functions. The activity in placenta was about 6-fold higher than that in amnion and chorion/decidua (Fig. 4). These results are consistent with expression pattern of CYP3A4 mRNA in these tissues, but not CYP3A5 and CYP3A7. CYP3A4, CYP3A5 and CYP3A7 actively catalyzed testosterone 6β-hydroxylation, however, with different velocities among the isoforms.[43—45] In the previous study, Ohmori *et al.* clearly indicated that the rate of the reaction catalyzed by CYP3A4 was about 6-fold higher than that catalyzed by CYP3A5 or CYP3A7.[43] These results indicate that CYP3A4 is a major isoform responsible for testosterone 6β-hydroxylase activity in amnion, chorion/decidua and placenta.

CYP3A isoforms have broad substrate specificity and are responsible for the oxidative metabolism of more than 50% of clinically used drugs.[46] Our results suggested that CYP3As in fetal membranes (amnion and chorion/decidua) have the metabolic function to protect the fetus from exposure to drugs that transfer from the maternal side to the placenta, and entry into amniotic fluid *via* uterus. However, further studies are needed to conclude whether CYP3A isoforms are also functionally active *in vivo* and have con-

sequences to the maintenance of integrity of the maternal-fetal barrier in the *in vivo* situation.

Acknowledgments This work was partly supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 18590138). We are grateful to Professor M. Kitada (Division of Pharmacy, University Hospital, Chiba University School of Medicine, Chiba, Japan) for the generous gift of anti-P-450 HFLa (CYP3A7) IgG. We also thank Ms. K. Aikawa for technical assistance.

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