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Effects of Finasteride, a Type 2 5-Alpha Reductase Inhibitor, on Fetal Development in the Rhesus Monkey (*Macaca mulatta*)

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ABSTRACT In genetic male fetuses, dihydrotestosterone (DHT) plays an important role in normal prostatic and external genital differentiation. The enzyme steroid 5-alpha reductase (5αR) catalyzes the conversion of testosterone (T) to DHT. The importance of 5αR in sexual differentiation is evident from the study of human genetic males who congenitally lack this enzyme and consequently develop ambiguous genitalia. These individuals are specifically deficient in the type 2 isozyme, whereas the normal type 1 isozyme activity has been found. The purpose of this study was to determine 1) the suitability of the rhesus monkey for testing the safety of 5αR inhibitors when administered during pregnancy and 2) the potential risk of administering a known type 2 5αR inhibitor, finasteride, during the critical period of internal and external genital differentiation in rhesus monkeys. In vitro studies were also performed on selected rhesus monkey tissues to determine the distribution of the 5αR isozymes. Gravid monkeys were treated once daily from gestational days (GD) 20 to 100. Sonographic monitoring was performed during the course of gestation to monitor viability, growth, and organ system development. Detailed fetal evaluations for developmental abnormalities were performed at term (GD 152 ± 2). A group of 13 pregnant monkeys ("positive control") were given a high oral dose (2 mg/kg/day) of finasteride to demonstrate that inhibiting type 2 5αR results in specific external genital abnormalities in male fetuses. Thirty-two pregnant monkeys were administered an intravenous (IV) formulation of finasteride at doses of 8, 80, or 800 ng/day. The highest IV dose selected was at least 60–750 times the semen levels of finasteride in man given orally 5 or 1 mg/day, respectively. Seventeen vehicle-control pregnant monkeys were also included. Administration of a high oral dose (2 mg/kg/day) of finasteride resulted in external genital abnormalities characterized by hypospadias, preputial adhesions to the glans, a small underdeveloped scrotum, a small penis, and a prominent midline raphe in male fetuses; however, no developmental abnormalities were seen in female fetuses. Similarly, no abnormalities were observed in either male or female fetuses of mothers given IV doses (8, 80, or 800 ng/day) of finasteride during pregnancy. The in utero sonographic findings in fetuses correlated with the gross findings at term. These studies have shown that external genital abnormalities can be produced in male monkey fetuses when exposed to a high oral dose (2 mg/kg/day) of finasteride, whereas no abnormalities were observed in fetuses exposed to the IV formulation of finasteride. Detailed in vitro studies demonstrated that the rhesus monkey also has two 5αR isozymes (types 1 and 2) with a tissue distribution similar to that seen in man and, furthermore, that finasteride is a potent, mechanism-based inhibitor with selectivity for both human and rhesus type 2 5αR. These studies have demonstrated that the monkey is a suitable model for assessing the safety of 5αR inhibitors administered during pregnancy.
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Finasteride (PROSCAR™, Merck & Co., West Point, PA) is a selective inhibitor of human type 2 5-alpha reductase (5αR), the enzyme responsible for the conversion of testosterone (T) to dihydrotestosterone (DHT). The selective pharmacological effect of finasteride has been well demonstrated in in vitro and in vivo studies (Brooks et al., '86; Cukierski et al., '91; Liang et al., '85; Rasmusson et al., '86; Prahalada et al., '94; Wise et al., '91). In men, oral administration of finasteride results in a marked decrease in circulating as well as intraprostatic DHT levels (Geller, '90; Gormley et al., '90, '92; McConnell et al., '92; Vermeulen et al., '89). Clinically, finasteride is indicated for the treatment of benign

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prostatic hyperplasia and is being developed to treat and prevent male pattern hair loss.

During sexual differentiation, DHT plays an important role in the development of male external genitalia (reviewed in George and Wilson, '88). In the human, genetic deficiency of 5αR results in feminization of male external genitalia (Imperato-McGinley et al., '81). Two types of isoenzymes of 5αR are known: the type 1 and the type 2 isozymes have been identified (Andersson and Russel, '90; Levy et al., '95). It has been shown that fetal genital skin and accessory sex glands have a predominance of the type 2 isozyme and that children with 5αR deficiency are deficient in this isozyme (Thigpen et al., '93).

The effect of pharmacologically induced DHT deficiency as a result of 5αR inhibition has been studied in rats. In these investigations, 5αR inhibition occurred as a result of finasteride administration during gestation; external genital malformations were observed in male fetuses (Clark et al., '90, '93), but no such effects were noted in female fetuses. Although the rodent model was invaluable for evaluating the potential risk of exposure to finasteride during gestation, significant developmental differences such as the timing of external genital differentiation and development, as well as differences in the finasteride selectivity for inhibition of the two isozymes (types 1 and 2), exist between humans and rats. In the rat, finasteride inhibits both type 1 and type 2 isozymes, which must be considered when interpreting results of the in vivo studies (Normington and Russel, '92; Azzolina et al., '97). In contrast, finasteride is a potent and highly selective inhibitor of human type 2 but not type 1 5αR (Bull et al., '96). Although both human and rat have two isozymes, the amino acid sequence identities are only 60% and 77% for the type 1 and type 2 5αR, respectively (Russel and Wilson, '94). Additionally, comparison of the two cloned 5αR isozymes (types 1 and 2) has shown that there is a high degree of homology (93–95%) between the human and cynomolgus monkey 5αR isozymes (Levy et al., '95). Similarly, recent studies have shown that there is a high degree of amino acid homology (>94%) between the human and rhesus monkey type 1 and type 2 5αR isozymes (Harris, unpublished observations).

Based on these data, studies were designed with the goal of determining 1) the suitability of the rhesus monkey for testing the safety of 5αR inhibitors when administered during pregnancy and 2) the potential risk of administering a known type 2 5αR inhibitor, finasteride, during the critical period of external genital differentiation. In vitro studies were also performed on selected rhesus monkey tissues in order to determine the nature of 5αR isozyme distribution and the mechanism of inhibition of 5αR by finasteride.

MATERIALS AND METHODS

Animals

Normally cycling adult female rhesus macaques (*Macaca mulatta*) (N = 62) with a history of prior pregnancy were bred and identified as pregnant according to established methods. Positive matings were confirmed by the presence of sperm in vaginal smears, and pregnancy was confirmed by radioimmunoassay of chorionic gonadotropin (Tarantal et al., '93) and ultrasound (Tarantal and Hendrickx, '88a). Pregnant rhesus macaques ranging in weight from 4.97 to 9.03 kg and age from 4 years, 7 months to 14 years, 11 months, were assigned to the study.

All procedures employed in the study conformed to the requirements of the Animal Welfare Act, and study protocols were approved prior to implementation by the Institutional Animal Use and Care Committee at the University of California at Davis. Activities related to animal care (diet, housing) were performed as per standard California Regional Primate Research Center (CRPRC) operating procedures. This study was sponsored by Merck Research Laboratories (West Point, PA) and conducted in accordance with FDA Good Laboratory Practice (GLP) regulations.

Finasteride administration

Oral. Finasteride was administered daily by oral gavage as a suspension in 0.5% methylcellulose (Dow Chemical Co., Midland, MI); the suspensions were prepared daily. The purity of the finasteride used was greater than 99% as determined by high performance liquid chromatography (HPLC). A vehicle-control group of monkeys were dosed daily with 0.5% methylcellulose.

Intravenous (IV). A sterile formulation of finasteride was used for daily IV injection. A placebo solution was used to inject vehicle-control animals. The formulation contained ethanol, polysorbate 80, sodium chloride, and water. This formulation was tested in vitro prior to study initiation for potential hemolysis and the results were negative. The total dosing volume was 1 ml/day in the high- and mid-dose (800 and 80 ng) and vehicle-control groups. The low-dose group monkeys received 0.1 ml/day (8 ng) of the drug formulation. Appropriate tests were done to confirm stability of the formulation for the duration of the study.

Study design

Assignment of animals to the study was on a rotational basis beginning with the treatment groups and ending with the control groups. As shown in Table 1, 13 dams were administered finasteride as an oral suspension (2 mg/kg/day; dosing volume 5 ml/kg) from gestational days (GD) 20 through 100 which encompasses the major period of organogenesis and external genital differentiation (term = 165 ± 10 days). Nine animals were assigned to a vehicle-control group and received 0.5% methylcellulose according to the same treatment regimen. Similarly, 32 animals were treated with the IV formulation of finasteride; 10 were administered 8 ng/day, 12 were administered 80 ng/day, and 10 were

TABLE 1. Overall study design for pregnant rhesus monkeys (*Macaca mulatta*) administered finasteride by the oral and IV routes

Compound	Dose	Route	Dosing volume	No. of animals
Control	0	Oral	5 ml/kg	9
Finasteride	2 mg/kg/day	Oral	5 ml/kg	13
Control	0	IV	1.0 ml	8
Finasteride	8 ng/day[1]	IV	0.1 ml	10
	80 ng/day[1]	IV	1.0 ml	12
	800 ng/day[1]	IV	1.0 ml	10

[1]Total dose per monkey.

administered 800 ng/day. Eight animals were assigned to an IV control group and received placebo only (buffered solution) according to the same treatment regimen (GD 20–100).

Maternal and fetal evaluations

The dams were observed twice daily during the treatment period for evidence of adverse physical signs, once in the morning and once approximately 2 hr after treatment. Maternal body weights were recorded once pretreatment then weekly until pregnancy termination. Ultrasound examinations were performed on all animals pretreatment (GD 18 ± 2), approximately every 5 days from GD 20 to 80, then approximately every 15 days from GD 90 to 150 (all ± 2) (Tarantal and Hendrickx, '88b). Included in the evaluations were assessments of viability, growth, and organ system development. Standardized measures of the head, abdomen, and limbs were obtained and compared to established colony reference values for the rhesus monkey (Tarantal and Hendrickx, '88b). For external genital formation, examinations during the period of differentiation consisted of assessments of the phallus, and subsequent development of the penis and scrotum in the male and clitoris and labia in the female (Tarantal and Hendrickx, '88c). Typically, the first indication of gender in the monkey can be detected approximately GD 55–60, with formation of the midline raphe and ventral migration of the phallus in the male. In the female, the phallus is typically observed in a more caudal orientation. By GD 65–70, the midline raphe and scrotum are clearly evident, and distinction between the penis and clitoris can be made.

All females with viable pregnancies were scheduled for hysterotomy on GD 152 ± 2. Fetal evaluations posthysterotomy consisted of a standard teratologic examination including external measurements of selected dimensions; a gross examination of the brain, in addition to thoracic, abdominal, and pelvic organs; and a determination of selected organ weights. Skeletal structures were analyzed after radiographs of the carcass were obtained. Placentas were weighed, measured, and examined for gross abnormalities. All aborted tissues or nonviable fetuses removed by hysterotomy were also evaluated for any abnormalities.

Finasteride plasma levels

Maternal blood samples (~2 ml) were collected from a peripheral vessel on GD 98 ± 2 at the following approximate time points from all animals administered oral finasteride: 0.5, 1, 2, 4, 6, 8, and 24 hr postdosing. An equivalent volume of blood (~2 ml) was collected at comparable time points from all concurrent vehicle controls. Blood samples were collected into heparinized syringes and plasma was recovered, frozen, and stored at ≤−70°C until shipped frozen for analyses. The samples were analyzed at Biochemical Toxicology Laboratory, Merck Research Laboratories (West Point, PA). No blood samples were taken from pregnant monkeys given IV doses of finasteride because the plasma concentrations were expected to be below the limit of quantitation.

Plasma concentrations of finasteride were determined by atmospheric pressure chemical ionization tandem mass spectrometry using a PE Sciex API III Mass Spectrometer. The assay was based on liquid extraction of the drug from plasma and multiple reaction monitoring of the parent and product ion. The method was accurate and precise to within ±20%. The method was validated for quantification of plasma level from approximately 0.25 to 50 ng/ml.

C_{max} was the peak plasma concentration, T_{max} was the time when C_{max} was observed, and AUC (or $AUC_{0\rightarrow 24hr}$) was the 0–24 hr area under the plasma concentration vs. time curve as calculated by the trapezoidal rule. A pharmacokinetic steady state was assumed, so the 24-hr plasma concentrations were used for the 0-hr values in AUC calculations. A value of zero (0) was used for calculations when plasma concentrations were below the limit of quantitation (LOQ). C_{max}, T_{max}, and AUCs were determined for individual animals and then these values were used to calculate mean toxicokinetic parameters. Group mean plasma concentrations for each sampling time point were calculated from the individual animal plasma concentrations.

In vitro studies of 5αR

Rhesus tissues were provided by New Iberia Research Center (New Iberia, LA). Specimens were collected from males (N = 4) and females (N = 2) ranging in age from 1 to 10 years of age. Tissues were also collected from a single male fetus on GD 100 (CRPRC, Davis, CA). Tissues were pulverized using a freezer mill and homogenized in 20 mM potassium phosphate, pH 6.5, 5 mM magnesium sulfate, 25 mM potassium chloride, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT, 5 μM NADPH, and 0.25 M sucrose using a Potter-Elverhjem homogenizer. The homogenate was centrifuged at 100,000*g* for 60 min. The membrane fraction was resuspended in 3–5 volumes of buffer containing 20% glycerol. Enzyme activity was measured in a reaction mixture containing 33 mM succinic acid, 44 mM imidazole, 33 mM diethanolamine (SID), [7-^{3}H]-T, 1 mM DTT, and 0.5 mM NADPH in a final volume of 100 μl. Typically, tissues containing type 1 5αR were assayed at pH 6.5 with 5 μM T. Conditions were altered

to pH 5.0–5.5 and 1 µM T for the type 2 isozyme. The assay was initiated by the addition of enzyme and incubated at 37°C for 30–90 min. T was separated from DHT using normal phase HPLC (Harris et al., '92). K_m values were determined as previously described (Harris et al., '92).

Inhibitor studies

IC_{50} values represent the concentration of inhibitor required to reduce the conversion of T to DHT by 50%. A human type 1 selective 5αR inhibitor (MK-0386, Merck & Co.) and finasteride (a type 2 selective 5αR inhibitor) were dissolved in 100% ethanol and diluted to the appropriate concentration in 10% ethanol. The concentration of inhibitor ranged from 0.1 to 1,000 nM for the IC_{50} determinations. Time-dependent inactivation by finasteride was determined by incubating 660 µg rhesus prostate homogenate in a mixture containing 0.5 mM NADPH and 5 nM finasteride at pH 5.5 in a final volume of 1.3 ml. Aliquots of 90 µl were withdrawn at selected times and ^{3}H-T and NADPH added to final concentrations of 1.5 µM and 1 mM, respectively.

RESULTS

Maternal evaluations

Physical signs/body weight. Administration of finasteride (oral or IV) during pregnancy resulted in no significant adverse maternal physical signs. The IV formulation was well tolerated with no evidence of local irritation at the injection site. A slight decrease in body weight was observed in some finasteride-treated dams when compared to concurrent controls; the magnitude of body weight changes was minimal and the individual animal body weights returned to within pretreatment values either during or posttreatment (data not shown). The body weight gains during the posttreatment period were similar among all groups.

Reproductive outcome

Pregnancy outcome for all treated and control groups is shown in Table 2. Administration of finasteride (oral, IV), did not result in a drug-related increase in the incidence of fetal loss. Although an apparent increase in the fetal loss rate was observed in the 2 mg/kg/day (23.1%) and 800 ng/day (20%) finasteride-treated groups when compared to concurrent controls, the overall incidence of fetal loss in the drug-treated groups (7/43; 16.3%) was within the range of historical incidence of fetal loss in the CRPRC rhesus colony during GD 18–100 [6.2% (8/129) to 16.5% (14/85)] (Hendrie et al., '96).

Ultrasound evaluations

Normal growth patterns were consistently observed for fetuses in all control and treated groups. For external genital development, only male fetuses in the oral finasteride group showed atypical developmental features. These consisted of the phallus positioned in a more caudal, female-like orientation (Fig. 1), with aberrant development of the penis and scrotum over time (Fig. 2). Additionally, the relationship of the phallus/penis to the scrotal sac was abnormal (Fig. 3). The midline raphe was noted in all male fetuses exposed to oral finasteride at the anticipated time point, but was more prominent when compared to normally developing males during the third trimester. Overall, although all male fetuses in the oral finasteride group were clearly males, they showed evidence of genital ambiguity during the early stages of differentiation, and abnormalities of the penis and scrotum that were relatively consistent when comparing fetuses.

All male fetuses in all other treated (IV finasteride) and control groups and all female fetuses in all treated (oral and IV finasteride) and control groups showed normal external genital development with characteristic features at the appropriate and anticipated time periods.

During the course of the study, other ultrasound observations unrelated to drug treatment included the following: one animal in the control group was noted with a nonviable conceptus on GD 31, with subsequent abortion of all products of conception. In the oral finasteride group, three animals were detected with fetal loss, two with nonviable fetuses on GD 50 and 72, respectively, and a third animal had a nongravid uterus on GD 25 indicating a complete abortion. For the 8 ng/day IV finasteride dose group, there was one placental abruption which resulted in removal of tissues surgically. For the 80 ng/day dose group, two animals were detected with fetal loss. One showed limited growth of the gestational sac (GS) on GD 26 and 30 with no evidence of embryonic viability. A nongravid uterus

TABLE 2. Pregnancy outcome for rhesus monkeys (*Macaca mulatta*) administered finasteride by the oral and IV routes

	Control		Oral (2 mg/kg/day)	IV		
	Oral	IV		8 ng/day	80 ng/day	800 ng/day
Dams						
Total gravid	9	8	13	10	12	10
No. with pregnancy loss	1[1]	0	3[2]	1[3]	2[4]	3[5]
Fetuses						
Total no.	8	8	10	9	10	7
Males	4	4	6	3	6	4
Females	4	4	4	6	4	3
No. with anomalies						
Males	0	0	6	0	0	0
Females	0	0	0	0	0	0

[1]Nonviable embryo: GD 31.
[2]Abortion/nonviable fetus: GD 25, 50, 72.
[3]Fetus surgically delivered on GD 98 due to placental abruption.
[4]One embryonic loss GD ≤25; one fetus aborted GD 110.
[5]Two abortions GD ≤27; one fetus surgically delivered GD 119 due to maternal complications unrelated to treatment.

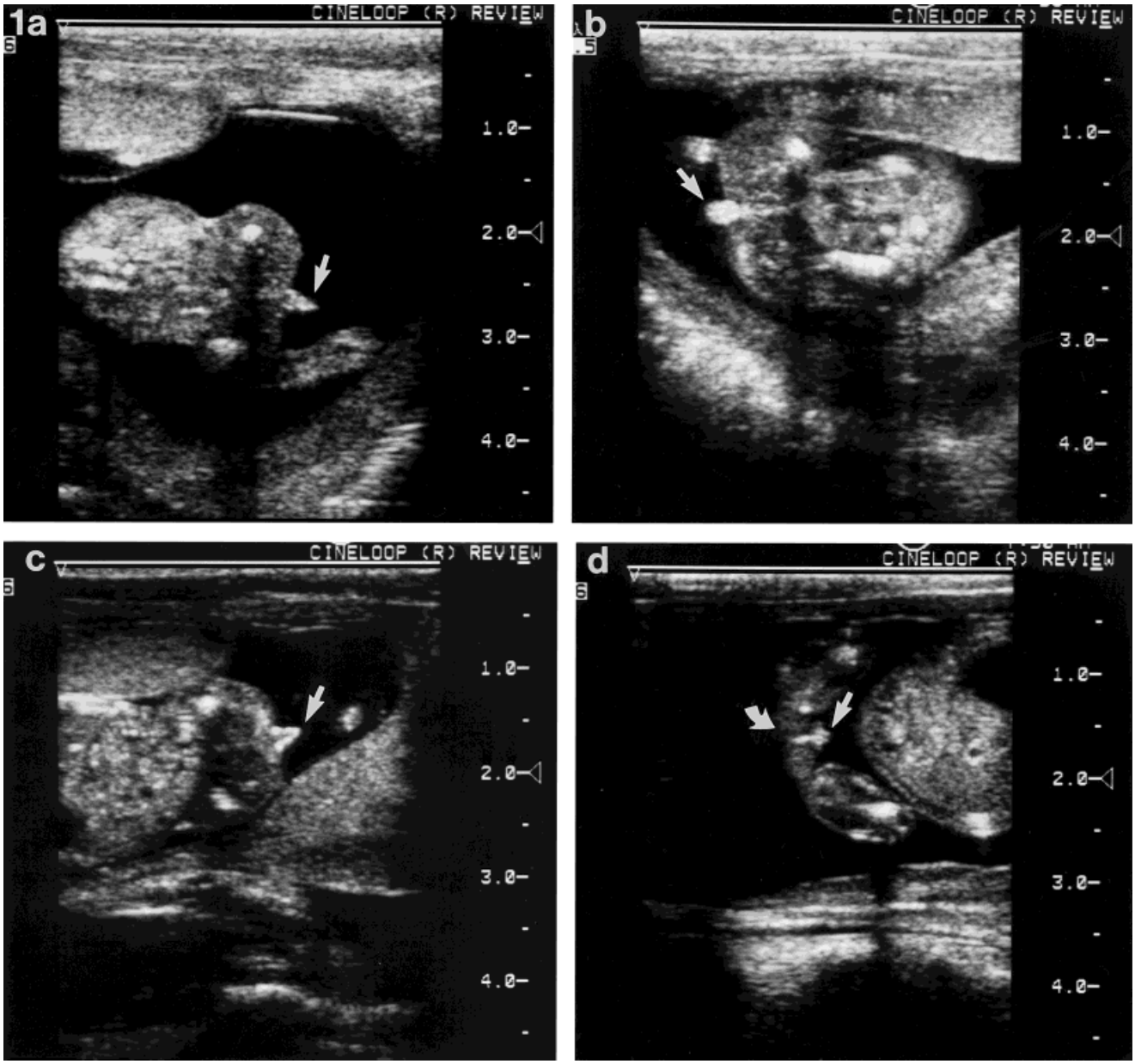

Fig. 1. Ultrasonographic examination of the phallus (arrow) of a male fetus of a dam exposed to a high oral dose (2 mg/kg/day) of finasteride [age: GD 60 (**a**) and 70 (**b**)]. Note anatomical similarities and differences, respectively, when compared to a normally developed female (**c**) and male fetus (**d**) at comparable gestational ages (curved arrow = scrotum). The numbers to the right of each sonogram represent cm.

was observed 3 days later confirming a complete abortion. Examination of the second animal in this dose group was unremarkable up through GD 108; a complete abortion was confirmed on GD 110. For the 800 ng/day dose group, two animals were detected with pregnancy loss. One showed no evidence of embryonic viability on GD 29 which was followed by a complete abortion, and the other was found to have a collapsing GS on GD 26 with a complete abortion confirmed on GD 27. A third animal was examined sonographically through GD 119, with all fetal assessments found to be within normal limits. However, due to maternal complications as a result of a peritoneal tear, the fetus was removed surgically at this time.

Fetal gross evaluations

Gross examinations. No abnormalities were observed in the control or IV finasteride-treated animals (Fig. 4a). In contrast, all male fetuses of dams administered a high oral dose of finasteride (2 mg/kg/day; 6/6) were observed with external genital abnormalities (Fig. 4b). The male external genital anomalies were characterized by hypospadias which was confined to the glans penis (5/6; 83.3%), preputial adhesions to the glans (6/6;

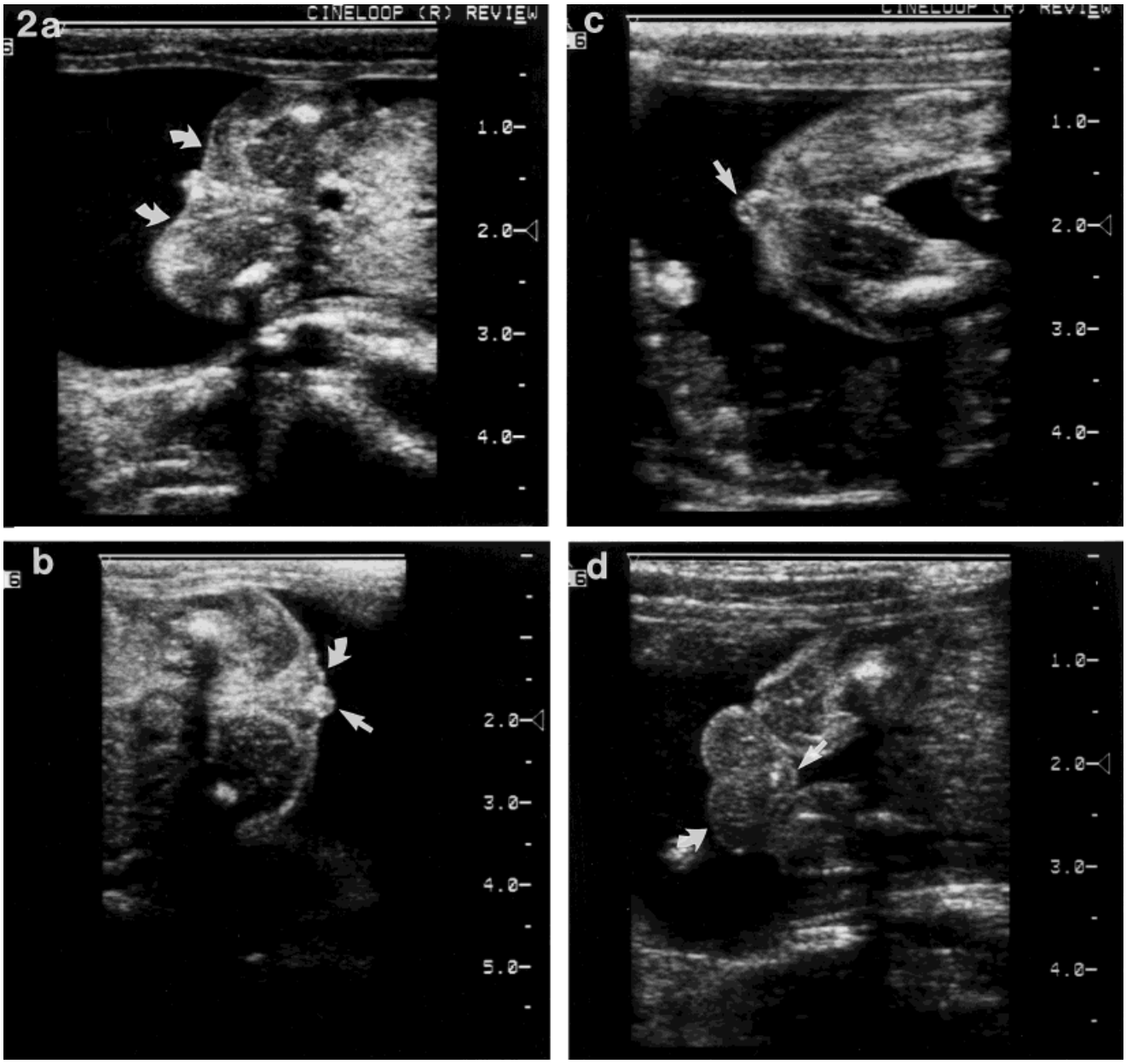

Fig. 2. For the fetus shown in Figure 1 (a,b), further development as monitored by ultrasound indicated an increase in size of the perineal folds (curved arrows; GD 80) (**a**) which developed into a small scrotal sac (curved arrow; GD 95) (**b**). Note abnormal position of the penis (arrow) in relation to the scrotum, which is more similar to the position of the clitoris (arrow) in the female of comparable gestational age (**c**), and markedly different when compared to a normally developed male (**d**).

100%), a small underdeveloped scrotum (6/6; 100%), a small penis (5/6; 83.3%), and a prominent midline raphe (6/6; 100%). No other developmental anomalies were noted in these fetuses and all female fetuses in this treatment group were normal.

External measurements and organ weights. There was no evidence of any finasteride-related effects on any external fetal measurements (Table 3) or fetal body or organ weights (Table 4). In spite of the genital abnormalities noted in the male fetuses exposed to oral finasteride, there were no apparent effects on anogenital distance or prostatic and seminal vesicular weights. Additionally, all placental measures were found to be within normal limits for all drug-treated groups when compared to concurrent controls (Table 5).

Plasma finasteride levels in pregnant rhesus monkeys

The mean (± SEM) plasma concentrations for each time point as well as the T_{max}, C_{max}, and AUC_{0-24hr}

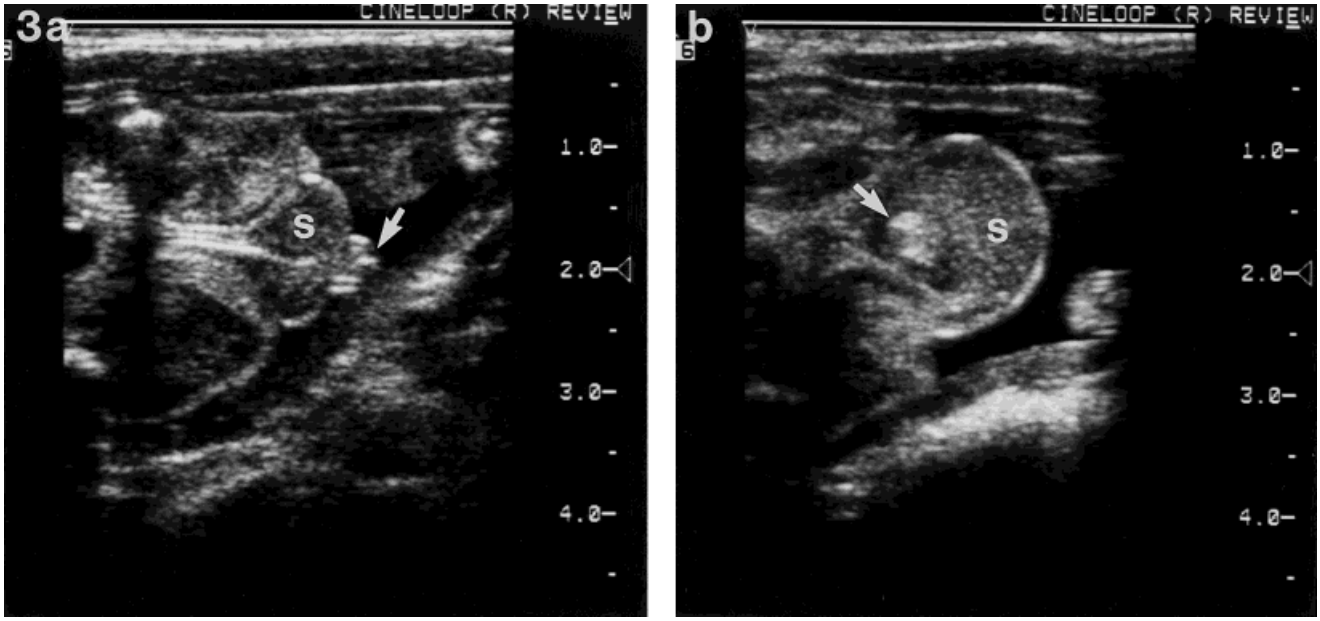

Fig. 3. An atypical relationship in the position of the penis (arrow) to the scrotum (s) was consistently noted sonographically in all male fetuses of dams exposed to a high oral dose (2 mg/kg/day) of finasteride (**a**) when compared to normally developed male fetuses of comparable gestational age (**b**). Note significant differences in the size of the scrotal sac.

values are presented in Figure 5. No attempt was made to quantify the plasma finasteride concentrations after the very low IV doses were given because the plasma concentrations were expected to be below the LOQ. Oral administration of finasteride at a dose of 2 mg/kg/day for approximately 78 days (± 2 days) to pregnant rhesus monkeys resulted in plasma drug levels that demonstrated systemic exposure. The plasma finasteride levels peaked at 2 hr, decreased approximately 6-fold by 8 hr, and fell to very low levels by 24 hr (Fig. 5). As discussed above, all male fetuses of dams in this group displayed external genital anomalies indicating that the maternal exposure was adequate to achieve biologically significant fetal exposure.

Steroid 5αR content of rhesus scalp and prostate

Human scalp skin is rich in type 1 5αR, whereas the prostate is rich in type 2 5αR (Harris et al., '92). For this reason, these tissues were chosen as possible sources of the isozymes of 5αR in the rhesus monkey. In general, there were low levels of enzyme activity present in all rhesus skin samples tested (0.2–0.8 pmol/min/mg) (Table 6). The level of activity did not depend on the region of skin (scalp, back, perineal), sex, or age of the animal (Table 6). Much higher levels of 5αR activity were found in rhesus prostate (6–40 pmol/min/mg) than in rhesus skin (Table 6).

The properties of the 5αR in rhesus scalp and prostate were compared to human type 1 (scalp skin) and type 2 (prostate) 5αR (Table 7). The enzyme activity in rhesus scalp has a broad pH profile with an optimum at neutral pH. At pH 6.5, a K_m of 6.8 for T was obtained which is in good agreement with that reported for human type 1 (scalp skin) 5αR (Harris et al., '92). Similar studies using rhesus prostate as a source of 5αR showed that this enzyme has an acidic pH optimum and a K_m of 1.5 μM for T.

Inhibitor studies were pursued in order to probe the similarities of the 5αR activity in rhesus skin and prostate with respect to human types 1 and 2 5αR, since it is now well established that the rat and human 5αR enzymes vary considerably with respect to inhibitor sensitivity (Normington and Russel, '92; Levy et al., '95). For these comparisons, MK-0386 and finasteride were chosen as types 1 and 2 5αR selective inhibitors, respectively (Harris et al., '92; Ellsworth et al., '96). The low levels of enzyme activity in rhesus skin resulted in some variability in the in vitro potency of the inhibitors. In order to minimize these variations, IC_{50} values represent the average of at least two determinations. As indicated in Table 7, IC_{50} values of 28 and >1,000 nM for MK-0386 were obtained with enzyme from rhesus scalp and prostate, respectively. In contrast, finasteride displayed >18-fold selectivity for the enzyme in rhesus prostate compared to skin with IC_{50} of 56 nM and 740 nM, respectively. Values obtained using human type 1 and type 2 5αR are also reported in Table 7 for comparison.

In order to extend the analysis, the time course for inhibition of the 5αR in rhesus prostate by finasteride was assessed. For these studies, enzyme and NADPH were preincubated in the presence and absence of 5 nM

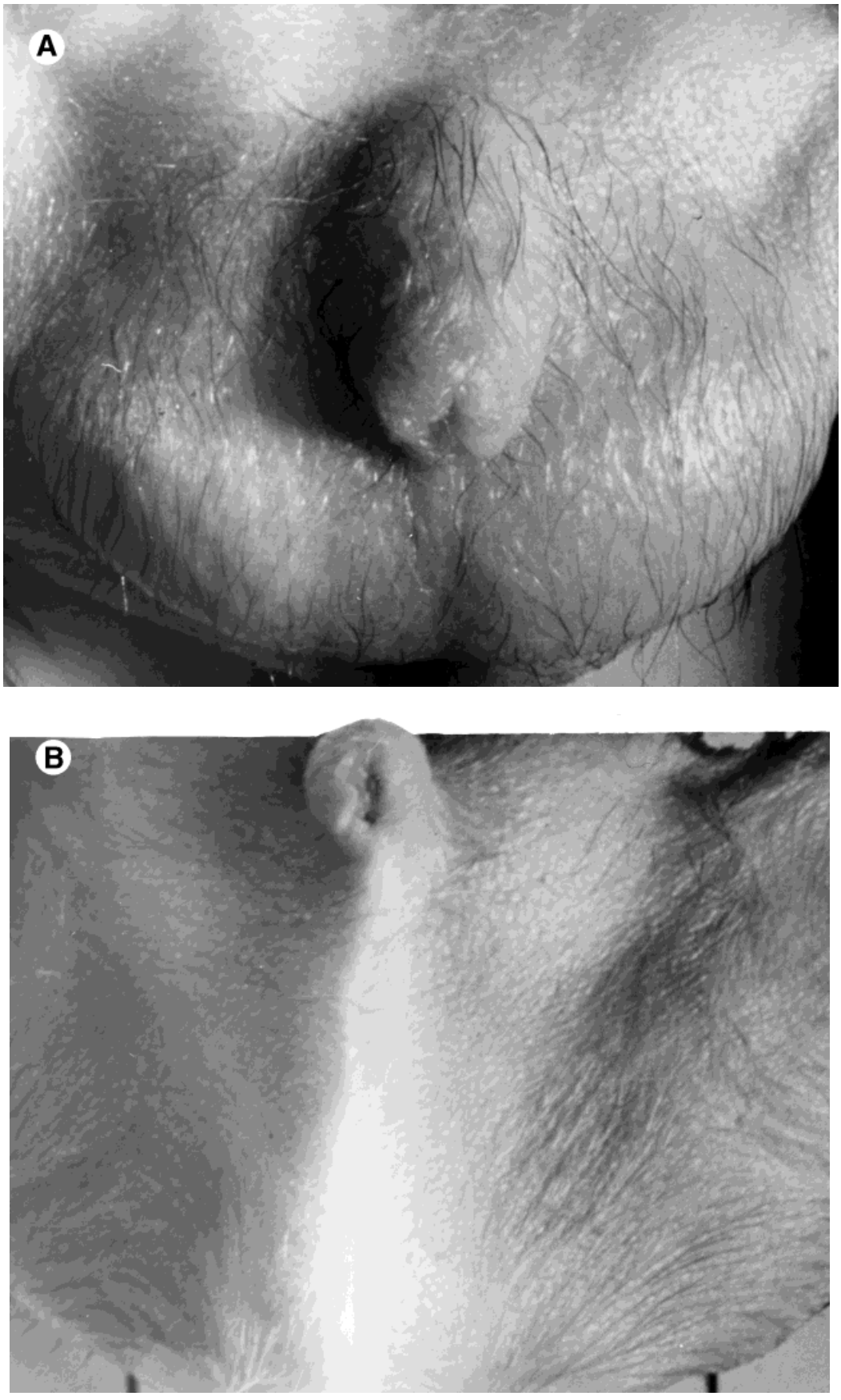

Figure 4

TABLE 3. Fetal external measurements (mm): Mean ± SD

	Control		Oral finasteride (2 mg/kg/day)	IV finasteride		
	Oral [N]	IV [N]	[N]	8 ng/day [N]	80 ng/day [N]	800 ng/day [N]
Crown-rump length	191.4 ± 5.6 [8]	192.4 ± 7.6 [8]	191.8 ± 6.5 [10]	196.3 ± 4.0 [9]	191.1 ± 5.0 [10]	194.3 ± 5.6 [7]
Crown-hip length	181.0 ± 5.9 [8]	181.3 ± 8.2 [8]	181.4 ± 6.5 [10]	185.4 ± 4.2 [9]	181.1 ± 4.5 [10]	183.1 ± 6.0 [7]
Femur length	58.8 ± 3.1 [8]	57.1 ± 2.8 [8]	59.2 ± 1.6 [10]	58.0 ± 1.7 [9]	56.9 ± 1.3 [10]	57.7 ± 2.4 [7]
Foot length	73.8 ± 1.3 [8]	73.3 ± 3.4 [8]	75.7 ± 2.4 [10]	75.2 ± 2.5 [9]	73.8 ± 2.4 [10]	76.9 ± 3.5 [7]
Biparietal diameter	52.0 ± 2.3 [8]	51.2 ± 1.4 [8]	52.4 ± 1.1 [10]	52.3 ± 1.7 [9]	52.3 ± 1.4 [10]	51.9 ± 1.4 [7]
Occipitofrontal diameter	66.9 ± 2.1 [8]	66.8 ± 2.3 [8]	67.4 ± 1.1 [10]	67.6 ± 1.2 [9]	66.7 ± 1.4 [10]	67.3 ± 1.4 [7]
Head circumference	192.9 ± 6.1 [8]	188.8 ± 5.9 [8]	192.8 ± 4.8 [10]	190.9 ± 4.0 [9]	189.9 ± 5.9 [10]	193.0 ± 4.6 [7]
Chest circumference	148.0 ± 7.8 [8]	139.4 ± 9.8 [8]	146.4 ± 6.1 [10]	141.9 ± 3.6 [9]	140.3 ± 6.3 [10]	143.0 ± 7.2 [7]
Anogenital distance						
Male	40.3 ± 3.9 [4]	39.5 ± 1.7 [4]	39.7 ± 7.8 [6]	40.0 ± 1.0 [3]	41.2 ± 2.8 [6]	40.3 ± 4.7 [4]
Female	19.5 ± 2.7 [4]	18.5 ± 1.0 [4]	19.8 ± 1.3 [4]	18.8 ± 1.5 [6]	17.8 ± 1.9 [4]	18.3 ± 1.5 [3]

TABLE 4. Fetal body and organ weights (g): Mean ± SD

	Control		Oral finasteride (2 mg/kg/day)	IV finasteride		
	Oral [N]	IV [N]	[N]	8 ng/day [N]	80 ng/day [N]	800 ng/day [N]
Body weight	468.31 ± 51.27 [8]	447.03 ± 76.19 [8]	467.20 ± 19.87 [10]	472.13 ± 32.81 [9]	445.17 ± 32.81 [9]	479.96 ± 40.13 [7]
Brain	55.76 ± 3.83 [8]	53.00 ± 4.76 [8]	54.87 ± 4.20 [10]	55.06 ± 5.24 [9]	55.13 ± 4.12 [10]	55.13 ± 2.17 [7]
Thymus	1.79 ± 0.42 [8]	1.26 ± 0.39 [8]	1.63 ± 0.52 [10]	1.56 ± 0.25 [9]	1.14 ± 0.36 [10]	1.58 ± 0.40 [7]
Liver	13.12 ± 2.42 [8]	11.93 ± 2.53 [8]	13.15 ± 2.09 [10]	13.15 ± 1.41 [9]	11.56 ± 1.41 [10]	12.80 ± 2.09 [7]
Spleen	0.70 ± 0.16 [8]	0.66 ± 0.14 [8]	0.65 ± 0.13 [10]	0.63 ± 0.08 [9]	0.59 ± 0.13 [10]	0.66 ± 0.12 [7]
Adrenal						
Right	0.12 ± 0.02 [8]	0.12 ± 0.02 [8]	0.13 ± 0.02 [10]	0.13 ± 0.04 [9]	0.11 ± 0.03 [10]	0.14 ± 0.01 [7]
Left	0.16 ± 0.03 [8]	0.15 ± 0.03 [8]	0.16 ± 0.02 [10]	0.17 ± 0.03 [9]	0.14 ± 0.03 [10]	0.18 ± 0.02 [7]
Kidney						
Right	1.22 ± 0.14 [8]	1.12 ± 0.21 [8]	1.19 ± 0.06 [10]	1.24 ± 0.17 [9]	1.12 ± 0.17 [10]	1.18 ± 0.16 [7]
Left	1.24 ± 0.12 [8]	1.13 ± 0.23 [8]	1.22 ± 0.09 [10]	1.30 ± 0.18 [9]	1.13 ± 0.16 [10]	1.21 ± 0.18 [7]
Gonads						
Male—right	0.05 ± 0.01 [4]	0.06 ± 0.02 [4]	0.06 ± 0.01 [6]	0.07 ± 0.00 [3]	0.06 ± 0.01 [6]	0.06 ± 0.01 [4]
Male—left	0.05 ± 0.01 [4]	0.06 ± 0.01 [4]	0.06 ± 0.01 [6]	0.07 ± 0.00 [3]	0.06 ± 0.01 [6]	0.06 ± 0.01 [4]
Female—right	0.03 ± 0.00 [4]	0.05 ± 0.02 [4]	0.04 ± 0.00 [4]	0.04 ± 0.01 [6]	0.04 ± 0.02 [4]	0.05 ± 0.02 [3]
Female—left	0.03 ± 0.01 [4]	0.05 ± 0.03 [4]	0.03 ± 0.00 [4]	0.04 ± 0.01 [6]	0.04 ± 0.01 [4]	0.04 ± 0.02 [3]
Prostate	0.16 ± 0.02 [4]	0.12 ± 0.04 [4]	0.14 ± 0.04 [6]	0.16 ± 0.01 [3]	0.17 ± 0.03 [6]	0.14 ± 0.01 [4]
Seminal vesicles	0.30 ± 0.04 [4]	0.28 ± 0.05 [4]	0.28 ± 0.04 [6]	0.36 ± 0.07 [3]	0.30 ± 0.03 [6]	0.29 ± 0.05 [4]

finasteride at 37°C. At selected times, aliquots were withdrawn and residual enzyme activity was determined by the addition of ^{3}H-T. As indicated in Figure 6, a time-dependent loss of enzyme activity was observed in the presence of 5 nM finasteride compared to the absence of finasteride. From these data it is apparent that the $t_{1/2} \leq 10$ min for inactivation of the rhesus prostate 5αR by 5 nM finasteride. Under identical conditions, there is little loss of enzyme activity in the control (Fig. 6). These data are in good agreement with a $t_{1/2} < 10$ min for the human type 2 5αR (Bull et al., '96; Faller et al., '92). A complete analysis of the kinetic characteristics and mechanism of inhibition of the rhesus type 2 5αR is in progress using recombinant enzyme.

Fig. 4. External genitalia of male rhesus monkey fetuses examined at term (GD 150 ± 2 days). **a:** A control male fetus with well-developed scrotum and penis. The urethral opening is at the center of the penis. **b:** A male fetus of a dam exposed to a high oral dose (2 mg/kg/day) of finasteride; the midline raphe is prominent, the scrotal sac is not apparent, and the penis is small with a ventral urethral opening (hypospadias). The male fetuses born to dams given IV doses of finasteride had external genitalia similar to vehicle controls (a).

5αR content in fetal tissues from rhesus monkey

The distribution of 5αR in 12 tissues from a GD 100 male rhesus fetus was determined using enzyme assays. In order to assess the predominant form of 5αR present in these tissues, samples were tested at pH 5.5 and 7.5. Under these assay conditions, the ratio of activity at the two pHs can predict isozyme content. This analysis is based on the difference in pH optimum of 7.0 and ≤5.5 for the type 1 and type 2 5αR, respectively (Harris et al., '92; Normington and Russell, '92; Levy et al., '95). The results of the pH studies are included in Table 8. A ratio of 1.5 or lower suggests that the predominant isozyme in the tissue is type 1, while a higher ratio (5–125) indicates that the predominant isozyme is type 2. For those samples where activity was detected at one pH but not the other, the ratio is not reported, but the isozyme determination is based on the pH at which activity was detected. High levels (5–9 pmol/min/mg) of 5αR activity were found in prostate,

TABLE 5. Summary of placental weights (g) and measurements (mm), umbilical cord lengths (mm), and amniotic fluid volumes (ml): Mean ± SD

	Control		Oral finasteride[3] (2 mg/kg/day)	IV finasteride		
	Oral[1]	IV[2]		8 ng/day[4]	80 ng/day[3]	800 ng/day[2]
Placental weight	128.01 ± 19.82	136.66 ± 21.14	138.85 ± 17.21	135.59 ± 19.26	125.51 ± 19.06	132.90 ± 19.52
Primary disc length	90.4 ± 14.1	90.6 ± 12.1	97.7 ± 14.2	99.9 ± 7.8	93.7 ± 13.1	90.6 ± 9.4
Primary disc width	81.3 ± 14.4	81.3 ± 9.2	93.3 ± 11.9	87.4 ± 11.0	86.6 ± 12.8	82.6 ± 9.4
Secondary disc length	82.0 ± 19.7	79.0 ± 8.8	81.1 ± 6.5[2]	76.9 ± 12.0[1]	72.1 ± 17.0[4]	76.8 ± 12.7[5]
Secondary disc width	70.4 ± 20.2	73.1 ± 9.1	72.7 ± 9.0[2]	70.1 ± 12.1[1]	62.6 ± 16.6[4]	73.2 ± 13.1[5]
Umbilical cord length	157.8 ± 33.9	180.6 ± 39.9	181.1 ± 33.0	175.8 ± 24.0	174.6 ± 31.9	160.1 ± 19.8
Amniotic fluid volume	113.1 ± 66.2	100.0 ± 39.6	112.7 ± 46.6	128.1 ± 48.2	89.0 ± 29.6	142.7 ± 47.6

[1]N = 8.
[2]N = 7.
[3]N = 10.
[4]N = 9.
[5]N = 5.

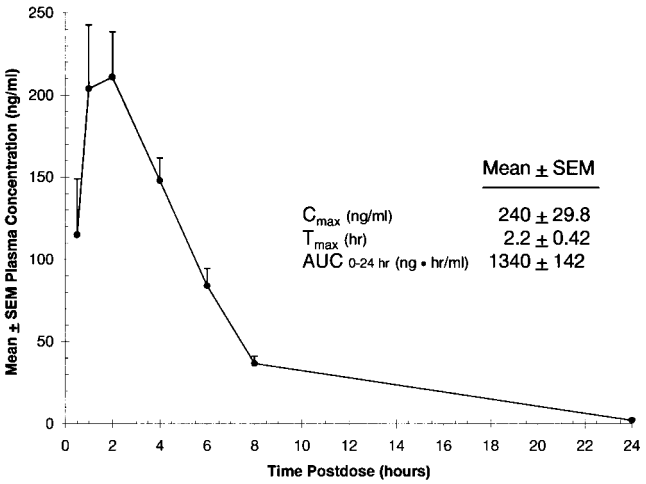

Fig. 5. Plasma drug levels on GD 98 ± 2 after oral administration of finasteride (2 mg/kg/day) from GD 20 to 100.

TABLE 6. 5αR activity in skin and prostate of rhesus monkeys[1]

	Age (years)	N	Sex	Specific activity (pmol/min/mg)[2]
Scalp	1	1	F	0.7
Back	1	1	F	0.7
Scalp	4	1	F	0.3
Back	4	1	F	ND
Scalp	2	3	M	0.4–0.8
Back	2	3	M	0.5–0.8
Perineal	2	3	M	0.3–0.5
Scalp	7	1	M	0.2
Genital skin	10	1	M	0.2
Prostate	NA	1	M	7.7
	7	1	M	40
	10	1	M	6

[1]N = number of samples; ND = none detected; NA = not available.
[2]Enzyme assays for skin and prostate were conducted at pH 6.5 and pH 5.5, respectively.

external genitalia, and seminal vesicle (Table 8) and lower levels (<~0.13 pmol/min/mg) of the activity were present in liver, scrotal skin, scalp, back skin, and brain.

DISCUSSION

The studies described here provide evidence for two forms of 5αR in rhesus monkey. The properties of the enzyme activity in rhesus scalp and back skin with respect to pH/activity profile, K_m for T, and IC_{50} values for finasteride agree closely with those of the human type 1 5αR. Furthermore, the properties of the 5αR activity in rhesus prostate resemble those of human type 2 5αR. Although the IC_{50} values suggest that there are differences in the affinity of the rhesus and human type 2 5αRs for finasteride (Table 7), it is likely that the value for rhesus type 2 underestimates the potency of this compound, since there is a close agreement in rate constants for inactivation of these two homologs by finasteride (Fig. 6; Bull et al., '96; Faller et al., '92). Furthermore, recent studies with the recombinant rhesus type 1 and type 2 5αRs (Harris, unpublished observations) confirm similarities between the rhesus and human type 5αRs. On the basis of these findings, we conclude that type 1 5αR predominates in rhesus skin, while type 2 is the predominant form in prostate, as has been previously reported for human tissues (Harris et al., '92; Thigpen et al., '93).

It is worth noting that the 5αR content of rhesus skin samples does not exhibit regional differences in enzyme activity as found in human (Thiboutot et al., '95). Similar levels of enzyme activity were obtained in rhesus scalp, back, and perineal skin regardless of age (1–10 years) or sex of the animal (Table 6). Most notable is the low level of enzyme in rhesus scalp skin compared to adult human scalp skin (Harris et al., '92). In contrast, there is good agreement in the level of enzyme activity in rhesus and human prostate.

The difference in pH optimum for rhesus type 1 and type 2 5αR obtained with both native and recombinant sources (Harris, unpublished observations) of enzyme

TABLE 7. Properties of 5αR in rhesus skin and prostate[1]

	Rhesus		Human[2]	
	Scalp skin	Prostate	Scalp skin	Prostate
pH optimum	Neutral	5.0	Neutral	5.5
K_m testosterone (μM)	6.8[3]	1.5[3]	7.5	0.3
Specific activity (pmol/min/mg)	0.2–0.8	6–40	10	20
IC_{50} MK-0386 (nM)	28	>1,000	20	>1,000
IC_{50} finasteride (nM)	740	56	670	4.2

[1]IC_{50} values represent the average of at least two determinations.
[2]Values reported previously for scalp skin (type 1) and prostate (type 2) (Harris et al., '92; Ellsworth et al., '96).
[3]Value for the rhesus scalp skin represents the average of three experiments; value for the prostate is from a single experiment.

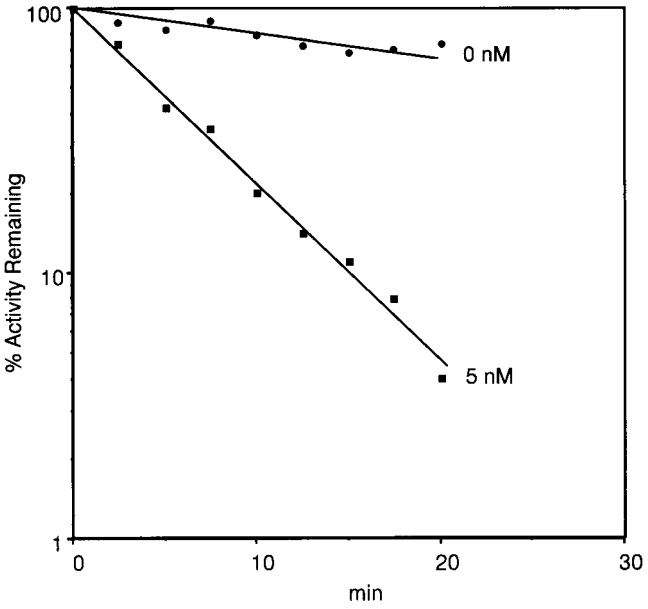

Fig. 6. Time course for inactivation of rhesus prostate 5αR by finasteride. Finasteride was incubated with prostate 5αR and NADPH. At selected times, samples were withdrawn and residual enzyme activity was measured by the addition of ^{3}H-T as described in Materials and Methods.

was exploited to define the isozyme distribution in rhesus fetal tissues. Results of these studies suggested that type 1 5αR is the predominant form in scalp, back skin, brain, liver, and testes, while type 2 5αR is present in prostate, external genitalia, seminal vesicle, mammary skin, kidney, and scrotal skin. The presence of high levels of type 2 5αR in fetal prostate and external genitalia as well as the similarities in the rhesus and human isozymes of 5αR make rhesus monkey a suitable species to evaluate the teratogenicity of finasteride.

Observations from the in vitro studies are supported by the detailed in vivo studies performed. First, a group of animals were administered a high oral dose of finasteride (2 mg/kg/day) as a "positive control" to establish that inhibition of type 2 5αR activity during the critical period of external genital formation results in specific male external genital abnormalities. All of six male fetuses in this "positive control" group had external genital anomalies in the absence of other nongenital developmental defects. No similar effects were observed in female fetuses in this "positive control" group. Determination of plasma drug levels showed that there was measurable maternal exposure to finasteride. The observations in male rhesus fetuses of dams given a high oral dose (2 mg/kg/day) of finasteride are consistent with the findings in male rat fetuses exposed in utero to finasteride (Clark et al., '90). However, the male external genital abnormalities observed were relatively less severe in both laboratory species compared to external genital abnormalities described in newborn males with genetic deficiency of type 2 5αR.

In order to better understand the physiologic role of type 1 5αR as well as explore the potential therapeutic use, type 1 specific 5αR inhibitors such as L-751,788 are being studied (Sahoo et al., '96). To evaluate the potential adverse effects of type 1 specific 5αR inhibition on fetal development in rhesus monkey, L-751,788 was administered orally to pregnant rhesus monkeys at doses of 2 or 10 mg/kg/day from GD 20 to 100. Administration of L-751,788 resulted in no external genital abnormalities in either male or female fetuses (Table 9). The observation in rhesus monkeys exposed to selective type 1 or type 2 5αR inhibitors is consistent with the findings in children with genetic deficiency of 5αR and supports the hypothesis that type 2 5αR plays an important role in external genital differentiation in mammals.

Finasteride is not indicated for use in women, although there is no apparent risk of exposure under nonpregnant conditions. Although administration of finasteride to pregnant women is contraindicated, indirect exposure to low levels of finasteride could theoretically result through semen from a partner who is on finasteride treatment. To address this question, the dosages chosen for the IV rhesus monkey study (8, 80, and 800 ng/day) were based on 1) the dose (5,000 ng/day) at which no effect on plasma DHT levels was observed following oral doses in men and 2) the levels of finasteride in semen of men on chronic finasteride treatment (Physicians' Desk Reference).

The highest IV dose administered to the monkey (800 ng/day or ~120 ng/kg/day) was similar to the oral dose (5,000 ng/day or ~100 ng/kg/day) that had no effect on plasma DHT levels in men. Furthermore, the semen concentration of finasteride is very low (0–21 ng/ml ejaculate; Physicians' Desk Reference). The semen lev-

TABLE 8. 5αR activity in fetal tissues of a rhesus monkey[1]

	% conversion		Specific activity		Predominant
	pH 5.5	pH 7.5	(pmol/min/mg)	Ratio	isozyme
Fetal					
Scalp skin	0.23	0.16	0.014	1.4	1
Back skin	0.15	0.14	0.006	1.1	1
Scrotal skin	5.22	0.0	0.13	—	2
Mammary skin	0.19	0.0	0.003	—	2
Brain	0.49	0.65	0.11	0.8	1
Liver	0.08	0.12	0.11	0.7	1
Testes	0	0.12	0.013	—	1
Kidney	1.07	.22	0.12	5	2
Epididymis	0	0	ND	—	ND
Prostate	96.6	1.13	5.19	85	2
External genitalia	78.6	0.63	7.23	125	2
Seminal vesicle	1.73	0.20	8.4	9	2
2 years					
Prostate	3.94	0.69	0.5	5	2
Scalp skin	0.31	0.31	1	1	1

[1]ND = none detected.

TABLE 9. Effect of L-751,788, a selective type 1 5αR inhibitor on pregnant rhesus monkeys[1]

Compound	Oral dose (mg/kg/day)	No. animals	Fetal outcome
Vehicle control	—	5	No abnormalities 5 males
L-751,788	2	5	No abnormalities 1 male, 4 females
	10	5	No abnormalities 3 males, 2 females

[1]The overall study design was the same as described for the finasteride study.

els of finasteride ranged from <0.1 to 10.54 and <0.1 to 1.52 ng/ml in men administered chronic (approximately ≥6 weeks) oral doses of finasteride at 5 and 1 mg, respectively (unpublished data). Assuming a total ejaculate volume of 5 ml, the highest possible level of finasteride exposure to a pregnant woman is therefore ≤100 ng (≤2 ng/kg) daily. This also assumes that there is 100% vaginal absorption. Despite such conservative estimates, there is a large margin of safety for purposes of risk assessment based on this animal model. The highest IV dose (800 ng/day) tested in the pregnant rhesus monkey is at least 60–750 times the highest semen finasteride levels in men administered 5 and 1 mg, respectively. Therefore, exposure of pregnant women through semen of men taking finasteride is not considered a risk for the human fetus.

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