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# Pharmacokinetic parameters and mechanisms of inhibition of rat type 1 and 2 steroid 5α-reductases: determinants for different *in vivo* activities of GI198745 and finasteride in the rat

J. Darren Stuart[a,*], Frank W. Lee[b,1], Deborah Simpson Noel[a], Sue H. Kadwell[a], Laurie K. Overton[a], Christine R. Hoffman[a], Thomas A. Kost[a], Timothy K. Tippin[b], Russell L. Yeager[b], Kenneth W. Batchelor[c], H. Neal Bramson[a]

[a]*Division of Biochemistry, Glaxo Wellcome Inc., 5 Moore Drive, Research Triangle Park, NC 27709, USA*
[b]*Division of Bioanalysis and Drug Metabolism, Glaxo Wellcome Inc., 5 Moore Drive, Research Triangle Park, NC 27709, USA*
[c]*US Drug Discovery, Glaxo Wellcome Inc., 5 Moore Drive, Research Triangle Park, NC 27709, USA*

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**Abstract**

The interaction of baculovirus expressed rat steroid 5α-reductase types 1 and 2 (r5AR1 and r5AR2) with 17β-*N*-(2,5-bis(trifluoromethyl)phenyl)carbamoyl-4-aza-5α-androst-1-en-3-one (GI198745) was investigated at pH 7 and 37°. This 5α-reductase inhibitor was found previously to be a time-dependent inhibitor of the two human 5α-reductase isozymes. In contrast, we demonstrate in the present study that although GI198745 is a potent time-dependent inhibitor of r5AR2, it is a classical rapid-equilibrium inhibitor of r5AR1. This type of behavior with human and rat 5α-reductases has been shown for the inhibitor 17β-(*N*-*tert*-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one (finasteride), a current therapy for benign prostatic hyperplasia. Inhibition of r5AR1 by GI198745 was competitive with testosterone and followed Michaelis–Menten kinetics with a $K_i$ value of 0.3 ± 0.02 nM. Data for the inhibition of r5AR2 by GI198745 were consistent with a two-step mechanism, where $K_i$ is the dissociation constant for an initial enzyme–inhibitor complex and $k_3$ is the rate constant for the second slow step. The pseudo-bimolecular rate constant ($k_3/K_i$) for the association of GI198745 with r5AR2 was (2.0 ± 0.4) × $10^7$ $M^{-1}$ $sec^{-1}$. The high affinity of this inhibitor for r5AR2 was further demonstrated by the inability of the enzyme–inhibitor complex to dissociate after approximately 7 days of dialysis at 4°. Both GI198745 and finasteride appear to inactivate r5AR2 by apparent irreversible modification, but are classical, reversible inhibitors of r5AR1. Therefore, we hypothesize that because of its pharmacokinetic parameters and increased potency against r5AR1, GI198745 is more effective than finasteride in preventing the growth of the rat prostate. 

*Keywords:* 5α-Reductase inhibitors; Testosterone 5α-reductase; Finasteride; Prostatic hyperplasia; Androgens; Pharmacokinetics of GI198745 and finasteride

## 1. Introduction

The steroid 5α-reductase (EC 1.3.99.5, 3-oxo-steroid $\Delta^4$-reductase, 5AR) is a microsomal enzyme that catalyzes the transfer of a hydride from NADPH to the $\Delta^{4,5}$ double bond of various steroids, e.g. the reduction of testosterone to form the more potent androgen DHT. In the human male, DHT is required for normal development of the prostate and is thought to be a causative factor in the condition known as benign prostatic hyperplasia. Two isozymes of 5α-reductase have been identified in both rats and humans, and these isozymes are referred to as type 1 and type 2 for the order in which they were cloned [1–4]. GI198745 (Fig. 1A), 17β-*N*-(2,5-bis(trifluoromethyl)phenyl)carbamoyl-4-aza-5α-androst-1-en-3-one, and finasteride (Fig. 1B), 17β-(*N*-*tert*-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one, are time-dependent inhibitors of both human 5α-reductases [5–9].

While many *in vitro* and *in vivo* studies have demonstrated the effects of finasteride on the rat 5α-reductases

* Corresponding author. Tel.: +1-919-483-4873; fax: +1-919-483-3895.
*E-mail address:* jds14989@gsk.com (J.D. Stuart).

[1]Present address: DuPont Pharmaceuticals Company, Newark, DE.

[2]Dr. Achintya K. Sinhababu, personal communication. Cited with permission.

*Abbreviations:* r5AR1, rat 5α-reductase 1; r5AR2, rat 5α-reductase 2; and DHT, dihydrotestosterone.

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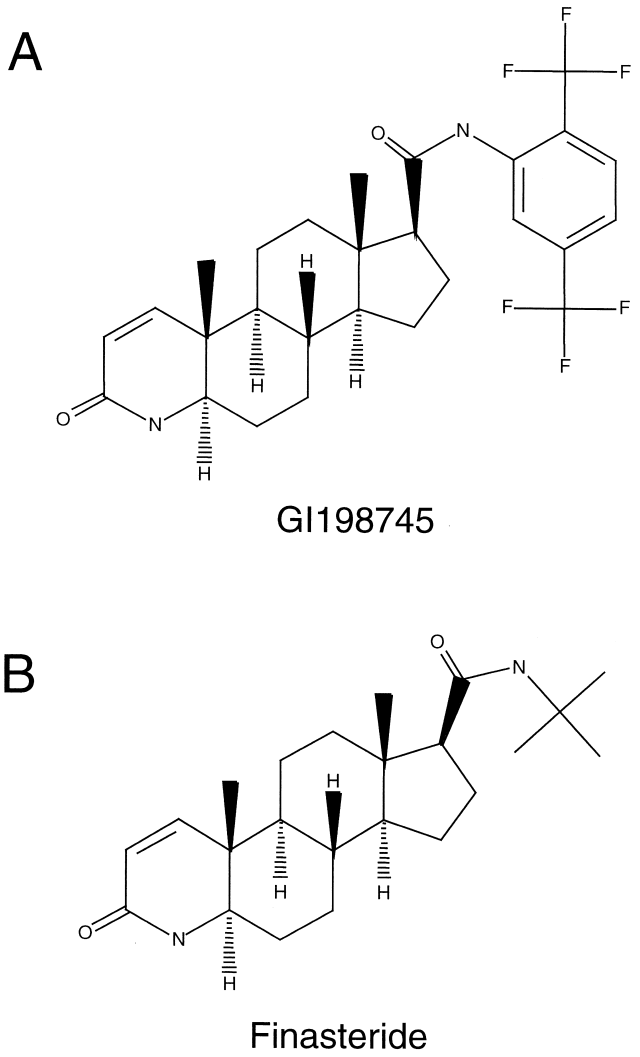


Fig. 1. Chemical structures: (A) 17$\beta$-*N*-(2,5-bis(trifluoromethyl)phenyl) carbamoyl)-4-aza-5$\alpha$-androst-1-en-3-one (GI198745); and (B) 17$\beta$-(*N*-*tert*-butylcarbamoyl)-4-aza-5$\alpha$-androst-1-en-3-one (finasteride).

[10,11], there is little information about how the mechanism for inhibition of these enzymes by finasteride affects *in vivo* activity. In the present study, we characterized the mechanism by which GI198745 inhibits the rat 5$\alpha$-reductases and used this information, along with the pharmacokinetic parameters of GI198745 and finasteride, to provide an explanation for the higher *in vivo* potency of GI198745 compared with finasteride in preventing prostatic growth in the rat.

## 2. Materials and methods

### *2.1. Materials*

[1,2,6,7-$^3$H(N)]Testosterone (85–101 Ci/mmol) was purchased from New England Nuclear (NEN). Unlabeled testosterone, NADPH, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were from the Sigma Chemical Co. Finasteride was synthesized according to Rasmusson *et al.* [12]. GI198745 was synthesized at Glaxo Wellcome Research and Development according to Batchelor and Frye [13].

### *2.2. Expression of rat 5α-reductases in the baculovirus expression system*

Plasmids containing the cDNAs for r5AR1 and r5AR2 were provided by Dr. David W. Russell. The coding region of r5AR1 was excised from the vector with *Eco*RI and *Bam*HI and directionally cloned into the corresponding restriction sites of the baculovirus transfer vector pVL1392 downstream from the viral polyhedron promoter. The coding region of r5AR2 was excised with *Eco*RI and *Hin*dIII. A *Hin*dIII/*Bam*HI linker was ligated to the 3′ end of the r5AR2 fragment, which was then directionally cloned into the *Eco*RI and *Bam*HI site of pVL1392. Recombinant virus production and expression of the 5$\alpha$-reductases by Sf9 cells were done as described previously [7] with the following exceptions. For 5$\alpha$-reductase production runs, Sf9 cells were grown in a stirred tank reactor to a density of $1.0 \times 10^6$ cells/mL and then infected with recombinant virus. The cells were harvested 48 hr post-infection.

### *2.3. Preparation of microsomal 5α-reductases 1 and 2*

Baculovirus-infected Sf9 cells expressing each rat 5$\alpha$-reductase were kept frozen at $-80^o$ until preparation of the microsomes. All homogenization steps were performed on ice, and all centrifugations were done at $4^o$. For the microsomal preparations, cells (35 mL) were thawed, diluted with 175 mL of homogenization buffer (50 mM potassium phosphate, pH 7.4, 0.25 M sucrose, 0.1 mM EDTA), and homogenized with a Brinkmann polytron for three, 15- to 20-sec blasts at 16,000 rpm. The homogenate was further homogenized by 10 strokes with a teflon/glass homogenizer attached to a Con-torque power unit at high speed or with a Dounce hand-held glass homogenizer. The homogenate was centrifuged at 600 *g* for 10 min, and the resulting supernatant was centrifuged at 10,000 *g* for 15 min. The 10,000 *g* pellet was resuspended in 32–40 mL of homogenization buffer using one of the two homogenizers mentioned above, and then was centrifuged again at 10,000 *g* for 15 min. The 10,000 *g* supernatants were combined and centrifuged at 100,000 *g* for 1 hr. The resulting microsomal pellets were resuspended as above in 45–100 mL of homogenization buffer and spun at 100,000 *g* for 1 hr. The washed microsomes were resuspended as described above in 7 mL of microsomal resuspension buffer [17.6 mM imidazole, 17.6 mM diethylamine, 14.2 mM succinic acid, 260 mM KCl, 0.005% Triton X-100, 1 mM dithiothreitol (DTT)] at pH 7 (r5AR1) or pH 6 (r5AR2) and then were stored in small aliquots ($<$200 $\mu$L) at $-80^o$ until used in the enzyme assays. The protein concentration of the stock microsomes was determined by the bicinchoninic acid (BCA) protein assay from Pierce, using bovine serum albumin as a standard.

### 2.4. Standard 5α-reductase activity assays

The assay was carried out at 37° in polystyrene 96-well plates (Costar 3797) in a total assay volume of 250 μL. A solution of the substrate [1,2,6,7-$^3$H]testosterone was prepared by evaporating ethanol from the stock solution in a Savant AES1000 SpeedVac and resuspending the residue in assay buffer (17.6 mM imidazole, 17.6 mM diethylamine, 14.2 mM succinic acid, pH 7, 260 mM KCl, 1 mM DTT) containing 2 to 9.2% ethanol or 1% DMSO. The assay buffer was heated to 37° in a circulating water bath prior to use. The assay consisted of 150 μL of assay buffer containing 1.7 mM glucose 6-phosphate and 22 U/mL of glucose 6-phosphate dehydrogenase, 4 μL of buffer:DMSO (1:1) with or without inhibitor, and 46 μL of substrate solution. Stock microsomes were thawed and diluted into 5 mM NADPH, and 50 μL of this solution was added to initiate the reaction. After the desired reaction time, an aliquot from the reaction was quenched through dilution into at least an equal volume of ethanol. The percent substrate and products (DHT and the secondary metabolites androstane-3α,17β-diol and androstane-3β,17β-diol) were quantitated by HPLC (Spectra-Physics pump; Bio-Rad or Spectra-Physics autosampler; Regis $C_{18}$ column, 15 cm × 4.6 mm) using an in-line radioisotope detector (Radiomatic). The mobile phase consisted of water:acetonitrile (59:41) at a flow rate of 2 mL/min.

### 2.5. Determination of the $K_m$ of testosterone

For r5AR1, enzyme activity was measured with various substrate concentrations (0.25 to 33 μM) as outlined above. Testosterone solutions were made in assay buffer containing 9.2% ethanol to alleviate solubility problems. The reactions were quenched at various times (2–39 min) to allow for the measurement of initial rates. For r5AR2, a reaction was run where the consumption of testosterone was monitored over time. The testosterone was dissolved in assay buffer containing 2% ethanol. Aliquots of the reaction mixture were quenched at various times until all of the substrate was consumed. It was necessary to use this method for r5AR2 because the $K_m$ value is in the low nanomolar range. Therefore, detection of product under initial rate conditions within the necessary testosterone concentration range would not have been possible.

### 2.6. Determination of inhibitor $K_i$ values and mechanism of inhibition against r5AR1

Enzyme activities were measured in the presence of various substrate concentrations (0.26 to 16.4 μM) and several inhibitor concentrations. Reaction aliquots were quenched into ethanol at various times to allow for the measurement of initial rates.

### 2.7. Preincubation experiments

A mixture consisting of 1.3 mM glucose 6-phosphate and 16.7 U/mL of glucose 6-phosphate dehydrogenase (195 μL), r5AR1 in 5 mM NADPH (50 μL), and inhibitor in assay buffer:DMSO (1:1; 5 μL) was preincubated at 37°. The preincubation was initiated by the addition of r5AR1, and then 15-μL aliquots were removed at various times and assayed for enzyme activity. The assay consisted of the preincubation aliquot (15 μL), buffer containing 1.25 mM NADPH, 1.25 mM glucose 6-phosphate, and 16.3 U/mL of glucose 6-phosphate dehydrogenase (180 μL), and [$^3$H]testosterone in assay buffer containing 9.2% ethanol (55 μL). After 1 min, the assay was quenched through the addition of a 100-μL reaction aliquot to 100 μL ethanol. Substrate and products in 50 μL of each sample were resolved using HPLC as described above.

### 2.8. Progress curve analysis

Reaction time courses in the presence and absence of inhibitors were analyzed in order to investigate the mechanism for time-dependent inhibition of r5AR2. Standard assays were run in the presence of various concentrations of inhibitor and enzyme along with 71 nM testosterone. The length of each reaction time course was varied depending upon the rate of enzyme–inhibitor association at each inhibitor concentration. Initial rate conditions were assumed to apply throughout these time courses since no more than 25% of the substrate was consumed in any case. Plots of product formed versus time were analyzed by nonlinear regression using SigmaPlot® for Windows™.

### 2.9. Recovery of r5AR activity after preincubation with inhibitor

Each preincubation was initiated by the addition of enzyme to an assay mixture (3.2 mL final volume) containing no inhibitor, 20 nM GI198745, or 100 nM finasteride, but no testosterone. Preincubations were allowed to incubate at 37° for 1.4 hr, then were transferred into dialysis tubing having a molecular weight cutoff of 10,000, and dialyzed against 1400-fold greater volumes of assay buffer containing 0.98% DMSO. The dialysis buffer was changed after 24, 47, and 97 hr. Enzyme activities were measured at the beginning of the preincubation period and throughout the dialysis, utilizing various enzyme concentrations in standard assays in which the concentration of testosterone was 100 nM.

### 2.10. Pharmacokinetics of GI198745 and finasteride in the rat

The research complied with national legislation and with company policy on the Care and Use of Animals and with related codes of practice. Male Sprague–Dawley or GLX-Han Wistar rats with body weights ranging from 250 to

302 g were purchased from Charles River Laboratories, Inc. Rats were assigned to groups for GI198745 or finasteride oral dosing. GI198745 was given to rats (N = 4) at 2.39 mg/kg, while finasteride was administered at 11.5 mg/kg. The GI198745 dose was prepared in PEG400 containing 1% Tween-80. Finasteride was dissolved in 100% propylene glycol. Blood samples were collected at specified times for 192 and 24 hr for GI198745 and finasteride, respectively. Serum was prepared from blood by centrifugation, and the parent compound concentration in serum was determined by LC/MS analysis. The GI198745 and finasteride serum concentrations versus time data were fit using the WinNonlin computer program (version 3.0, Pharsight Corp.). The GI198745 serum concentration versus time profile fit is best with a one-compartment model while a two-compartment model fit the finasteride serum concentration versus time profile best. Those model fit pharmacokinetic parameters were used to simulate repeat dosing serum profiles for both drugs.

## 3. Results

### *3.1. $K_m$ of r5AR1 and r5AR2 for testosterone*

For r5AR1 (0.9 $\mu$g of microsomal protein), initial rates were measured using several testosterone concentrations (0.1 to 46 or 0.26 to 33 $\mu$M) producing saturation curves such as the one shown in Fig. 2A. This experiment was repeated three times, and the mean $K_m$ was 2.3 ± 0.1 $\mu$M. As shown in Fig. 2B, the $K_m$ of r5AR2 (0.9 to 9 $\mu$g of microsomal protein) for testosterone was determined by measuring the consumption of testosterone during a reaction time course. $K_m$ measurements were performed seven times, and Fig. 2B shows a typical curve obtained from the best fit of the data to the equation:

$$t = \frac{-\ln \frac{S}{S_o}}{\frac{V_{\max}}{K_m}} + \frac{S_o - S}{V_{\max}} \quad (1)$$

where $t$ is time, $S_o$ is the initial substrate concentration, $S$ is the substrate concentration, and $V_{\max}$ is the maximum velocity. The mean $K_m$ from these different determinations was 0.8 ± 0.2 nM.

### *3.2. Classical inhibition of r5AR1 by GI198745 and finasteride*

Preincubation experiments were used to determine whether or not GI198745 and finasteride are time-dependent inhibitors of r5AR1. In these experiments, enzyme activities would decrease with increasing time of preincubation if exposed to sufficient concentrations of a time-dependent inhibitor. To test for such effects, enzyme (58 $\mu$g of microsomal protein) was preincubated with 100 nM GI198745 or

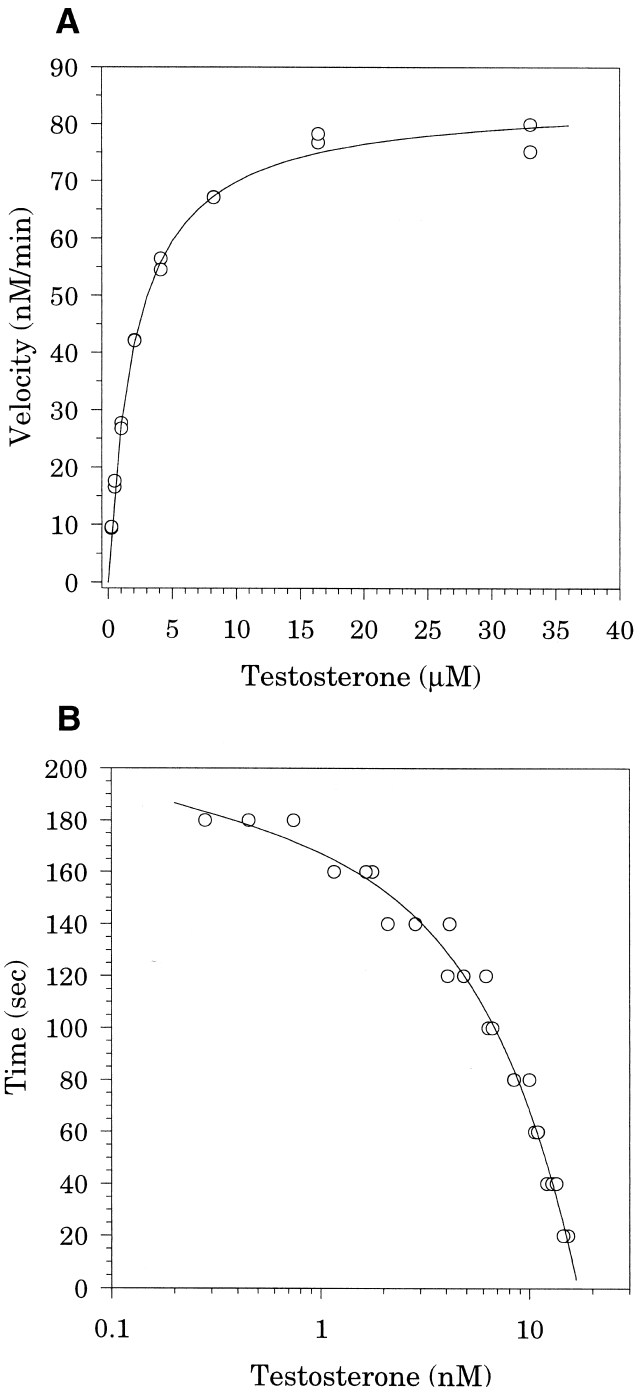


Fig. 2. Determination of the $K_m$ of r5AR1 and r5AR2 for testosterone. (A) Microsomes (0.9 $\mu$g protein) were assayed for r5AR1 activity in the presence of various testosterone concentrations (0.26 to 33 $\mu$M). The solid line represents the best fit to the Michaelis–Menten equation. This experiment was repeated three times with a $K_m$ of 2.3 ± 0.1 $\mu$M (mean ± SEM). (B) Microsomes (0.9 to 9 $\mu$g protein) were assayed for r5AR2 activity in the presence of testosterone (10.5 to 20 nM). The consumption of testosterone was monitored with time, and the solid line represents the best fit to the integrated Michaelis–Menten equation. This experiment was performed seven times, and the $K_m$ from these determinations was 0.8 ± 0.2 nM (mean ± SEM).

Table 1
Classical inhibition of r5AR1 by GI198745 and finasteride

| Preincubation time (min) | Percent of control activity | |
|---|---|---|
| | GI198745 | Finasteride |
| 1 | 14 | 27 |
| 3 | 13 | 28 |
| 5 | 12 | 29 |
| 10 | 11 | 28 |
| 20 | 11 | 30 |
| 35 | 12 | 30 |
| 50 | 13 | 31 |
| 70 | 13 | 30 |

Microsomes containing r5AR1 (58 $\mu$g of total protein) were preincubated with 100 nM GI198745 or 400 nM finasteride for the indicated times, then were diluted 17-fold, and assayed for enzyme activities. Control activity without compound was set to 100% (~15 nM DHT/min).

400 nM finasteride for various lengths of time and then diluted 17-fold and assayed in the presence of 100 nM testosterone as described in "Materials and methods." The results in Table 1 indicate that both GI198745 and finasteride reached rapid equilibrium with the enzyme, so these compounds are classical inhibitors, rather than time-dependent inhibitors, of r5AR1.

### *3.3. Competitive inhibition of r5AR1 by GI198745 and finasteride*

To determine the $K_i$ values and mechanism for inhibition of r5AR1 by GI198745 and finasteride, r5AR1 activity in 0.9 $\mu$g of microsomal protein was measured at a saturating NADPH concentration and in the presence of various concentrations of testosterone (0.26 to 16.4 $\mu$M) and GI198745 (0.078 to 2.5 nM) or finasteride (0.6 to 40 nM). Data for all concentrations of each inhibitor were simultaneously fit to each of the equations representing competitive, noncompetitive, and uncompetitive models of enzyme inhibition using SigmaPlot® for Windows™. The model that best fits the data for GI198745 and finasteride was chosen after visual inspection of the fits with the three models and comparison of the resulting Chi-square values, with the lower value representing the best fit. The data for both GI198745 and finasteride fit best with the competitive model, with resulting $K_i$ values of 0.3 ± 0.02 and 5.4 ± 0.2 nM, respectively (Fig. 3, A and B). The $K_i$ value for finasteride was in good agreement with the previously determined value of 10.2 ± 1.3 nM [9]. The $K_m$ for testosterone determined from the analysis of the GI198745 and finasteride data was 1.7 ± 0.1 and 2.1 ± 0.1 $\mu$M, respectively, which is in agreement with our previously determined value of 2.3 ± 0.1 $\mu$M.

### *3.4. Time-dependent inhibition of r5AR2 by GI198745 and finasteride*

Due to the rapid rate of inhibition of r5AR2 by GI198745 and finasteride, preincubation experiments could not be used to determine whether or not these inhibitors are time-dependent inhibitors of r5AR2. Instead, progress curves were utilized in which the formation of product was followed over time at several inhibitor concentrations. Figure 4A displays the inhibition of r5AR2 (0.9 $\mu$g of microsomal protein) by 4 nM GI198745. The hyperbolic shape of this progress curve indicates that GI198745 is a time-dependent inhibitor because maximum inhibition of enzyme activity was reached only after 50 min of reaction. Figure 4B illustrates that similar results were obtained for the inhibition of r5AR2 by 100 nM finasteride. A separate experiment showed that, in a reaction performed under similar condi-

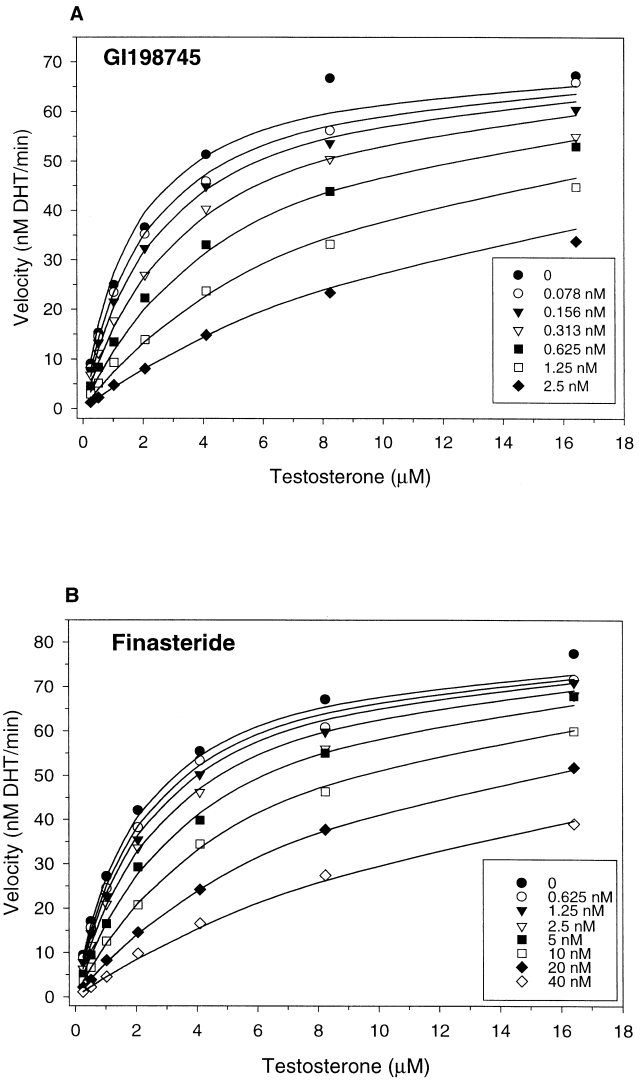


Fig. 3. Competitive inhibition of r5AR1 by (A) GI198745 and (B) finasteride. The initial rate of r5AR1 activity in 0.9 $\mu$g of microsomal protein was measured at various testosterone concentrations (0.26 to 16.4 $\mu$M) with and without several different inhibitor concentrations (indicated in the inset graph legends). The data were fit to competitive (shown above), noncompetitive, and uncompetitive models of inhibition. Analysis of this data using the competitive model provides the best fit with a $K_i$ value of 0.3 ± 0.02 nM for GI198745 and 5.4 ± 0.2 nM for finasteride. The solid lines represent the simultaneous fit of the data for all concentrations of each inhibitor to the equation representing the competitive model.

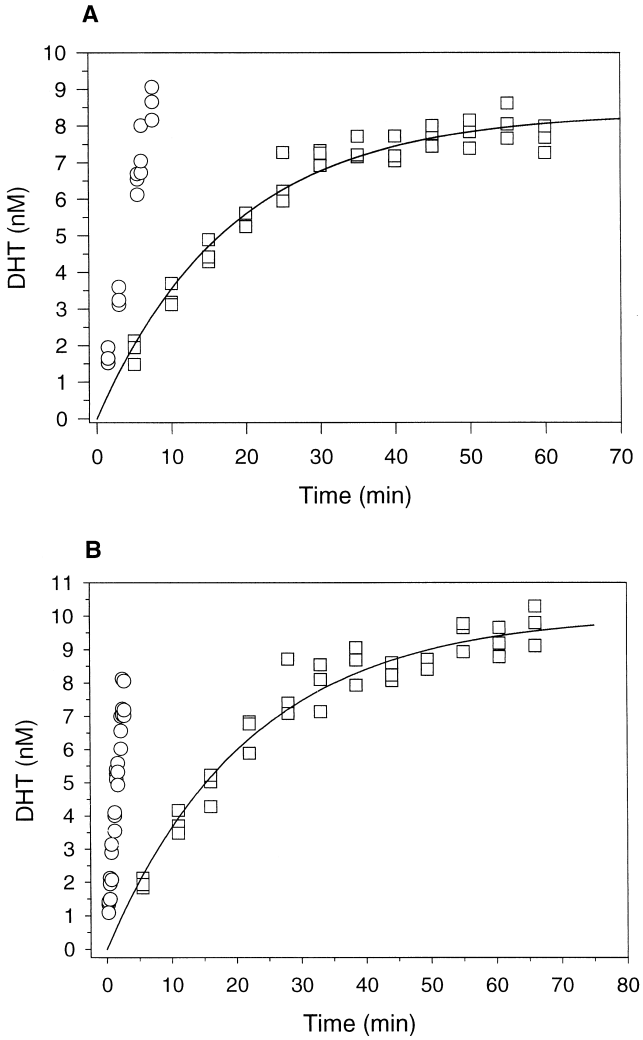


Fig. 4. Progress curves for the time-dependent inhibition of r5AR2 by (A) 4 nM GI198745 and (B) 100 nM finasteride. A standard assay was run in the presence of 0.9 $\mu$g of microsomal protein and 71 nM testosterone with (□) and without (○) inhibitor. The amount of DHT formed was monitored over time, and the solid line represents the best fit of these data to Eq. 3.

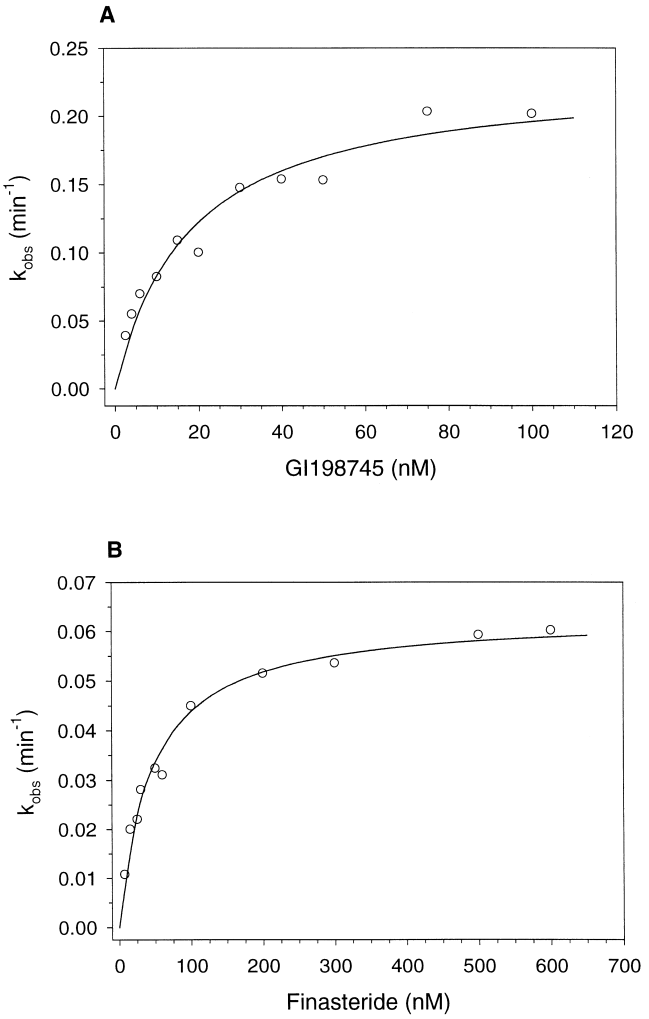


Fig. 5. Relationship between (A) GI198745 and (B) finasteride concentrations and the pseudo-first-order rate constant for the approach to equilibrium ($k_{obs}$). Progress curves obtained from reactions run in the presence of various enzyme (0.9 to 9 $\mu$g of microsomal protein) and inhibitor concentrations (2.5 to 100 nM GI198745 or 7.5 to 600 nM finasteride) were fit to Eq. 3 to determine $k_{obs}$ and $v_i$. These values of $k_{obs}$ were plotted versus inhibitor concentration and fit to Eq. 5 by nonlinear regression to determine $K_i$ and $k_3$, and the fit was used to generate the theoretical curve of this figure.

tions except lacking inhibitor, the enzyme activity was stable for at least 2 hr (data not shown), indicating that the deviation from linearity in panels A and B of Fig. 4 was due solely to time-dependent inhibition. For the data shown in Fig. 4 and similar reactions, the consumption of testosterone was less than 25%, so the resulting progress curves can be described by the equation [14]:

$$P = v_s t + \frac{(v_i - v_s)}{k_{obs}} (1 - e^{-k_{obs} t}) \quad (2)$$

where $P$ is the product formed, $v_i$ is the initial velocity, $v_s$ is the steady-state velocity, $t$ is time, and $k_{obs}$ is the pseudo first-order rate constant for the approach to steady state. Since the progress curve reaches a plateau indicating complete inhibition, the value of $v_s$ is zero and Eq. 2 can be simplified to:

$$P = \frac{v_i}{k_{obs}} (1 - e^{-k_{obs} t}) \quad (3)$$

Therefore, to analyze the time-dependent inhibition of r5AR2 by GI198745, a series of reactions were run in the presence of several inhibitor concentrations (2.5 to 100 nM), and each curve was allowed to reach a plateau. All curves were fit by nonlinear regression analysis to Eq. 3, and values for $v_i$ and $k_{obs}$ were determined at each inhibitor concentration. The effects of GI198745 concentration on $k_{obs}$ are shown in Fig. 5A. The hyperbolic shape of the data indicates that the inhibition process involves more than one step and is consistent with the following model:

$$E + I \underset{k_2}{\overset{k_1}{\rightleftarrows}} EI \xrightarrow{k_3} EI^* \quad (4)$$

where $K_i = k_2/k_1$ and $k_3$ is the rate constant for the second step. The combined data were fit by nonlinear regression to the equation [14]:

$$k_{obs} = \frac{k_3 I}{K_i\left(1 + \frac{S}{K_m}\right) + I} \quad (5)$$

to determine $k_3$ and $K_i$, which for GI198745 were (3.8 ± 0.3) × $10^{-3}$ $sec^{-1}$ and 0.2 ± 0.04 nM, respectively. Progress curves also were analyzed for 7.5 to 600 nM finasteride, and the results show that inhibition of r5AR2 by finasteride was also described well by a two-step mechanism (Fig. 5B). The constants $k_3$ and $K_i$ for inhibition by finasteride were (1.1 ± 0.03) × $10^{-3}$ $sec^{-1}$ and 0.5 ± 0.05 nM, respectively. These values are in good agreement with previously determined values of (1.62 ± 0.22) × $10^{-3}$ $sec^{-1}$ and 1.19 ± 0.10 nM [9]. The rate constants for the association of enzyme and inhibitor ($k_3/K_i$) calculated from our results were (2.0 ± 0.4) × $10^7$ $M^{-1}$ $sec^{-1}$ and (2.2 ± 0.2) × $10^6$ $M^{-1}$ $sec^{-1}$ for GI198745 and finasteride, respectively.

If GI198745 and finasteride compete with testosterone for binding to the active site of r5AR2, a reciprocal plot of $1/k_{obs}$ versus testosterone concentration according to [14]:

$$\frac{1}{k_{obs}} = \frac{K_i\left(1 + \frac{S}{K_m}\right) + I}{k_3 I} \quad (6)$$

should yield a straight line with a slope of $K_i/(k_3 K_m[I])$ for each inhibitor. Reaction time courses were performed with 20–120 nM testosterone in the presence of 15 nM GI198745 and 4.5 μg of microsomal protein or 60 nM finasteride and 0.9 μg of microsomal protein. As can be seen in Fig. 6A for GI198745, a plot of $1/k_{obs}$ versus testosterone concentration does yield data consistent with Eq. 6. Substituting the known values of $k_3$, $K_i$, and [I] for the slope from this plot yields a $K_m$ of 0.4 ± 0.1 nM, which is close to the value of 0.8 ± 0.2 nM in this work determined from a reaction time course. The same analysis in the presence of finasteride produced data that were described well by Eq. 6 and which yielded a $K_m$ of 0.6 ± 0.1 nM (Fig. 6B). These results strongly suggest that GI198745 binds to the active site of r5AR2 as has been shown for finasteride [9]. An attempt was made to analyze the inhibition of initial rates ($v_i$) as a more accurate method for the determination of the $K_i$ values of these compounds. Unfortunately, we were unable to adopt this approach because the high rates at which these compounds associate with r5AR2 prevented accurate calculations of $v_i$ for the analysis.

To provide further evidence for tight-binding of r5AR2 by GI198745 and finasteride, an attempt was made to dissociate enzyme from inhibitor by dialysis. Microsomes containing r5AR1 and r5AR2 (9 μg of total microsomal protein for each isozyme) were incubated with 20 nM GI198745 or 100 nM finasteride for 1.4 hr and then dialyzed against assay buffer as described in "Materials and methods." Following incubation, the enzymes were assayed for residual activity in the presence of 100 nM testosterone. Compared to controls with no inhibitor, r5AR1 was inhibited 98% by GI198745 and 94% by finasteride under the preincubation conditions. After a 10-fold dilution of each inhibited enzyme in the assay, r5AR1 regained 41 and 49% of its activity in the presence of GI198745 and finasteride, respectively, compared to the control with no inhibitor. After dialysis for 22 hr at 4°, r5AR1 regained 100% of its activity

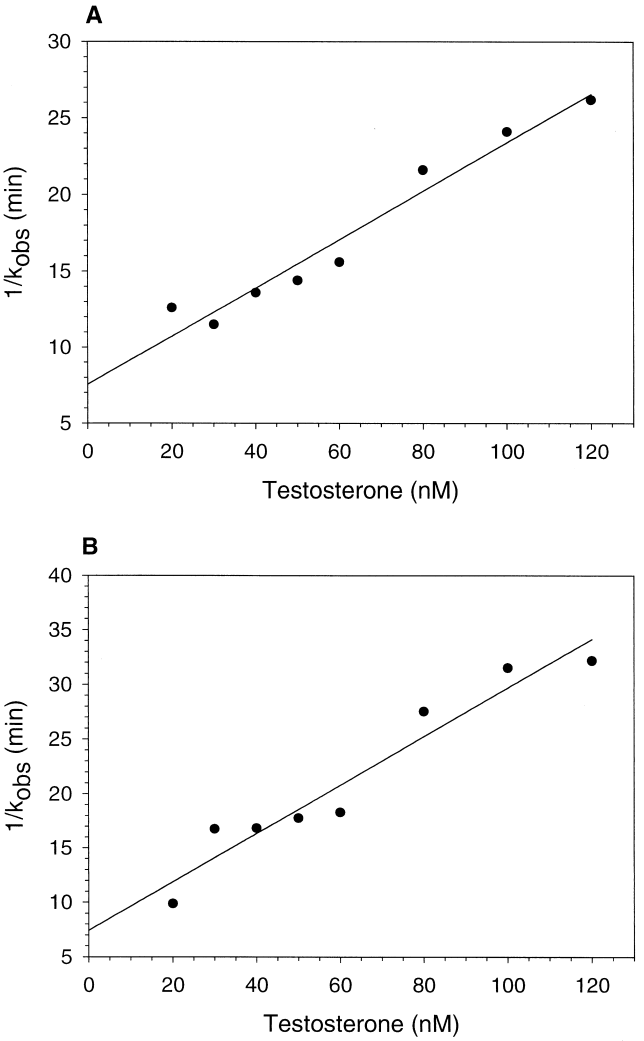


Fig. 6. Effect of testosterone concentration on the pseudo-first-order rate constant $k_{obs}$ for (A) GI198745 and (B) finasteride. Reaction time courses were run with 20–120 nM testosterone in the presence of 15 nM GI198745 and 4.5 μg of microsomal protein or 60 nM finasteride and 0.9 μg of microsomal protein. The resulting data were fit to Eq. 3 to determine the $k_{obs}$. The results from these experiments were used to generate the plot of $1/k_{obs}$ versus testosterone concentration shown in this figure. The solid line represents the best fit to a linear model from which known values of $k_3$, $K_i$, and [I] were substituted for the slope to calculate $K_m$.

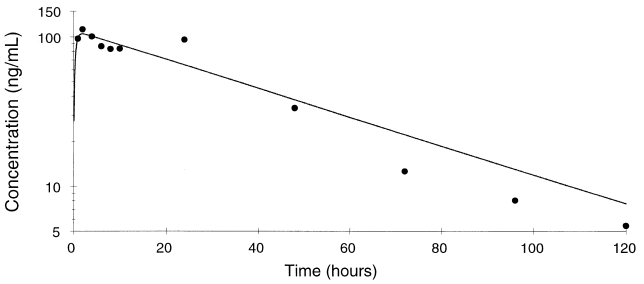


Fig. 7. Mean GI198745 serum concentration versus time profile following administration of an oral dose (2.39 mg/kg) in rats. Dosing (N = 4) and serum preparation were as described in "Materials and methods." The GI198745 serum concentration versus time profile was fit using the WinNonlin computer program using a one-compartment oral dosing model.

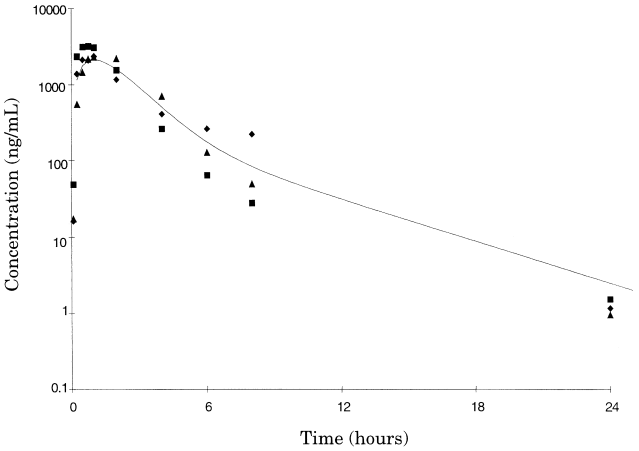


Fig. 8. Finasteride serum concentration versus time profile following administration of an oral dose (11.5 mg/kg) in rats. Dosing (N = 3, represented by ▲, ◆, ■) and serum preparation were as described in "Materials and methods." The finasteride serum concentration versus time profile was fit using the WinNonlin computer program using a two-compartment oral dosing model.

compared with the control. In comparison, the preincubation of r5AR2 with 20 nM GI198745 or 100 nM finasteride resulted in inactive enzyme, and no activity was detected following a 10-fold dilution of the inhibited enzyme or 22, 39, or 159 hr of dialysis. In contrast, r5AR2 preincubated in the absence of inhibitor retained activity following all of these treatments. It should be noted that the r5AR2 enzyme activity gradually decreased over the course of the dialyses resulting in a 75% loss of activity by the end of the 7-day dialysis compared with activity before the dialysis began. For the purpose of determining the amount of enzyme inhibition by GI198745 and finasteride, the r5AR2 enzyme activity in the presence of inhibitor was always compared to the control activity without inhibitor. Thus, similar to previous data for finasteride [9], our data suggest that GI198745 is a reversible inhibitor of r5AR1 and that r5AR2 treated with GI198745 remains inhibited completely for a period of at least 7 days following treatment.

### *3.5. Pharmacokinetics of GI198745 and finasteride following oral administration in rats*

The serum concentration versus time profiles for GI198745 and finasteride following administration of a single oral dose to rats are presented in Figs. 7 and 8, respectively. The pharmacokinetic parameters of GI198745 and finasteride in rats are listed in Table 2. GI198745 showed a long terminal half-life of 31 hr, compared with 2.2 hr for finasteride, that resulted from a lower total body clearance (485 mL/hr/kg) and higher volume of distribution (21,793 mL/kg). The simulated finasteride serum concentration versus time profile with repeat dosing for 15 days at 0.7, 7.2, 10, and 72 mg/kg/day is presented in Fig. 9. A similar simulated profile for GI198745 at 1, 10, and 100 mg/kg/day (equimolar with the 0.7, 7.2, and 72 mg/kg/day dosages of finasteride, respectively) is presented in Fig. 10. The concentrations of finasteride in the plasma decreased to very low levels over the 24-hr post-dose period, while GI198745 accumulated due to its long half-life. The accumulation of GI198745 in the rat was estimated by the simulation to be complete in 7 days, after which the nadir of GI198745 concentration with administration of 1 mg/kg/day was estimated to be approximately 66 ng/mL (125 nM).

## 4. Discussion

In this study, we demonstrated that GI198745 was a potent inhibitor of both r5AR1 and r5AR2 but that the mechanism of inhibition was different for the two isozymes. This compound, as well as finasteride, is a classical competitive inhibitor of r5AR1 and rapidly forms a dissociable complex with the enzyme. These characteristics were shown by preincubation experiments and by analysis using the competitive, noncompetitive, and uncompetitive models of inhibition. A comparison of the $K_i$ values of GI198745 and finasteride (Table 3) indicates that GI198745 ($K_i$ = 0.3 nM) is nearly a 20-fold more potent inhibitor of r5AR1 than finasteride ($K_i$ = 5.4 nM). This $K_i$ for finasteride is in good

Table 2
Pharmacokinetic parameters of GI198745 and finasteride following administration of a single oral dose to rats

| Parameter | GI198745 (one-compartment model) | Finasteride (two-compartment model) |
|---|---|---|
| Dose (mg/kg) | 2.39 | 11.5 |
| $C_{max}$ (ng/mL) | 105 | 2607 |
| AUC [(hr.ng)/ml)] | 4931 | 7357 |
| $T_{1/2}$ (hr) | 31 | 2.2 |
| V/F (mL/kg) | 21,793 | 1807 |
| $K_{01}$ ($hr^{-1}$) | 2.423 | 0.931 |
| $K_{10}$ ($hr^{-1}$) | 0.02224 | 0.965 |
| $K_{12}$ ($hr^{-1}$) | | 0.161 |
| $K_{21}$ ($hr^{-1}$) | | 0.254 |

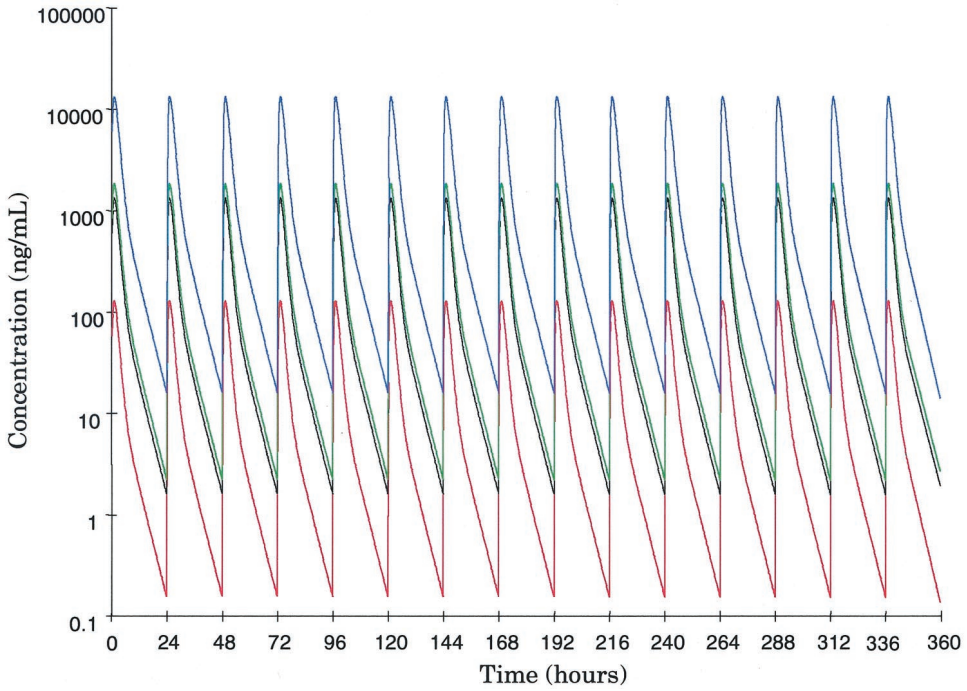


Fig. 9. Computer simulation of finasteride pharmacokinetics following repeated daily oral dosing for 15 days. Estimated pharmacokinetic parameters from Table 2 were used to simulate repeat dosing for 15 days with 0.7 (red line), 7.2 (black line), 10 (green line), and 72 (blue line) mg/kg/day.

agreement with the previously published value of 10.2 nM [9]. In contrast, the inhibition of r5AR2 by both GI198745 and finasteride was more complicated and required a detailed analysis. We demonstrate here that, like finasteride, GI198745 was a time-dependent inhibitor of r5AR2 and that GI198745 was approximately 10-fold more potent than finasteride at inhibiting r5AR2 *in vitro* (Table 3). Dialysis experiments indicated that the binding of r5AR2 with GI198745 and finasteride is extremely tight. Although irreversible in our hands with dialysis, the results of Azzolina *et al.* [9] with finasteride suggest that GI198745 would dissociate from the enzyme under denaturing conditions.

Using pharmacokinetic simulations (Fig. 9), one can calculate the plasma concentration of finasteride over a 24-hr period after a dose of 0.7 mg/kg and then calculate what percentage of the r5AR1 is active using the previously determined $K_i$ value of 5.4 nM (2 ng/mL). It is assumed that all of the r5AR2 is inactive due to the tight binding between r5AR2 and finasteride or GI198745, as demonstrated earlier. The calculation reveals that approximately 8% of the r5AR1 is active 1 hr after the administration of finasteride but that approximately 75% of the r5AR1 is active after 10

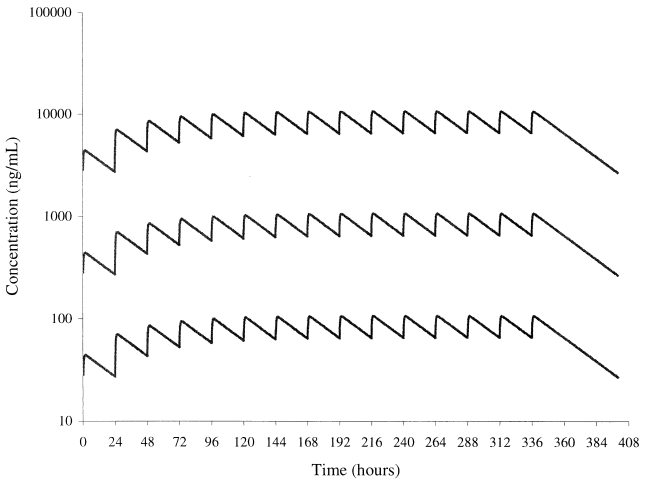


Fig. 10. Computer simulation of GI198745 pharmacokinetics following repeated daily oral dosing for 15 days. Estimated pharmacokinetic parameters from Table 2 were used to simulate repeat dosing for 15 days with 1 (bottom line), 10 (middle line), and 100 (top line) mg/kg/day.

Table 3
Summary of kinetic parameters of the inhibition of rat 5α-reductase (r5AR) by GI198745 and finasteride

| | $k_3$ ($sec^{-1}$) | $K_i$ (nM) | $k_3/K_i$ ($M^{-1}$ $sec^{-1}$) |
|---|---|---|---|
| r5AR1 | | | |
| GI198745 | NA[a] | 0.3 ± 0.02[b] | NA |
| Finasteride | NA | 5.4 ± 0.2[b] | NA |
| | | 10.2 ± 1.3[c] | |
| r5AR2 | | | |
| GI198745 | $(3.8 \pm 0.3) \times 10^{-3}$[b] | 0.2 ± 0.04[b] | $(2.0 \pm 0.4) \times 10^{7}$ |
| Finasteride | $(1.1 \pm 0.03) \times 10^{-3}$[b] | 0.5 ± 0.05[b] | $(2.2 \pm 0.2) \times 10^{6}$[d] |
| | $(1.62 \pm 0.22) \times 10^{-3}$[c] | 1.19 ± 0.10[c] | $(1.37 \pm 0.31) \times 10^{6}$[c] |

[a] Not applicable to this isozyme.
[b] These values are from a representative experiment, and the error reported is the standard error of the fit.
[c] From Azzolina *et al.* [9].
[d] The error was computed using a function assuming that the error from each component propagated independently.

hr, taking into account that approximately 82% of the drug is serum protein bound [15]. Based on pharmacokinetic simulations for GI198745 (Fig. 10) at a dose of 1 mg/kg (equimolar to 0.7 mg/kg of finasteride), the nadir after the first dose is 27 ng/mL (51 nM) and after seven doses the drug accumulates so that the nadir is 66 ng/mL (125 nM). Taking into account that serum protein binding is at least 99%,[2] then the concentration of free GI198745 available for binding to r5AR1 is 0.66 ng/mL (1.25 nM). Therefore, at the most, 19% of the r5AR1 is active from day 7 to day 15 based on a $K_i$ of 0.3 nM.

Alone, neither the pharmacokinetic profiles of GI198745 and finasteride nor information regarding their mechanisms of inhibition of both rat 5$\alpha$-reductases is adequate for a complete understanding of their *in vivo* potencies. Bramson *et al.* [10] report data showing that GI198745 is at least 100-fold more potent than finasteride in preventing the growth of the rat prostate. The suggestion was made that these results were due largely to the longer half-life and total body clearance of GI198745. Now our data show that the mechanism of inhibition of the rat 5$\alpha$-reductases by these drugs is also extremely important in understanding their *in vivo* potencies. Both GI198745 and finasteride are capable of inactivating r5AR2 because of the apparent irreversible modification of the enzyme by both inhibitors. Since the association of GI198745 and finasteride with r5AR1 is reversible, the *in vivo* inhibition of r5AR1 will vary during the elimination of the drugs from the body. At equimolar dosing of GI198745 and finasteride, GI198745 with its longer half-life and greater potency is more effective than finasteride in providing reversible inhibition of r5AR1 and, therefore, preventing rat prostatic growth [10].

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