Impact of Physicochemical and Structural Properties on the Pharmacokinetics of a Series of $\alpha_1_L$-Adrenoceptor Antagonists

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ABSTRACT:

A rational drug discovery process was initiated to design a potent and prostate-selective $\alpha_1_L$-adrenoceptor antagonist with pharmacokinetic properties suitable for once-a-day administration after oral dosing, for the treatment of benign prostatic hyperplasia. Two series of compounds based on a quinoline or quinazoline template were identified with appropriate pharmacology. A series of high molecular weight cations with high hydrogen-bonding potential had extensive in vivo clearance, despite demonstrating metabolic stability. Studies in the isolated perfused rat liver and fresh rat hepatocytes indicated that active transport protein-mediated hepatobiliary elimination is an efficient clearance process for these compounds. A reduction in molecular weight and hydrogen-bonding potential resulted in a second series of compounds with in vivo hepatic clearance predictable from in vitro metabolic clearance. Initially, lipophilicity was reduced within this second series to reduce metabolic clearance and increase elimination half-life. However, this strategy also resulted in a concomitant reduction in volume of distribution and a negligible effect on prolonging half-life. An alternative strategy was to increase the intrinsic metabolic stability of the molecule by careful structural modifications while maintaining lipophilicity. Replacement of the metabolically vulnerable morpholine side chain resulted in identification of UK-338,003, $(N-[2-(4-amino-5-(4-fluoro-phenyl)-6,7-dimethoxy-quinolin-2-yl)-1,2,3,4-tetrahydro-isoquinolin-5-yl]-methanesulfonamide)$, which fulfilled the objectives of the discovery program with suitable pharmacology (human prostate $\alpha_1_L$ $A_{2\alpha}$ of 9.2 with 25-fold selectivity over rat aorta $\alpha_1_L$) and sufficiently long elimination half-life in human volunteers (11–17 h) for once a day administration.

$\alpha_1$-Adrenoceptor antagonists are well precededent in the effective treatment of both hypertension and benign prostatic hyperplasia (BPH); e.g., doxazosin (Fulton et al., 1995), terazosin (Jonler et al., 1994), and tamsulosin (Wilde and McTavish, 1996). However, because many of the $\alpha_1$-adrenoceptor antagonists clinically used in the treatment of BPH were originally developed for hypertension, they are nonselective for $\alpha_1$-adrenoceptor subtypes. These nonselective $\alpha_1$-adrenoceptor antagonists have similar efficacy and are generally well tolerated in BPH patients, but some patients experience dizziness and postural hypotension, which may be minimized by dose titration. It has been postulated that a drug that blocks the $\alpha_1$-adrenoceptor subtype would specifically target the prostate and thus have a better ratio of efficacy to safety, with fewer cardiovascular side effects than currently available nonselective $\alpha_1$-adrenoceptor antagonists.

The aim of the drug discovery program was to identify a novel and potent $\alpha_1_L$-adrenoceptor antagonist that was functionally selective for the prostate gland over the cardiovascular system and thus have the potential to be effective in the treatment of BPH with fewer dose-limiting side effects. To assess potency for the prostate gland, functional antagonist potency of project compounds at human $\alpha_1$-adrenoceptors was determined against noradrenaline-mediated contractions of an isolated human prostate preparation.

To assess selectivity over the cardiovascular system, antagonist potency of project compounds versus the vascular $\alpha_1_B$- and $\alpha_1_D$-adrenoceptors was considered. Antagonist potency against noradrenaline-induced contraction of rat spleen and rat aorta was determined as a functional index of activity at $\alpha_1_B$- and $\alpha_1_D$-adrenoceptors, respectively. The compounds evaluated in the drug discovery program achieved selectivity versus $\alpha_1_B$-adrenoceptors relatively easily, and therefore, structure-activity relationships focused on selectivity of $\alpha_1_L$-adrenoceptors in human prostate versus $\alpha_1_D$-adrenoceptors in rat aorta.

Terazosin has essentially balanced prostate and blood pressure...
effects in the clinic (Kirby, 1998). In vitro, terazosin has a pA2 value of 9.8 versus α1D-adrenoceptors in human prostate and a pA2 value of 10.0 versus α1L-adrenoceptors in rat aorta (in-house data) and thus is not selective for prostate over blood pressure effects. The objectives of the drug discovery program were to provide symptomatic relief for BPH equivalent to that provided by established agents such as terazosin, with at least a 10-fold improvement in cardiovascular side effects. To achieve this, only compounds with pA3 values greater than 8.3 versus α1L-adrenoceptors in human prostate were considered to be sufficiently potent to meet project objectives. Since current α1-adrenoceptor antagonists exhibit little or no selectivity in vitro for the cloned α1-adrenoceptor subtypes and no prostate selectivity in the clinic (Kirby et al., 2000) there was no clinical marker to provide guidance for in vitro vs. in vivo correlations of selectivity. Therefore, a strategy based on the knowledge of antagonist pharmacology was used to design a compound that lacked dose-limiting cardiovascular side effects. From antagonist pharmacology it was assumed that 75% receptor occupancy is required for efficacy of α1L-adrenoceptor antagonists in vivo for BPH (Smith, 1997). To provide selectivity over the cardiovascular system, a value of 10-fold selectivity was chosen versus α1L-adrenoceptors, as this would represent a receptor occupancy of much less than 50%, thus diminishing the risk of α1D pharmacology and cardiovascular side effects.

Two structurally distinct series of compounds, based on a quinoline or quinazoline central template, were identified and shown to be potent and prostate-selective α1L-adrenoceptor antagonists (Collis et al., 1997; Fox, 1998). In addition to finding a drug candidate possessing the desired pharmacology, the aim of the drug discovery program was to identify a drug candidate with pharmacokinetic properties suitable for once a day administration to humans after oral dosing. Design of a successful drug candidate incorporates both good pharmacological activity and appropriate pharmacokinetic properties, and the study of absorption, distribution, metabolism, and excretion properties and pharmacokinetics is now well established in the drug discovery phase (Smith et al., 1996).

In addition to the requirement for drug efficacy, optimal pharmacokinetic properties ensure that a drug works in clinical practice when lack of patient compliance has to be taken into consideration. This aspect of drug behavior is termed “forgiveness” (Urquhart, 1998) and reflects the actual duration of effect after administration. To be suitable for oral administration, it was ideal to design a drug with high oral bioavailability. To achieve the desired once daily dosing regimen, a compound was required with sufficient half-life to drive efficacy and minimize variability in concentration by reducing peak to trough ratio. A long half-life would also enable forgiveness.

In this article, the balance that needed to be struck between pharmacodynamics and pharmacokinetics to select a compound for development with suitable overall properties is discussed. In addition, the differences in absorption, distribution, metabolism, and excretion properties between the two series of compounds in the context of their physicochemical properties are discussed.

Materials and Methods

Materials. The α1-adrenoceptor antagonists studied in series 1 and 2 were synthesized by the Discovery Chemistry Department, Pfizer Global Research and Development (Sandwich, UK). The chemical purity of the compounds was determined using a range of methods including mass spectrometry, NMR and microanalysis. Chemical purity of all compounds was >95% (Collis et al., 1997; Fox, 1998). All other chemicals and reagents, unless stated otherwise, were obtained from Sigma-Aldrich Research (St. Louis, MO) and were of the highest grade available.

Lipophilicity Determination. Distribution of the α1-adrenoceptor antagonists between octanol and 0.1 M potassium phosphate buffer, pH7.4, was determined by the method of Stopher and McClean (1990). Approximately 2 mg of compound was dissolved in 2 ml of octanol (octan-1-ol, specially pure; BDH, Poole, UK) and mixed with 2 ml of 0.1 M potassium phosphate buffer, pH 7.4 on a rotary mixer at 390rpm for 60 min. After centrifugation, the two phases were separated and 10 μl of a 1:10 dilution of the octanol and 100 μl of buffer were directly injected onto the HPLC systems described for the analysis of plasma samples. The distribution coefficient (log D7.4) was calculated from the log of the ratio of the concentration of compound in octanol to the concentration of compound in buffer. Duplicate determinations were carried out for each compound. Calculated log P values (clogP) were calculated using the MedChem computer program (version 3.55; BioByte Corp., Claremont, CA).

pKa Determination. The ionization constant (pKa) values of the α1-adrenoceptor antagonists were determined by potentiometric titration in water/methanol using a GlpKa apparatus (Sirius Analytical Instruments, East Sussex, UK). All titrations were conducted under argon to exclude atmospheric CO2 and at 25°C. Six separate semi-aqueous solutions in methanol/distilled water of compound 0.15% KCl were acidified with 0.1 M HCl to pH 2. The solutions were then titrated with standardized KOH to pH 12. Bjerrum difference plots were deduced from each titration curve and used to calculate approximate values of the apparent ionization constant in the mixed solvent. These approximate values were used in a weighted nonlinear least-squares procedure to refine the pKa values by including previously determined values of citric acid as unrefined constituents. The refined values were extrapolated to zero cosolvent by the Yasuda-Shedlovsky procedure (Gobry et al., 2000).

Plasma Protein Binding Determination. Protein binding of the α1-adrenoceptor antagonists in plasma was determined in vitro by equilibrium dialysis. Samples of rat, dog, or human plasma (1 ml, n = 3–5) containing compound at 1 μg/ml were dialyzed (Spectrapor-1 dialysis membrane, 6000–8000 mol. wt. cutoff; Spectrum, Laguna Hills, CA) against isotonic Krebs-Ringer buffer (1 ml, pH 7.4) for 4 h at 37°C in a rotating dialyzer (Dianorm; NBS Biologicals, Huntingdon, UK). After dialysis, the compounds were extracted and the concentrations of drug in plasma and buffer were determined by HPLC analysis, as described for the analysis of plasma samples.

Plasma protein binding values were determined using the following equation:

\[
\text{Plasma protein binding (％)} = 100 - \left(\frac{\text{buffer concentration}}{\text{plasma concentration}}\right) \times 100
\]

In Vitro Metabolism Studies in Liver Microsomes. Human, rat, and dog liver microsomal incubations were carried out at 1 μM substrate concentration and 0.5 μM cytochrome P450 concentration. The final incubation volume was 400 μl for the human incubations and 1.2 ml for the rat and dog incubations. Microsomes were purchased from BD Biosciences (Franklin Lakes, NJ) and stored at −80°C. The concentrations of protein and cytochrome P450 were determined by standard methods (Lowry et al., 1951; Omura and Sato, 1964). The incubation volume was made up of 50 mM potassium phosphate, pH 7.4, and 5 mM MgCl2. The reducing equivalents required by cytochrome P450 were provided by NADPH which was generated in situ from NADP (1 mM) using an isocitric acid (5 mM) and isocitrate dehydrogenase (1 unit/ml) system. All the components were preincubated at 37°C except NADP, which was added at time 0 to start the reaction. Aliquots of the human liver microsomal incubation (25 μl) and the rat and dog liver microsomal incubation (100 μl) were removed over a 60-min period and immersed in ice-cold acetonitrile (50 μl for the human microsomes and 200 μl for the animal microsomes) containing internal standard (0.2 μg/ml eltriprant or fluconazole) to terminate the reaction. The resulting samples were centrifuged at 1700g for 30 min.

For the human liver microsomes, 10 μl of sample was analyzed by a specific LC-MS/MS method. Samples were injected onto an HPLC system consisting of a cartridge column (OPTI-LYNX cartridge C18, 15 × 2.1 mm, porous 40 μM; Jay Tee Biosciences Ltd., Whistable, Kent, UK) and an isocratic Jasco pump (Jasco, Tokyo, Japan) operating at 2 ml/min. The eluting mobile phase was 90% methanol, 10% water, 2 mM ammonium acetate, and 0.03% formic acid.

For the animal liver microsomes, 80 μl was analyzed by a specific LC-MS/MS method. Samples were injected onto a gradient HPLC system comprising a Chromolith Speed ROD column (50 × 4.6 mm; Merck KGaA,
Darmstadt, Germany) eluted by a gradient of 10 to 90% methanol in water containing 2 mM ammonium acetate and 0.03% formic acid, over 3 min at a flow rate of 1 ml/min.

Detection was by multiple reaction monitoring using a Sciex API2000 or API4000 triple quadrupole mass spectrometer (PerkinElmerSciex, Boston, MA) in a positive atmospheric pressure chemical ionization mode.

Microsomal Binding Determination. The extent of binding of the α1-adrenoceptor antagonists in series 2 (compound 1–7) to dog and human microsomes was determined in vitro by equilibrium dialysis using a 96-well micro-equilibrium Teflon dialysis device (HTDialysis, LLC, Gales Ferry, CT). Regenerated cellulose dialysis membrane strips with a molecular mass cutoff of 12,000 to 14,000 Da (HTDialysis, LLC) were used.

Dog and human microsomal incubations were prepared as described previously for in vitro metabolism studies, but without the addition of compound or NADP. The incubations were left to stand at room temperature for 12 h before addition of compound at 1 μM. Aliquots of microsomal incubation (150 μl) were added to one side of the dialysis device and dialyzed against 50 mM phosphate buffer (pH 7.4, 150 μl) by placement of the dialysis block on an oscillating platform for 4 h at 37°C. After this period, microsome samples (100 μl) and buffer samples (100 μl) were removed from the apparatus and diluted with the opposite phase (100 μl of control dialyzed microsomal incubation or buffer). Compound and internal standard were extracted from the samples basified with 0.2 M sodium borate buffer, pH 9.0 (1 ml) into tert-butyl methyl ether (2 ml). The samples were mixed by inversion for 10 min and centrifuged, and the ether was removed. The ether extract was evaporated to dryness under nitrogen at 37°C and dissolved in 200 μl of 10:90 methanol/water containing 2 mM ammonium acetate and 0.03% formic acid. Samples (180 μl) were analyzed by a specific LC-MS/MS system as described previously for the dog liver microsome in vitro metabolism studies.

Microsomal binding values were determined using the following equation:

\[
\text{Microsome binding (\%)} = 100 - \frac{(\text{buffer compound:IS ratio} \times 100)}{\text{microsome compound:IS ratio}}
\]

Calculation of Unbound Intrinsic Clearance In Vitro. Using the assumption that a substrate concentration of 1 μM is below the apparent \( K_u \), disappearance half-life values were then calculated from the negative slope of a plot of the natural log of the substrate/initial standard peak area ratio versus time. This was scaled to determine the clearance expected in vivo using the following equation (Obach et al., 1997):

\[
\text{CL}_{\text{in vivo}} = \frac{0.693 \times \text{weight}}{\text{In vitro f}_{u(\text{inc})} \times \text{in vivo f}_{u(\text{inc})}}
\]

where in vivo \( f_{u(\text{inc})} \) is in minutes, liver weight is in grams per kilogram body weight, and “liver in incubation” refers to the grams of liver per milliliter in the incubation, resulting in units of ml/min/kg for \( \text{CL}_{\text{in vivo}} \). The liver in incubation value was calculated from the amount of microsomal protein in the incubation and a scale-up factor from microsomal protein to grams of liver. \( f_{\text{inc}} \) is the unbound fraction in the incubation, which is determined from the microsomal binding value. Thus, the CL' \( f_{\text{inc}} \) calculated is based on free concentrations in the incubation.

The following values were taken from the literature. Human: 325 pmol of P450/mg of microsomal protein (Iwatsubo et al., 1997); 33 mg of microsomal protein/g of liver (Naritomi et al., 2001); 2 g of liver/kg of body weight (Davies and Morris, 1993).

Hepatocyte Transport Assay. Rat hepatocytes were isolated using a modified two-step collagenase perfusion (Seglen, 1993) from livers of male Sprague-Dawley rats. Fresh hepatocytes (1 \( \times \) 10⁶ cells/ml) were incubated with UK-191,005 (0.2–100 μM) in Krebs-Henseleit buffer at 4°C and 37°C for 10 min. Aliquots (1 ml) were immediately placed on ice and hepatocytes separated by low-speed centrifugation (50g at 4°C). Hepatocytes were then washed twice with 1 ml of ice-cold buffer. The hepatocytes were then pelleted by centrifugation at 50g, 200 μl of acetonitrile was added, and the samples were stored frozen until analysis. At the time of analysis, 25 μg of internal standard (in 25 μl of methanol) was added to each sample followed by 100 μl of Milli-Q water (Millipore Corporation, Billerica, MA). Samples were vortex-mixed and centrifuged at 4°C for 1 h in a 96-well block. Samples were analyzed by turbulent flow chromatography/tandem mass spectrometry with multiple reaction monitoring as described in detail previously (Chassaing et al., 2001). Initial experiments were completed to assess the linearity of UK-191,005 uptake with time, and uptake was found to be linear for 10 min.

Animal Experimentation. All studies involving animals were carried out in accordance with the requirements of United Kingdom national legislation and conducted under necessary authorities. At all stages, consideration was given to experiment refinement, reduction in animal numbers, and replacement with in vitro techniques.

Isolated Perfused Rat Liver Preparation. Livers of male Sprague-Dawley rats (250–300 g) were perfused in situ as described previously (Lennard et al., 1993) with some modifications. Experiments were performed using the recirculation mode with a flow rate of 15 ml/min. The perfusion medium (150 ml) consisted of Krebs-Henseleit bicarbonate buffer, bovine serum albumin (2.6% w/v), and washed human erythrocytes (13% w/v), adjusted to pH 7.4 and oxygenated with 95% O2/5% CO2. After a 5-min equilibration period, taurocholate (1 μmol/min) was infused into the reservoir of the isolated perfused rat liver (IPRL). After a further 10 min, test α1-adrenoceptor antagonist (1-mg dose; 1 ml added of a 1-mg/ml solution) was added to the reservoir. Perfuse samples (1 ml) were taken from the reservoir at regular intervals out to 90 min postdosing and centrifuged (3000 rpm, 10 min); the supernatant was collected and stored frozen at −20°C until assay. Bile was collected at approximately 15-min intervals and sample volumes were recorded. Concentrations of drug in perfuse and bile were determined by HPLC analysis, as described for the analysis of plasma samples.

Intravenous and Oral Pharmacokinetics in the Rat. UK-191,005 (series 1) was administered intravenously at 3 mg/kg to male Sprague-Dawley rats. UK-294,315 and UK-338,003 (series 2) were administered intravenously at 0.5 mg/kg and orally at 4 mg/kg and 1 mg/kg, respectively, to male Sprague-Dawley rats. Intravenous doses were prepared by dissolving the compounds in 0.1 M HCl, which was diluted with saline and back-titrated to pH 7.4 with 0.1 M NaOH. Oral doses were prepared in the same way but used purified water instead of saline. Male Sprague-Dawley rats (−250 g, Charles River, Manston, UK) were surgically prepared with an indwelling jugular vein cannula at least 2 days before administration of dose. Before and during the intravenous and oral studies, rats had free access to food (rat diet pellets) and water.

Rats (n = 4 per compound and n = 2 per route) received intravenous doses of each compound via the caudal vein (0.5 mg/kg, 1 ml/kg) or oral doses of UK-294,315 (4 mg/kg, 2 ml/kg) and UK-338,003 (1 mg/kg, 2 ml/kg) by gavage tube. Blood samples (175 μl) were collected from the jugular vein catheterer at specific time points and the cannula was flushed with heparinized saline after each sample. All blood samples were collected into heparinized tubes and plasma was prepared by centrifugation. Plasma was stored frozen at −20°C until analysis.

Intravenous and Oral Pharmacokinetics in the Dog. Intravenous doses of the α1-adrenoceptor antagonists studied were freshly prepared by dissolving in 0.1 M HCl, which was diluted with saline and back-titrated to pH 7.4 with 0.1 M NaOH. Oral doses were prepared in the same way but used purified water instead of saline. Dose levels were selected based on pharmacological activity. Intravenous doses administered were 0.3 mg/kg for UK-338,003 (series 2) and 0.5 mg/kg for all other α1-adrenoceptor antagonists studied. Oral doses administered were 0.5 mg/kg for compounds 2, 5, and 6, 0.6 mg/kg for UK-338,003, 2.0 mg/kg for compounds 3 and 4, and 4 mg/kg for UK-294,315.

Male or female beagle dogs (12–16 kg) from the colony at Pfizer Global Research and Development were used for these studies. At least two animals received each of the eight compounds. Animals were fasted overnight before administration of either intravenous or oral doses and for 7 h after dosing.

Intravenous doses were administered into the left saphenous vein as an infusion at 1 ml/min for a duration of 15 min via a temporary indwelling catheter. Dogs were restrained in canvas slings from just before dosing to 7 h after dosing, at which point they were returned to their pens. Blood samples (5 ml) were taken from the right saphenous vein via a temporary indwelling catheterer for the first 7 h and then successively by vena puncture of the cephalic vein. Blood samples were collected into heparinized tubes and plasma was prepared by centrifugation. Plasma was stored frozen at −20°C until analysis.

Seven days after intravenous administration, the dogs were given an oral dose of the same compound by gavage. Again, dogs were restrained in canvas
slings for the first 7 h after oral dosing and then returned to their pens. Plasma samples were prepared and stored as above. When not in slings, the dogs had free access to food (at regular feeding times) and water throughout the study.  

Administration to Human Volunteers. The pharmacokinetics of UK-294,315 and UK-338,003 from series 2 were determined in healthy human volunteers in two separate studies. The human pharmacokinetic studies were conducted according to the Association of the British Pharmaceutical Industry guidelines and to the revised Declaration of Helsinki (Hong Kong, 1989). The clinical study protocols (single-blind placebo-controlled single escalating oral doses) were approved by a local Ethics Review Committee, and the studies were carried out at the Kent and Canterbury Hospital (Canterbury, UK). Written consent was obtained from 24 healthy male volunteers (aged 18–45 years, weighing 60–100 kg) who took part in each study.  

For UK-294,315, the pharmacokinetic study was divided into two cohorts of 12 subjects. Cohort A was a three-way crossover study investigating 1 mg, 3 mg, and 10 mg of active compound, incorporating placebo substitution. Cohort B was a four-way crossover study investigating 30 mg, 60 mg, and 100 mg of active compound and placebo.  

For UK-338,003, the pharmacokinetic study was divided into three cohorts of eight subjects. Doses were administered in an escalating fashion in cohort 1 (0.25 mg, 0.75 mg, and 1.5 mg of active compound plus placebo) and cohort 2 (0.75 mg, 1.0 mg, and 1.25 mg of active compound plus placebo). Dosing in cohort 3 was fully randomized (0.1 mg, 0.5 mg, and 1.25 mg of active compound plus placebo).  

In each pharmacokinetic study the subjects were fasted for 12 h before dosing and for 4 h after dosing. UK-294,315 was administered as a solution in water (250 ml) and UK-338,003 was administered as a solution in 0.01 M hydrochloric acid. There was a minimum of 7 days between each dose. Blood samples (6–7 ml) were collected in heparinized tubes up to 24 to 48 h postdose for UK-294,315 and up to 96 h postdose for UK-338,003. Samples were centrifuged (~1500 × g for 4°C for 10 min) within 60 min of sample collection and plasma was removed and stored in screw-capped propylene tubes at approximately −20°C.  

Analysis of Rat, Dog, and Human Plasma Samples. Concentrations of the α1-adrenoceptor antagonists in rat, dog, or human plasma samples were determined using the following procedures.  

Series 1 α1-adrenoceptor antagonists and compound 2 (series 2). Compound and internal standard were extracted from rat or dog plasma (1 ml) basified with 0.2 M sodium borate buffer, pH 9.0 (1 ml) into tert-butyl methyl ether (6 ml). The samples were mixed by inversion for 10 min and centrifuged, and the ether was removed. The ether extract was evaporated to dryness under nitrogen at 37°C and dissolved in 100 μl of mobile phase for injection onto the HPLC column. The mobile phase comprised acetonitrile/10 mM potassium dihydrogen phosphate containing 10 mM octane sulfonic acid, pH 3.0 (40:60 by volume), and the column was a reverse phase basic 10 cm × 4.6 mm (HiChrom, Reading, UK). Detection was by UV absorbance at 254 nm. The approximate retention time for UK-338,003 was 5 min. The concentration of compound 5 was determined by fluorescence (λex 248 nm, λem 388 nm). UK-294,315 had a retention time of approximately 7 min. The limit of detection was 0.1 ng/ml from 1 ml of plasma.  

Compounds 3 and 4 (series 2). Compound and internal standard were extracted from dog plasma (1 ml) basified with 0.2 M sodium borate buffer, pH 9.0 (1 ml) into tert-butyl methyl ether (6 ml). The samples were mixed by inversion for 10 min and centrifuged, and the ether was removed. To the ether was added 100 μl of 0.1 M orthophosphoric acid, and again the samples were mixed for 10 min and centrifuged. The ether was removed and the orthophosphoric acid was injected onto HPLC. The mobile phase comprised acetonitrile/0.1% trifluoroacetic acid (14:86 by volume for compound 3 and 20:80 by volume for compound 4), and the column was reverse phase basic 10 cm × 4.6 mm (HiChrom). Detection was by UV absorbance at 254 nm. The approximate retention time was 5 min for compounds 3 and 4.  

Compound 5 (series 2). The concentrations of compound 5 were determined in dog plasma using a liquid-liquid extraction method followed by LC-MS/MS quantification. Compound and internal standard were extracted from dog plasma (1 ml) basified with 0.1 M NaOH (1 ml) into tert-butyl methyl ether (4 ml). The samples were mixed by inversion for 10 min and centrifuged, and the ether was removed. The ether extract was evaporated to dryness under nitrogen at 37°C and dissolved in 200 μl of 2 mM ammonium acetate buffer, pH 4.0, in methanol/water (30:70 v/v). Samples (180 μl) were injected onto the HPLC column (Hypersil C18, 50 × 4.6 mm) with a mobile phase consisting of 2 mM ammonium acetate, pH 4.0, in methanol/water (90:10 v/v) at 1 ml/min. Detection was by multiple reaction monitoring for the transitions of 483 to 396 using a Sciex API III+ triple quadrupole mass spectrometer (PerkinElmer-Siex) in a positive atmospheric chemical ionization mode.  

Compound 6 (series 2). Compound and internal standard were extracted from dog plasma (1 ml) by solid phase extraction using C18 cartridges (Bond Elut, 100 mg/1 ml), which had been pretreated with methanol (1 ml) and water (1 ml). The cartridges were washed sequentially with water (1 ml) and 20% acetonitrile (1 ml). The extracts were eluted with methanol, which was evaporated to dryness under nitrogen at 37°C and dissolved in 100 μl of mobile phase for injection onto the HPLC column. The mobile phase comprised acetonitrile/0.1% trifluoroacetic acid (13:87 by vol) and the column was reverse phase basic 10 cm × 4.6 mm (HiChrom). Detection was by UV absorbance at 254 nm. The approximate retention time for compound 6 was 4 min.  

UK-338,003 (series 2). Compound and internal standard were extracted from rat or dog plasma (0.1- to 1-ml aliquots, respectively) by solid phase extraction using PH cartridges (Bond Elut, 100 mg/1 ml), which had been pretreated with methanol (1 ml) and water (1 ml). The cartridges were washed sequentially with water (1 ml) and 40% methanol (1 ml). The extracts were eluted with methanol, which was evaporated to dryness under nitrogen at 37°C and dissolved in 100 μl of mobile phase for injection onto the HPLC column. The mobile phase comprised acetonitrile/0.1% potassium dihydrogen phosphate containing 10 mM octane sulfonic acid, pH 3.0 (34:66 by volume) and the column was a reverse phase basic 10 cm × 4.6 mm (HiChrom, Reading, UK). Detection was by fluorescence at excitation wavelength 340 nm and emission wavelength 410 nm. The approximate retention time for UK-338,003 was 5 min.  

The method for analyzing UK-338,003 in human plasma used PH solid phase extraction followed by column-switched trace enrichment HPLC with fluorescence detection. Plasma samples (1 ml) were mixed with internal standard and buffered with 0.75 ml (pH 7) of 0.05 M phosphate buffer and applied to previously conditioned PH solid phase extraction sorbent packed in a 96-well format (50 mg of sorbent per well). The sorbent was washed with water and UK-338,003, and internal standard was selectively eluted two times with 0.25 ml of propan-2-ol into a 2-ml 96-well block. The eluent was evaporated under nitrogen at 37°C and redissolved in 0.2 ml of water/acetonitrile/trifluoroacetic acid (80:20:0.1). Extracted samples were centrifuged and 0.15 ml was injected onto an HPLC apparatus connected to a 1-cm SAS trace enrichment column (Phenomenex, Macclesfield, Cheshire, UK). A second HPLC apparatus using a mobile phase of acetonitrile/water/trifluoroacetic acid (72:28:0.1) was used to wash the compounds off of the trace enrichment column and through a HiRPB column to a fluorescence detector. Detection wavelengths were set at 330 nm (excitation) and 394 nm (emission). The approximate retention time for UK-338,003 was 7.6 min.  

Internal Standards. The dimethyl quinoline version of UK-191,005 was used as the internal standard for UK-191,005 (series 1). For all other com-
pounds UK-191,005 or the dimethyl quinoline version of UK-191,005 was used as internal standard. In each case the internal standards used were more lipophilic than parent compounds and not expected to be metabolites.

Pharmacokinetic Analysis of Data. Standard algorithms were used to perform the pharmacokinetic analysis of the data. $C_{\text{max}}$ and $T_{\text{max}}$ values were obtained directly from the recorded data. The terminal phase rate constant ($k_{\text{el}}$) was determined by linear regression of the log plasma concentration-time profile. The terminal elimination half-life ($t_{1/2}$) was calculated from $0.693/k_{\text{el}}$. Area under the plasma concentration-time curve (AUC 0-$T$) was calculated using the linear trapezoidal rule and extrapolated to infinity (AUC0-$\infty$) using $k_{\text{el}}$. Clearance (CL) was calculated from intravenous data using the relationship $\text{dose} / \text{AUC}_{0-\infty}$. Oral bioavailability ($F$) was calculated from the ratio of AUC0-$\infty$ values after oral and intravenous doses, after normalizing for dose. The extent of first pass extraction ($E$) was estimated by reference to the well stirred model of hepatic clearance and assuming hepatic blood flow of 100 ml/min/kg in the rat and 50 ml/min/kg in the dog. Based on the bioavailability observed and the estimate of hepatic extraction, the extent of absorption ($A$) was estimated [$A = F/(1 - E)$]. This method assumes that clearance is solely hepatic, which is supported by observations of low renal clearance (data not reported).

Free Drug Considerations. Only free drug in plasma is in equilibrium with free drug in the tissues, and the relative equilibria between free and protein-bound drug in both plasma and tissues will affect the extent of tissue distribution. In the same way, only free drug is available for clearance by metabolism and excretion (Wilkinson, 1983; Smith et al., 1996). It is therefore appropriate to correct clearance and volume of distribution for free fraction before considering the impact of physicochemical properties on these processes. Therefore, unbound intrinsic clearance and unbound volume of distribution values were considered. Unbound volume of distribution and clearance values were calculated by dividing the values of total volume of distribution and clearance obtained from intravenous pharmacokinetic studies by the free fraction of the compound in the plasma of that species.

Results

Physicochemical Properties of the $\alpha_1$-Adrenoceptor Antagonists. The structures and physicochemical properties of the $\alpha_1$-adrenoceptor antagonists in series 1 and 2 are presented in Figs. 1 and 2 and in Table 1. Physicochemical properties were determined by experimental methods (log $D_{7.4}$ and $pK_a$) or by computational analysis.
TABLE 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Mass</th>
<th>clogP</th>
<th>Log D&lt;sub&gt;7.4&lt;/sub&gt;</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
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<th>HBA</th>
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<tr>
<td>UK-191,005</td>
<td>609</td>
<td>4.4</td>
<td>4.0</td>
<td>6.2</td>
<td>2</td>
<td>10</td>
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<tr>
<td>Series 2</td>
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<td>6.9</td>
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<td>3.0</td>
<td>6.7</td>
<td>3</td>
<td>9.8</td>
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</table>

HBD, hydrogen bond donor; HBA, hydrogen bond acceptor.

(molecular mass, clogP, and number of hydrogen bond donors and acceptors). All compounds are lipophilic and basic in nature with molecular mass values ranging from 416 to 609 Da. The higher the pK<sub>a</sub>, the larger the difference between clogP and log D<sub>7.4</sub>. The compounds containing a quinoline ring structure were more basic than the quinazoline-containing compounds and therefore have the largest discrepancy between clogP and log D<sub>7.4</sub>. All compounds have the potential for hydrogen bonding with 2 to 3 hydrogen bond donor and 7 to 10 hydrogen bond acceptor groups per molecule. The compounds in series 1 had a higher molecular mass and log D<sub>7.4</sub> than the compounds in series 2.

**IPRL, in Vitro Metabolism, and Rat Hepatocyte Transport Data for Series 1 α1-Adrenoceptor Antagonists.** The IPRL data for the compounds in series 1 are summarized in Table 2 and Fig. 3. UK-191,005 and analogs showed high hepatic extraction ratios in the IPRL (>0.7) with the majority of the dose excreted unchanged in the bile (>90%). These data are consistent with the high plasma clearance observed in vivo for UK-191,005 in the rat. UK-191005 is taken up rapidly into the liver (Fig. 3) with a liver to perfusate ratio of 11:1 after 5 min postdose.

Studies in rat liver microsomes showed that the compounds in series 1 are all slowly metabolized with disappearance half-life values of >120 min (Table 2). This finding suggests that metabolic clearance is negligible for these compounds and correlates with IPRL data, which demonstrate high biliary excretion of unchanged compound rather than metabolism as the principal route of clearance.

In agreement with the rapid hepatic uptake observed in IPRL experiments was the demonstration that UK-191,005 underwent temperature-dependent, saturable active uptake in isolated fresh rat hepatocytes (Fig. 4) with a K<sub>m</sub> of 6.5 ± 0.9 μM and V<sub>max</sub> of 481 ± 37.5 pmol/min/10<sup>6</sup> cells.

**Pharmacokinetics of UK-191,005 in Rat and Dog.** The pharmacokinetic parameters estimated for UK-191,005 (series 1) after intravenous administration to rat and dog are summarized in Table 3. After intravenous administration to male rats, UK-191,005 exhibited a plasma clearance of 135 ml/min/kg, which is greater than liver blood flow in the rat (Boxenbaum, 1980). The volume of distribution was 9.3 l/kg and the resulting elimination half-life was 1.4 h. In the dog, the intravenous clearance of UK-191,005 was 47 ml/min/kg, which approximates to liver blood flow in this species (Boxenbaum, 1980). The volume of distribution was 10 l/kg and plasma concentrations declined with an apparent half-life of 2.4 h.

After oral administration of UK-191,005 to both rat and dog, drug levels were below the limit of quantitation. This suggests very low bioavailability and is in keeping with the high systemic clearance observed in both species and complete first pass extraction by the liver.

**Choice of Animal Species for Pharmacokinetic Studies of α1-Adrenoceptor Antagonists in Series 2.** Although the rat is typically the species used for initial pharmacokinetic evaluation of a compound, the clearance of UK-294,315 (series 2) and similar analogs was limited by blood flow in the rat. This resulted in short half-life values in the rat and low bioavailability due to complete first pass effect at the liver. Therefore, the rat could not be used to rank compounds since...
unbound clearance was always limited by liver blood flow and bio-
availability could not be observed.

In vitro and in vivo studies have shown that UK-294,315 and
UK-338,003 are metabolized almost exclusively by CYP3A (data not
reported). The high clearance values observed in the rat may occur
because of the activity of CYP2C (Smith, 1991), which has substrate
specificity that overlaps with CYP3A in this species.

In general, the pharmacokinetics of substrates of CYP3A appear
similar in dog and human (Smith, 1991). In addition, studies in vitro
in hepatic microsomes showed that the rate of metabolism of these
compounds was similar in dog and human (see Fig. 6). Dog was also
the species used in in vivo pharmacology studies. For these reasons
dog was chosen as the most appropriate species to evaluate the
pharmacokinetics of the compounds in series 2 and most likely to
be representative of the pharmacokinetics of the α1L-antagonists in
human.

In Vitro Metabolism of Series 2 α1-Adrenoceptor Antagonists
in Dog and Human Liver Microsomes. The disappearance half-life
values for series 2 compounds in dog and human liver microsomes at
1 μM substrate concentration and 0.5 μM P450 concentration are
shown in Table 4. No disappearance was detected in the absence of
NADP. The extent of binding in the incubation was also determined.
In general, the most lipophilic and basic compounds have the highest
microsomal binding. In addition, binding is greater in the human liver
microsomal incubation than the dog, which is consistent with a higher
protein concentration (1.5 mg/ml protein concentration in the human
and 1.0 mg/ml protein concentration in the dog microsomal incuba-
tions). The disappearance half-life values and unbound fraction in the
incubation were used to scale up to unbound intrinsic clearance in
ml/min/kg. In dog, unbound intrinsic clearance values ranged from 23
to 162 ml/min/kg (Fig. 5). Assuming phase 1-mediated metabolism is
the major route of clearance for compounds in series 2, these values
could be used to predict unbound intrinsic clearance in the dog.
In human, unbound intrinsic clearance values ranged from 12 to 154
ml/min/kg. The values of unbound intrinsic clearance in dog and
human were in general within 2-fold of each other, indicating a similar
rate of metabolism in dog and human liver microsomes for these
compounds (Fig. 6).

Pharmacokinetics of Series 2 α1-Adrenoceptor Antagonists in
Dog, Rat, and Human. Pharmacokinetic data after intravenous ad-
ministration of 7 α1L-adenreceptor antagonists in series 2 to the dog
are shown in Table 5.

UK-294,315 (compound 1) is a quinoline with a homopiperaziney
morpholine 2-substituent (Fig. 2). After intravenous administration to
the dog, UK-294,315 had a plasma clearance of 15 ml/min/kg, which
is approximately one third of liver blood flow (Boxenbaum, 1980),
and a volume of distribution of 4.8 l/kg. These values contributed to
an elimination half-life of 6.3 h in the dog.

Compound 2 is similar in structure to UK-294,315. The clearance
of compound 2 after intravenous administration to the dog was lower
at 9 ml/min/kg. However, the volume of distribution was also lower at
2.8 l/kg and, thus, compound 2 had a shorter half-life of 3.6 h.

Compounds 3 and 4 are quinazoline compounds with homopiperaza-
zinyl morpholine 2-side chains (Fig. 2). They had higher plasma
clearance values than the quinoline compounds of 23 and 20 ml/min/
kg, respectively, and similar volumes of distribution of 4.3 and 2.2
l/kg, respectively, after intravenous administration to the dog. There-
fore, compounds 3 and 4 had short elimination half-life values in the
dog of 2.3 and 1.6 h, respectively.
Compound 5 is a quinoline compound with a homopiperazinoyl morpholine 2-side chain (Fig. 2). This compound had a low plasma clearance of 5.9 ml/min/kg after intravenous administration to the dog. However, the resulting elimination half-life was short at 1.9 h because of a drop in the volume of distribution to 1 l/kg. A higher volume of distribution of 5.1 l/kg was measured for the quinazoline compound 6, but its high plasma clearance of 40 ml/min/kg resulted in a short half-life of 1.9 h.

The desired pharmacokinetics were obtained with UK-338,003 (compound 7), which is a quinoline compound with a methane sulfonamide tetrahydroisoquinolyl 2-side chain. This compound had a low plasma clearance of 5.6 ml/min/kg and a volume of distribution of 4.1 l/kg after intravenous administration to the dog. UK-338,003 therefore had a long elimination half-life in the dog of 9.2 h.

After oral administration to the dog, the majority of compounds in series 2 were determined in rat and in human as well as in the dog, and these data are summarized in Table 6. After intravenous administration to the rat, UK-294,315 (compound 1) exhibited a mean elimination half-life of 1.0 h. This half-life was due to a high systemic clearance of 91 ml/min/kg and a relatively high volume of distribution of 5.6 l/kg. Oral bioavailability in the rat was 11%, which is consistent with an oral absorption of approximately 30%.

After intravenous administration of UK-338,003 (compound 7) to the rat, plasma clearance was lower than that of UK-294,315 at 22 ml/min/kg. However, volume of distribution was also lower at 1.7 l/kg, and as a result, the elimination half-life of UK-338,003 in the rat was the same as that of UK-294,315 at 1.0 h. The oral bioavailability of UK-338,003 was 42% in the rat, which is consistent with an oral absorption of 72%.

In humans, across an oral dose range of 1 to 100 mg, UK-294,315 (compound 1) exhibited a mean elimination half-life of 11.6 ± 1.4 h. AUC<sub>0-T</sub> increased proportionally with dose from 1 to 100 mg; peak plasma concentration (C<sub>max</sub>) increased superproportionally up to a dose of 50 mg, with a 220-fold increase in C<sub>max</sub> for a 50-fold increase in dose. Between 50 and 100 mg, C<sub>max</sub> increased in a dose-proportional manner. T<sub>max</sub> was variable, decreasing from 3.8 h at 1 mg to a plateau of 0.6 ± 0.2 h for all doses above and including 30 mg.

UK-338,003 (compound 7) was administered to human as oral doses of 0.25 to 1.5 mg. UK-338,003 was rapidly absorbed after administration in solution with peak concentrations occurring 1 to 2 h after dosing and had a mean estimated oral bioavailability of 73%. C<sub>max</sub> and AUC increased approximately proportionally with dose. The terminal plasma elimination half-life was 11 to 17 h.

### Table 5
Mean pharmacokinetic parameters of the α<sub>1</sub>-adrenoceptor antagonists in series 2 following single intravenous and oral administration to the dog

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<thead>
<tr>
<th>Parameter</th>
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<tr>
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<td>Human (n = 5)</td>
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<td>Intravenous dose (mg/kg)</td>
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<tr>
<td>Elimination half-life (h)</td>
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<tr>
<td>Plasma clearance (ml/min/kg)</td>
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<tr>
<td>Unbound intrinsic plasma clearance (ml/min/kg)</td>
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</tr>
<tr>
<td>Volume of distribution (l/kg)</td>
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<tr>
<td>Unbound volume of distribution (l/kg)</td>
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<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
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<tr>
<td>Oral bioavailability (%)</td>
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<table>
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<td>Elimination half-life (h)</td>
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<td>Oral bioavailability (%)</td>
<td>11</td>
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a N.A., intravenous data not available; n/a, not available.
b Administered as 60 mg in solution.
c Administered as 0.5 mg in solution.
human plasma for UK-294,315 (compound 1) and UK-338,003 (compound 7) at 1 μg/ml (n = 3). UK-294,315 had a plasma protein binding value of 92% in rat and 94% in human plasma. Rat and human plasma protein binding of UK-338,003 was 93%.

**Discussion**

In this article we discuss the pharmacokinetic considerations in the design of an α1L-adrenoceptor antagonist for the treatment of BPH. In doing so, we highlight the balance required between pharmacokinetics and pharmacodynamics to design a drug with optimum characteristics. The primary focus is the impact of physicochemical properties and structural modifications on pharmacokinetics and, in particular, clearance, which is a major determinant of overall clearance for these compounds, and hepatic extraction can be considered as a three-step process involving hepatic uptake, metabolism, and biliary clearance.

The first series of compounds investigated was a series of lipophilic bases, with large 2- and 4-substituents on a core quinoline or quinazoline ring template (Fig. 1). These compounds are exemplified by UK-191,005, which is a weak base with a log D7.4 of 4, high mol. wt. quinoline ring template (Fig. 1). These compounds are exemplified by UK-191,005, which is a weak base with a log D7.4 of 4, high mol. wt. (609), and a high propensity for hydrogen bonding (Table 1). The compounds were potent α1-adrenoceptor antagonists, with selectivity over the receptors in the cardiovascular system. Also, in vitro studies in rat, dog, and human liver microsomes indicated that the compounds were metabolically stable (Table 2). As a consequence, these compounds represented an attractive series for further study from both a pharmacological and pharmacokinetic perspective.

However, in rat and dog pharmacokinetic studies, UK-191,005 was found to have high hepatic clearance approximating to liver blood flow (Table 3). As a result, UK-191,005 was not orally bioavailable in both species. Further studies were completed in the IPRL to try to understand the mechanism of high hepatic extraction. In the IPRL, UK-191,005 and analogs showed high hepatic extraction ratios (>0.7) and levels of unchanged drug excreted in the bile were high (>90%) (Table 2). These data agree with the high clearance observed for UK-191,005 in the rat. UK-191005 was taken up rapidly into the liver with a liver to perfusate ratio of 11:1 after 5 min. Uptake of drugs from the blood into the hepatocyte can occur via passive diffusion or via active uptake across the sinusoidal membrane. The physicochemical properties of this series of compounds (high mol. wt. cations with high hydrogen bonding potential) suggest that passive diffusion across membranes is relatively slow and the rapid uptake of UK-191,005 into the liver is therefore more likely to be an active process, facilitated by carrier proteins on the sinusoidal membrane of the hepatocyte. A wide variety of active carrier systems are known to be present at the sinusoidal membrane facilitating the uptake of a diverse range of drugs (Hagenbuch, 1997; Meijer et al., 1997). The involvement of active transport processes in the hepatic uptake of UK-191,005 was confirmed in isolated rat hepatocytes at 4°C and 37°C, where uptake of UK-191,005 was found to be temperature-dependent and saturable (Km 6.5 μM; Fig. 4). Therefore, active transport protein-mediated uptake in rat hepatocytes is likely to be a key determinant for entry of UK-191,005 into the liver.

For this series of compounds, rapid hepatic uptake was followed by extensive biliary excretion of unchanged drug with negligible metabolic clearance. UK-191,005 was shown to be a substrate for apical transport proteins in Caco-2 cells (data not shown) and such extensive biliary excretion is likely to be an active process mediated by canalicular (apical) transport proteins. As a consequence, this series of compounds had inadequate pharmacokinetics for further progression despite the attractive pharmacological properties of this series.

Compounds with high mol. wt. and high hydrogen bonding potential seem to be particularly susceptible to high hepatic extraction and biliary excretion (Ayrton and Morgan, 2001). Our studies showed that replacement of the large 2- and 4-substituents on the core quinoline or quinazoline template resulted in compounds with lower hepatic extraction ratios (<0.3) in the IPRL and low levels excreted in the bile. As a result, the project focus shifted to investigation of lower molecular weight (<510) quinoline or quinazoline compounds, exemplified by series 2 (Fig. 2) in which metabolism is the predominant clearance route.

Indeed, a significant difference between series 1 and 2 is that the rate of in vitro metabolism of the compounds in series 2 in liver microsomes translates to in vivo metabolic clearance. Unbound intrinsic clearance values for seven compounds in dog range from 23 to 536 ml/min/kg. In general, these values can be predicted within 2-fold from unbound intrinsic clearance values scaled from microsomal half-life in dog liver microsomes (Fig. 7). This relationship is in agreement with similar analyses in rat and human reported in the literature (Obach, 1997; Austin et al., 2002). However, due to the assumptions made in such predictions, it is not unusual to find outliers. Here, two compounds (UK-294,315 and compound 6) have a much higher unbound intrinsic clearance in vivo in dog compared with that predicted in vitro from dog liver microsoma data. A possible explanation is that these compounds are subject to phase 2 metabolism or a mechanism of nonmetabolic clearance. Dog liver has a high efficiency/capacity for glucuronidation (Soars et al., 2001), which may account for the high unbound intrinsic clearance observed for compound 6 in the dog. UK-294,315 has been demonstrated to be a substrate for the efflux transporter P-glycoprotein (Harrison et al., 2004), which may contribute to its clearance in the dog, either by clearance into the bile or via direct secretion across the gut wall (Ayrton and Morgan, 2001).

The quinazoline compounds within series 2 had higher unbound intrinsic clearance values than the quinoline analogs, reflecting their increased lipophilicity. For example, comparison of the quinoline UK-294,315 (compound 1), with its direct quinazoline counterpart, shows that the quinazoline compound has approximately a 5-fold higher rate of metabolism in human liver microsomes (Fig. 8). The quinazoline analog is less basic than the quinoline compound (pKₐ values of 6.7 and 8.5, respectively), which results in an approximately 1 log unit higher log D₇.₄ and thus a higher propensity for metabolism.
Optimizing Half-Life via Modifying Physicochemical Properties. Within series 2, the quinoline compound UK-294,315 exhibited a long half-life and high oral bioavailability in the dog. This compound was progressed to phase 1 studies in human volunteers, where it exhibited a mean elimination half-life of 11.6 h (Harrison et al., 2004). However, the potency and selectivity of UK-294,315 for the prostate versus the cardiovascular system was not optimal, giving rise to an inadequate therapeutic window. Therefore, project effort focused on the compounds containing a quinazoline ring system, which were more potent antagonists of the human $\alpha_1$-adrenoceptors found in the prostate, with greater selectivity over the cardiovascular system.

However, as discussed, the quinazoline compounds were more lipophilic than the quinolines and therefore were predisposed to a higher metabolic clearance. Within the quinazoline series, the challenge was therefore to design a compound with sufficient pharmacokinetic half-life. To balance pharmacodynamic and pharmacokinetic requirements, the initial strategy was to reduce log $D_{7.4}$ in the quinazoline series of compounds to lower metabolic clearance and increase half-life.

To reduce lipophilicity within the quinazoline series, the 5'-phenyl analogs of the quinoline compounds 1 and 2 were replaced with a 6'- or 5'-pyridine in the quinazoline compounds 3 and 4. Compound 3 has moderate lipophilicity (log $D_{7.4}$ 2.1). This lipophilicity results in an unbound volume of distribution of 20 l/kg in the dog, but also a high unbound intrinsic clearance of 200 ml/min/kg. The elimination half-life of compound 3 is therefore short (2.3 h). Compound 4 is less lipophilic (log $D_{7.4}$ 1.5), and this results in a decrease in unbound intrinsic clearance to 60 ml/min/kg. However, the elimination half-life is still short (1.6 h), and this is due to a drop in unbound volume of distribution to 4 l/kg. This net effect of reducing unbound clearance and unbound volume of distribution after lowering log $D_{7.4}$ was also observed in the quinoline series, for example, compound 5, resulting in equally short half-life values (Table 5).

The approach of increasing volume of distribution and, thus, half-life, by increasing basicity has been shown to be successful (e.g., amlodipine) (Stopher et al., 1988). However, this approach was discounted with the quinazoline series due to deleterious effects on potency.

Optimizing Half-Life via Structural Changes. To improve the half-life of the quinazoline series it was necessary to design compounds with less metabolic lability for a given lipophilicity. This can be achieved by removing or altering the functionality associated with metabolism while maintaining lipophilicity to increase volume of distribution. It was necessary to do this in a low molecular weight series to avoid clearance via active hepatic uptake as encountered in series 1 and to maintain complete absorption to obtain high bioavailability. Steric and electron stabilization of a function to oxidative attack by cytochrome P450 is well preceded in the literature (van De Waterbeemd et al., 2001). For example, the metabolically vulnerable methoxyethyl substituent of metoprolol is replaced by a cyclopropyl group in bextaxolol, which is much more stable to hydrogen abstraction (Manoury et al., 1987).

The 2' homopiperazinyl morpholine side chain of compounds 1 to 5 was found to be the major site of metabolism. Metabolism studies in liver microsomes have shown that this is susceptible to hydroxylation and ring opening (Fig. 9), which is consistent with other piperazine- and morpholine-containing compounds (Jauch et al., 1990; Cocchiara et al., 1991; Denissen et al., 1994). Efforts were made to replace this moiety with a less metabolically vulnerable 2' side chain to decrease metabolic clearance.

Initially, the homopiperazinyl morpholine was replaced with a tetrahydronaphthyridine group (compound 6), which had moderate unbound intrinsic clearance from in vitro metabolism studies in dog liver microsomes. However, this clearance was not reflected in the pharmacokinetics as compound 6 has a high unbound intrinsic clearance in the dog of 450 ml/min/kg and a short half-life of 1.5 h. As discussed previously, this may be because compound 6 is subject to phase 2 metabolism or a nonmetabolic route of clearance in vivo.

UK-338,003 (compound 7) has a methanesulfonamido tetrahydrisoquinolyl side chain. This compound is weakly basic ($pK_a$ 6.7) and is...
lipophilic (log D$_{2,4}$ 3.0) and, thus, is typical of the quinazoline series of compounds. The high log D$_{2,4}$ results in an unbound volume of distribution of 21 l/kg and the more metabolically stable 2'-side chain facilitates a low unbound intrinsic clearance of 29 ml/min/kg (Fig. 5).

As a result, UK-338,003 has a long elimination half-life of 9.2 h in the dog. In addition, it has an oral bioavailability of 89% in the dog, which is consistent with complete transcellular absorption from the gastrointestinal tract.

UK-338,003 fulfilled the objectives of the discovery program as it had suitable pharmacology (human prostate α1A, P402 of 9.2 with 25-fold selectivity over rat aorta α1A). In addition, pharmacokinetic studies in the dog predicted a long elimination half-life and high oral bioavailability in human. UK-338,003 was progressed to phase 1 studies in young healthy volunteers, to assess pharmacokinetics and safety. Single doses of UK-338,003 in solution were administered at oral doses of up to 1.5 mg. As predicted from the pharmacokinetics in dog, UK-338,003 was rapidly absorbed with peak concentrations occurring 1 to 2 h after dosing and a mean bioavailability of 73%. The terminal plasma half-life was 11 to 17 h and was superior to the prototype quinoline compound UK-294,315 (Harrison et al., 2004) and suitable for a once a day administration to human (Table 6).

In conclusion, these data demonstrate the complex effect on pharmacokinetics and pharmacodynamics of change in structure or physicochemical properties of compounds. Understanding these relationships is essential to drug discovery so that drugs can be designed with optimum characteristics. In the program described here, ultimately a compound was identified with suitable pharmacokinetic-pharmacodynamic properties for progression to clinical development.

Acknowledgments. We gratefully acknowledge many Pfizer colleagues for their scientific and practical input into the BPH drug discovery program that is described in this article. In particular, we thank Patrick Johnson, Brian Kenny, and John Davis from the Departments of Discovery Chemistry, Discovery Biology, and Clinical Research & Development, respectively.

References

Austin RP, Barton P, Cockroft SL, Wenlock MC, and Riley RJ (2002) The influence of compound was identified with suitable pharmacokinetic-pharmacodynamic properties of change in structure or physicochemical properties of compounds. Understanding these relationships is essential to drug discovery so that drugs can be designed with optimum characteristics. In the program described here, ultimately a compound was identified with suitable pharmacokinetic-pharmacodynamic properties for progression to clinical development.

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