JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 795–802

Received November 14, 2006
Accepted February 13, 2007
DOI 10.1211/jpp.59.6.0006
ISSN 0022-3573

Pharmacokinetics of letrozole in male and female rats: influence of complexation with hydroxybutenyl-β-cyclodextrin

Michael F. Wempe, Charles M. Buchanan, Norma L. Buchanan, Kevin J. Edgar, Gregory A. Hanley, Michael G. Ramsey, Jennifer S. Skotty and Peter J. Rice

Abstract

Cyclodextrins (CDs) are one of the most successful solutions to the problem of poor drug solubility. In this study, we examined the in-vitro effects of three CDs on the solubility of letrozole, a breast cancer drug that is practically insoluble in water. The most promising, hydroxybutenyl-β-cyclodextrin (HBenβCD), was used for in-vivo studies in male and female Sprague-Dawley rats. Letrozole is a drug with dramatic gender-based differences in pharmacokinetics. For example, the terminal half-life (t½) of letrozole following intravenous administration in male rats was 11.5 ± 1.8 h (n = 3), while in female rats it was 42.3 ± 2.9 h (n = 3). HBenβCD increased the solubility and enhanced the dissolution rate of letrozole. Complexation of letrozole with HBenβCD improved oral absorption in male rats and maximized absorption in female rats. Regardless of gender, the presence of HBenβCD in the formulation increased the in-vivo rate of absorption. When administered in a capsule formulation with letrozole, HBenβCD resulted in a higher C_{max} (61% in male rats, 42% in female), shorter T_{max} values (8.4 to 6.3 h in male, 16.4 h to 5.4 h in female) and increased absolute oral bioavailability (46 ± 2 vs 38 ± 3 in male, 101 ± 3 vs 95 ± 2 in female). Thus, solubility limits both rate and extent of letrozole absorption in male rats, but limits only the rate of absorption in female rats.

Introduction

Many drug candidates (ca. 40%) developed by the pharmaceutical industry suffer from poor water solubility (Naseem et al 2004; Wong et al 2006). Poor drug solubility in a physiological environment may substantially reduce drug absorption across biological barriers, and thus limit drug bioavailability. Improving bioavailability may reduce variability (Rowland & Tozer 1994) and enable dosage reduction, which may in turn reduce drug side effects and expense. Therefore, developing drug delivery systems that mitigate solubility, dissolution and bioavailability issues is important. Various drug delivery techniques have been developed to overcome these limitations, such as pro-drugs, addition of surfactants, salt selection, particle size reduction and inclusion complexes with cyclodextrins (CDs) (Malmsten 2002; Stahl & Wermuth 2002).

CDs are cyclic glucose oligomers connected via α-1,4 linkages. Commonly used natural CDs contain 6, 7 or 8 glucose monomers and are typically referred to as α-CD, β-CD and γ-CD, respectively. CDs form a torus; most synthetic CDs have a hydrophobic interior and a hydrophilic exterior. CDs have an intrinsic ability to form specific inclusion complexes (Szejtli 1991, 1995; Connors 1997; Uekama et al 1998; Hirayama & Uekama 1999); their unique physicochemical characteristics allows for exploitation in various applications. For example, chemists have included β-CD to enhance hydrophobic effects and increase Diels-Alder reaction rates (Rideout & Breslow 1980; Sternbach & Rossana 1982). Selected pharmaceutical formulations have incorporated CDs to enhance drug stability, solubility and bioavailability (Loftsson et al 1991, 2004; Redenti et al 2000; Buchanan et al 2007). Modified CDs, such as hydroxypropyl-β-cyclodextrin (HPβCD), used in clinical formulations may overcome poor solubility issues and enhance bioavailability (Barone et al 1998).

Eastman Chemical Company, Kingsport, TN, USA

Michael F. Wempe, Charles M. Buchanan, Norma L. Buchanan, Kevin J. Edgar, Michael G. Ramsey, Jennifer S. Skotty

Department of Pharmacology, East Tennessee State University, Johnson City, TN, USA

Michael F. Wempe, Gregory A. Hanley, Peter J. Rice

Correspondence: P. J. Rice, Department of Pharmacology, East Tennessee State University, P. O. Box 70577, Johnson City, TN 37614, USA. E-mail: rice@etsu.edu

Acknowledgement: We thank James Little at Eastman Chemical Company for consultation on MS.

Despite successes, narrow inclusion selectivity and restricted commercial alternatives make it imperative to investigate novel CDs and demonstrate their ability to enhance solubility, dissolution and bioavailability. Previous work from our laboratories described the preparation and characterization of just such a novel CD, the highly water-soluble hydroxybutenyl-β-cyclodextrin (HBenβCD) (Buchanan et al 2002). HBenβCD is successful at solubilizing diverse drugs (Buchanan et al 2002, 2006).

In Western countries, breast cancer is a major cause of death in women (Haynes et al 2003). Approximately 25% of women diagnosed with breast carcinoma eventually die from the disease (Howe et al 2001). It is well known that some breast carcinomas are estrogen dependent and reducing estrogen levels can successfully combat the disease (Buzdar et al 2002). Estrogen receptor antagonists, such as tamoxifen, may alter estrogen effects in-vivo (Dixon & Bundred 2006; Buchanan et al 2007).

Tamoxifen is a non-steroidal anti-estrogen for first-line endocrine treatment as well as adjuvant therapy of early and metastatic breast cancers in postmenopausal women. Work from our laboratories has shown that HBenβCD complexes are a highly effective way to enhance tamoxifen solubility (Buchanan et al 2006) and bioavailability in a rat model (Buchanan et al 2007). More recently, estrogen biosynthesis inhibitors, such as aromatase inhibitors, have been developed. Letrozole (Figure 1) – manufactured by Novartis under the trade mark Femara – is a potent third-generation type 2 aromatase inhibitor and may be superior to tamoxifen in postmenopausal women with endocrine-sensitive breast cancer (Breast International Group 2006). Letrozole is a non-steroidal competitive inhibitor that interacts with cytochrome P-450 (Buzdar et al 2002; Haynes et al 2003) and inhibits the conversion of androgens to estrogens. However, letrozole has very poor water solubility (vide-infra). Poor solubility, its structural similarity to tamoxifen and status as a new treatment in the same therapeutic category led us to investigate whether complexes with HBenβCD could enhance letrozole solubility and bioavailability. Herein we describe the preparation, isolation, in-vitro solubility and dissolution testing of a solid letrozole–HBenβCD complex, and its comparison with un-complexed letrozole in pharmacokinetic studies in male and female Sprague-Dawley rats.

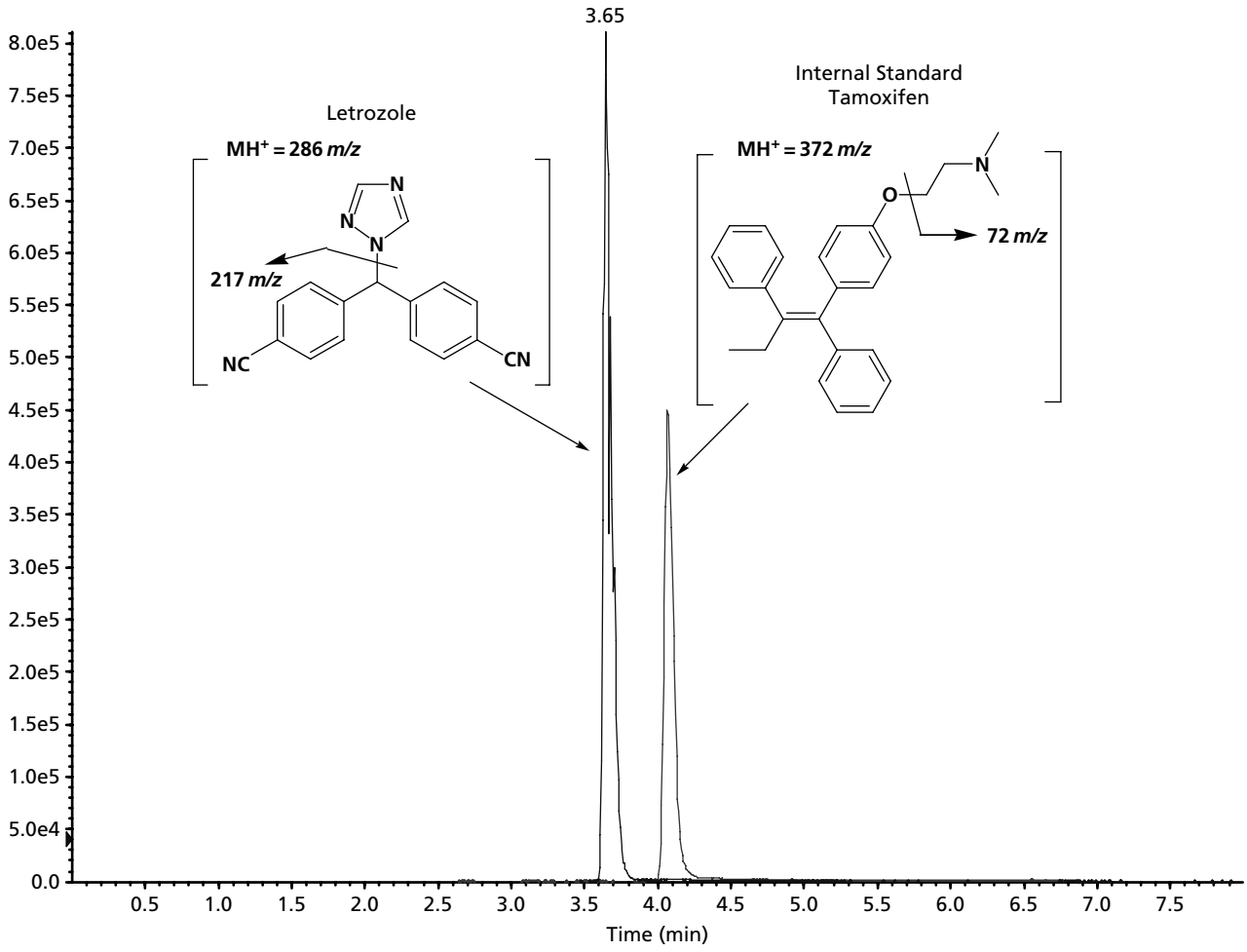

Figure 1 Representative chromatogram of letrozole and internal standard, tamoxifen.

Materials and Methods

Materials

Letrozole (> 99% purity) was purchased from Apin Chemicals Ltd (Abingdon, Oxon, UK). Tamoxifen base (≥ 99% purity), HPLC-grade water, carboxymethyl cellulose (CMC; microgranular, 25–60 μm), L-tartaric acid, HPLC-grade methanol, HPLC-grade acetonitrile, ethanol, isopropyl alcohol, ammonium acetate, formic acid and hydroxypropyl-β-cyclodextrin (HPβCD, MS = 4.4) were purchased from Sigma-Aldrich (St Louis, MO). HBenβCD (MS = 4.7) (Buchanan et al 2002) and SulfoHBenβCD (MS = 0.3) (Buchanan et al 2003) were prepared at Eastman. Isoflurane, USP (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane, was obtained from Butler Animal Health Supply (Dublin, OH).

Letrozole–HBenβCD complex formed in ethanol

Letrozole (100 mg) was weighed into a vial that contained ethanol (5.8 g). The material was heated (60 ± 2°C) and sonicated (20 min) to afford a hazy solution. HBenβCD (1.9 g) was added and the mixture sonicated a second time (20 min) to give a clear solution. The solution was frozen and lyophilized (28 h) to afford a letrozole–HBenβCD complex that was 5.0% letrozole by weight via HPLC analysis.

Letrozole/CMC formulation

Letrozole (160 mg) was weighed out and the particle size was reduced manually using a mortar and pestle; 144 mg of this material was transferred to a vial (20 cc). CMC (1870 mg) was added and the vial was capped and placed onto a mechanical roller (1 h). To help remove clumps or aggregates that might have formed while mixing, the material was removed and passed through a 35-mesh sieve screen. After additional mixing via the mechanical roller (45 min), the resulting blend contained 7.1% letrozole by weight via HPLC analysis.

Subsequently, to conduct an appropriate in-vivo study comparison, a 5.0% mixture of letrozole/CMC was required. A sample of the letrozole/CMC formulation (1.0 g) was transferred into a vial (20 cc). CMC (425 mg) was added. The vial was capped and placed onto a mechanical roller (1 h). To help remove clumps or aggregates that might have formed while mixing, the material was removed and passed through a 35-mesh sieve screen. After additional mixing via the mechanical roller (45 min), the resulting blend contained 5.0% letrozole by weight via HPLC analysis.

In-vitro dissolution studies

Using a filling funnel, letrozole/CMC and letrozole–HBenβCD were encapsulated into hard shell Torpac Lock ring gel (size 0, in-vitro dissolution; and size 9, in-vivo dosing) capsules (Torpac, USA). The in-vivo capsules contained 1.0 mg of letrozole and the in-vitro dissolution capsules contained 1.2 mg. In-vitro dissolution testing was performed in triplicate using a Varian VK 7000 dissolution tester (Cary, NC) according to USP 28-NF 23 711 (United States Pharmacopeia, 2004) with buffer solutions (900 mL) at 37°C and 50 rev min^{-1}.

Equilibrium solubility determination of letrozole

The equilibrium solubility of letrozole was determined in 50 mM L-tartaric acid (pH 2.0) in the presence of 0–40 wt% CD. Water filtered through a Milli-Q Water System (Millipore Corporation, Bedford, MA) was used for the solubility determinations. All CD derivatives were dried at 10–15 mmHg at room temperature for 14–60 h before use. Letrozole (ca. 5–10 mg) was added to each well of a 2-mL 96-well polypropylene mixing plate. Water, buffer (blanks with no CD) or appropriate CD solution (300–500 μL) was added; each determination was done in triplicate. Blanks were used to determine the intrinsic solubility (S_o) of letrozole in the corresponding solution, while the wells containing CD solutions were used to determine the solubility of letrozole due to CD (S_t).

After stock solution additions, the plate was sealed and shaken (Helidolph Titramax 1000) at 800–1200 rev min^{-1} at 23 ± 2°C for 48–72 h. Samples were transferred to a 96-well 2-mL multiscreen filter plate and filtered using a vacuum manifold. Letrozole concentration was determined using UV spectroscopy. Letrozole sample solutions (10–20 μL) were transferred to a 96-well plate (UV-STAR plates; Greiner, 190–400 nm spectral range) and diluted with water–ethanol (1:1) to afford an absorbance reading that was in the linear response range. Measurements were made using a SpectraMax Plus 384 Molecular Devices multi-well plate reader. Absorbance was converted to letrozole concentration.

In-vivo absorption studies

The in-life portion was conducted at East Tennessee State University – Quillen College of Medicine in an AAALAC accredited facility. All procedures were reviewed and approved by the ETSU Committee on Animal Care.

Male (260–294 g) and female (218–262 g) Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). For each dose group (oral and intravenous administration), three rats were used to investigate letrozole pharmacokinetics. Rats were housed in groups of three at 22.2 ± 1.1°C and 55 ± 15% humidity with 12-h dark–light cycles. Dosing occurred 2.0–2.5 h after the beginning of a light cycle. All rats had free access to water and were fasted for 14–16 h before dosing; food was returned 4 h post dose.

Rats were dosed in one of three ways: oral gavage (intragastric administration) using a 1.0-mL syringe with 0.01-mL measurement capability; orally using a Torpac capsule syringe (Torpac, USA) followed by an immediate water (500 μL) bolus; or intravenously via the ophthalmic venous plexus (orbital sinus) using a 1.0-mL syringe with a 27G needle. Rats were anaesthetized with isoflurane and subsequently infused (300 μL over < 30 s) letrozole formulation through the ophthalmic venous plexus (orbital sinus). Oral dose solutions were prepared as follows: letrozole (5.2 mg) was weighed into a sterile glass vial and, immediately before oral dosing, diluted with 12.5% aqueous ethanol with sonication to afford a 1.0 mg mL^{-1} suspension; and letrozole–HBenβCD complex (626.2 mg complex, 4.76 mg letrozole) was weighed out into

a vial and, immediately before oral dosing, diluted and mixed with 12.5% ethanol in water to afford a 1.0 mg letrozole mL^{-1} solution. The intravenous dose solutions were freshly prepared as follows: letrozole–HBenβCD complex (236.1 mg ≈ 11.8 mg letrozole) was weighed out into a glass vial, diluted with saline solution (Baxter HealthCare Co.) to afford a 1.0 mg mL^{-1} solution and without delay (< 2 min), the solution was filtered through a syringe filter (0.45 micron) and immediately dosed; and letrozole (9.9 mg) without HBenβCD was weighed into a glass vial, diluted with dimethyl sulfoxide (DMSO) (1.0 mL), and sonicated into solution; without ado, stock solution (300 μL) was added to saline (9.7 mL), mixed to afford a letrozole solution (0.297 mg mL^{-1}), which was filtered without delay (< 2 min), and immediately dosed.

Using tail-vein collection (i.e. the distal portion was transected, 2–3 mm), blood samples (125 μL) were collected using mini-capillary blood collection tubes that contained EDTA di-potassium salt (SAFE-T-FILL; RAM Scientific Inc., Yonkers, NY). Immediately after filling the individual samples, the tubes were capped, mixed, stored on dry ice and kept frozen (−80 ± 10°C) until sample preparation and subsequent LC/MS/MS analysis.

Determination of letrozole in rat blood

Equipment

The samples were assayed using a Sciex 4000-QTrap mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a Shimadzu HPLC, a PEAK Scientific API Systems gas generator (Bedford, MA) and Leap auto-sampler (Carrboro, NC).

Extraction procedure, calibration and quality control samples

An extraction solution containing 66.5 nM tamoxifen as an internal standard solution was prepared in acetonitrile–methanol (1:1). In individual rat sets, the blood samples were removed from the freezer (−80 ± 10°C) and allowed to warm to ambient temperature (15–20 min). The tubes were vortexed (3–5 s) and extraction solution (250 μL) was added, vortex mixed (3–5 s) and sonicated (10 min; Branson 2510 Ultrasonic cleaner, Danbury, CT). The tubes were centrifuged at 13 200 rev min^{-1} (10 min) using an Eppendorf minispin centrifuge (Hamburg, Germany). The supernatant was transferred into individual wells of a 96-well plate. The 96-well plate was sealed and centrifuged at 3000 rev min^{-1} for 10 min at 10°C (Labofuge 400R Centrifuge; VWR, West Chester, PA). The 96-well plate was placed into the LEAP auto-sampler cool-stack (6.0 ± 0.1°C) and analysed via LC/MS/MS.

Control blood was used to prepare standard curve and quality control samples. Gender-specific control blood was collected via inferior vena cava bleeding and pooled from naive (no drug) rats (each gender n = 3). Using addition and complete mixing of stock aqueous letrozole solutions with control blood, gender-specific letrozole standard curves and quality-control solutions were prepared. Samples were frozen (−80 ± 10°C), extracted and processed in an analogous fashion to the in-vivo samples previously described.

Coupled to the LEAP autosampler, the chromatographic system consisted of a Shimadzu SCL-10A Controller, LC-10AD LC, and DGU-14A Degasser (Shimadzu Scientific Instruments, Inc., Norcross, GA) connected to the Sciex 4000-Qtrap. Analyst 1.4.1 was used for data acquisition. Prism 4.02 (GraphPad Software, Inc., San Diego, CA) was used for data analysis, graphing and statistical analysis. Ten microlitres of the extracted samples were injected onto a Zorbax extended-C18 50 × 4.6 mm, 5-micron 80 Å column (Agilent Technologies, UK). The column temperature was set at 40 ± 1°C using a Temperature Control Module (Analytical Sales and Services, Pompton Plains, NJ). A binary gradient was used: solvent A was a 10 mM ammonium acetate solution containing 0.1% formic acid and solvent B was methanol–acetonitrile (50:50). Using a flow-rate of 0.4 mL min^{-1}, the following gradient was used for the HPLC separations: 95% A for 1.0 min; brought to 5% A at 3.5 min and held for 2 min; returned to 95% A after 0.5 min and held at 95% A for 2 min (8 min total). Between samples, the autosampler was washed with acetonitrile–methanol–isopropanol–water (1:1:1:1) containing 0.1% formic acid (Little et al 2006).

Letrozole and tamoxifen were analysed using electrospray ionization operated in the positive mode (ESI+). The following mass spectrometer parameters were used: an ionspray voltage of 5500 V; temperature of 450°C; nitrogen was used for the curtain gas (CUR) and for the Collisionally Activated Dissociation (CAD) gas; the CAD gas was set at medium; Ion Source gas one (GS1) and two (GS2) were air and both set at 15.0; the entrance potential was set at 10.0; quadruple one (Q1) and three (Q3) were both set on Unit Resolution; dwell time was set at 200 ms; letrozole and tamoxifen were monitored using multiple reaction monitoring (MRM) employing a declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) of 36.0 V, 19.0 V, 4.0 V and 91.0 V, 47.0 V, 12.0 V, respectively; and the mass transitions of *m/z* 286 → 217 (letrozole) and *m/z* 372 → 72 (tamoxifen) were monitored (Figure 1).

Statistical methods

Statistical analysis on the effects of in-vitro equilibrium solubility for different CDs was performed using a non-parametric Kruskal–Wallis test followed by a Dunn's post-test at a 95% confidence interval. The effects of time and pH on drug solubility were analysed using a Friedman's test followed by a Dunn's post-test. Gender specific in-vivo data were analysed using the Mann–Whitney test with a two-tail *P* value test at the 95% confidence level. Gender differences were analysed using a Friedman's followed by a Dunn's post-test or, where the gender time-points were not identical, by using a one-way analysis of variance followed by a Tukey–Kramer comparison test. $P < 0.05$ denotes significance in all cases. The formulation group comparisons (Table 2), area under the curve (AUC), C_{max}, T_{max} and absolute bioavailability (F) were compared using unpaired *t*-tests with Welch correlations.

Results and Discussion

Bioanalytical pharmacokinetic summary

As illustrated in Figure 1, the LC-MS/MS method afforded a retention time (t_R) of 3.7 min and 4.1 min for letrozole and the internal standard, tamoxifen, respectively. To avoid endogenous

lipid matrix ionization effects, in-source multiple reaction monitoring (IS-MRM) was probed during method development (Little et al 2006). We observed no difference in analytical response for the calibration curve obtained using male versus female control blood. Letrozole calibration data was fitted to a $1/x^2$ weighted (x = analyte concentration) linear regression using nine standard curve concentrations ranging from 0.3 to 1426 ng mL^{-1}. The limit-of-detection (LOD) was ~0.3 ng mL^{-1} and the correlation coefficient (R) was 0.9996, computed using the Pearson correlation with a two tailed P value test ($P < 0.0001$) (not shown). As summarized in Table 1, five QC samples were used and the analytical method was accurate and precise; these bioanalytical results are analogous to those previously described (Marfil et al 1996).

In-vitro equilbrium solubility determination

The equilibrium solubility of letrozole in the presence of different CDs at concentrations of 0–40 wt% is summarized in Figure 2. In the absence of any CD derivative, letrozole has very poor aqueous solubility ($S_o = 62\ \mu g\ mL^{-1}$). All tested CD derivatives increased the solubility of letrozole, but at each concentration tested the differences between the CDs were not statistically different ($P = 0.37$).

Table 1 Letrozole accuracy and precision acquired from rat blood after organic solvent extraction and LC-MS/MS analysis

QC sample	Theoretical concn (ng mL^{-1})	Observed letrozole concn	Accuracy[a]	Precision[b]
Q1	1.43	1.58 ± 0.03	110.5 ± 1.9	± 1.7
Q2	14.3	13.4 ± 0.6	93.6 ± 4.0	± 4.3
Q3	142.7	135.1 ± 7.3	94.7 ± 5.1	± 5.4
Q4	713.3	725.6 ± 36.0	101.7 ± 5.1	± 5.0
Q5	1426.6	1416.2 ± 54.6	99.3 ± 3.8	± 3.8

Observed letrozole concentration represents mean ± s.d., n = 8. [a]Accuracy is given as percent of the theoretical value. [b]Precision (reproducibility of the measurement) is given as percent of the relative s.d.

In-vitro dissolution

The dissolution profiles for letrozole/CMC and letrozole–HBen-βCD are illustrated in Figure 3. Letrozole–HBenβCD showed a faster in-vitro dissolution rate than letrozole alone, with essentially all of the drug being released within 15 min from the letrozole–HBenβCD formulation. Even after 6 h, the maximum amount of letrozole dissolved from the letrozole/CMC formulation was only 70%. Significant effects were found for the effect of time and pH on drug solubility ($P < 0.0001$; Gaussian approximation), while there were no significant differences within each formulation type. The comparison of letrozole–HBenβCD and letrozole formulations were significant at pH 4.5, and 6.9.

In-vivo oral absorption

A previous report suggesting significant gender differences (Liu et al 2000) prompted us to investigate the pharmacokinetics of letrozole in fasted male and female Sprague-Dawley rats. Following oral and intravenous administration, blood samples were collected and assayed for letrozole via LC/MS/MS. Before analysis, and to facilitate the comparison, individual letrozole blood levels were normalized to a 1.0 mg kg^{-1} dose (Table 2).

In male rats, letrozole was eliminated from blood within 36 h following administration by oral gavage (Figure 4). The letrozole gavage dose with and without HBenβCD were statistically different ($P = 0.006$). The presence of HBenβCD resulted in an increase in letrozole apparent absorption rate ($k_a = 0.56$ and 1.6 for letrozole and letrozole–HBenβCD, respectively), calculated using the absorption time method $k_a = 4.61/t_a$ (h^{-1}) (Ritschel & Kearns 2004). Oral gavage of male rats with letrozole–HBenβCD solution resulted in about a two-fold increase in AUC_{0-36} (2323 ± 110 ng h mL^{-1}) relative to dosing male rats with the letrozole suspension ($AUC_{0-36} = 1061 \pm 166$ ng h mL^{-1}).

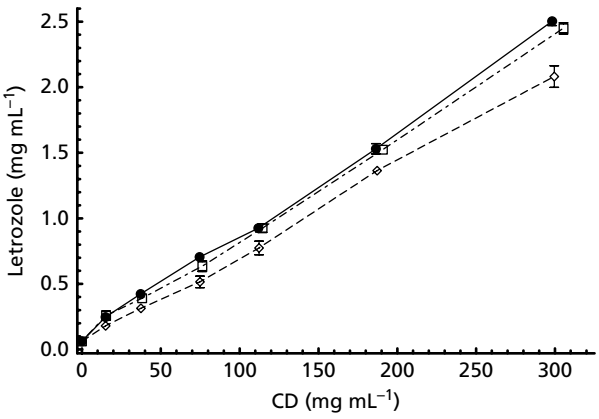

Figure 2 Mean letrozole equilibrium solubility concentration–CD profile at 23 ± 2°C (for each concentration and CD, n = 3 ± s.d.) are shown for HBenβCD (●), HPβCD (◇) and SulfoHBenβCD (□).

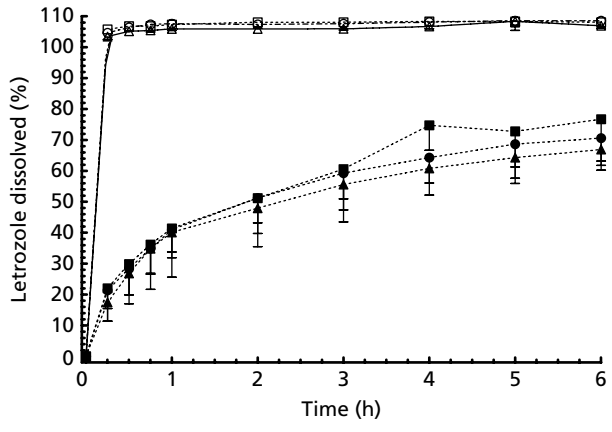

Figure 3 Mean dissolution profile of letrozole formulations at 37°C (each formulation type and pH, n = 3 ± s.d.). □, letrozole–HBenβCD pH 1.2; Δ, letrozole–HBenβCD pH 4.5; ○, letrozole–HBenβCD pH 6.8; ■, letrozole pH 1.2; ▲, letrozole pH 4.5; ●, letrozole pH 6.8.

Table 2 Letrozole and letrozole–HBenβCD groups of rats and summary of pharmacokinetic parameters

Gender	Letrozole formulation[b]	Route	Letrozole dose (mg kg^{-1})	AUC[a] (ng h mL^{-1})	T_{max} (h)	C_{max}[a] (ng mL^{-1})	F(%)
Male	Suspension	Oral	2.9–3.1	1061 ± 166[c]	6.0 ± 1.4	71 ± 13	42 ± 5
Male	HBenβCD solution	Oral	2.9–3.1	2323 ± 110[c]**	2.3 ± 0.3*	157 ± 7**	63 ± 3** 91 ± 2[g]**
Male	Solution	Intravenous	0.35–0.38	2541 ± 58[d]	1.5 ± 0.3	332 ± 20	100
Male	HBenβCD solution	Intravenous	1.1–1.3	3669 ± 333[d]*	1.8 ± 0.3 ns	489 ± 42*	100 ns
Female	Solution	Intravenous	0.37–0.40	24439 ± 504[e]	2.5 ± 0.5	493 ± 25	100
Female	HBenβCD solution	Intravenous	1.2–1.3	27366 ± 789[e]*	4.0 ± 0.0*	605 ± 24*	100 ns
Male	CMC capsule	Oral	3.6–3.8	970 ± 95[c]	8.4 ± 0.0	87 ± 2	38 ± 3
Male	HBenβCD capsule	Oral	3.6–4.1	1687 ± 83[c]**	6.3 ± 0.0**	140 ± 5**	46 ± 2* 66 ± 2[g]***
Female	CMC capsule	Oral	4.0–4.5	23217 ± 821[f]	16.4 ± 3.8	326 ± 13	95 ± 2
Female	HBenβCD capsule	Oral	4.0–4.7	27640 ± 1464[f]*	5.4 ± 1.7*	464 ± 39 ns	101 ± 3 ns

[a]AUC and C_{max} are normalized to a 1.0 mg kg^{-1} dose. [b]n = 3 for each formulation group. [c]AUC_{0-36}. [d]AUC_{0-24}. [e]AUC_{0-72}. [f]AUC_{0-126}. [g]F% computed using the AUC for intravenous letrozole in the absence of HBenβCD. Unpaired *t*-tests. Two-tailed: ns = not significant, *P <0.05, **P <0.01, ***$P < 0.001$.

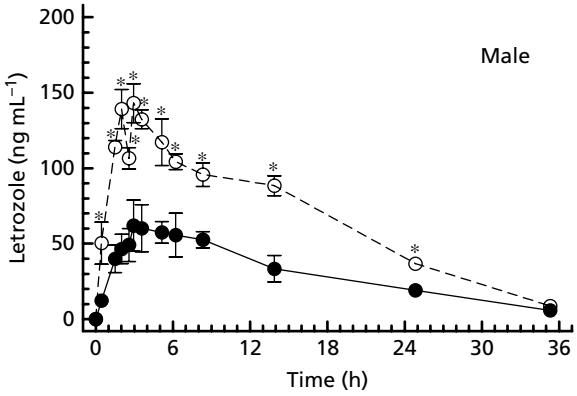

Figure 4 Pharmacokinetics of letrozole in solution administered to male rats by oral gavage in the absence (●) and presence (○) of HBenβCD. Samples were obtained at 0.45, 1.5, 2.0, 2.6, 2.9, 3.6, 5.1, 6.2, 8.3, 13.9, 24.8 and 36.3 h. *$P < 0.05$ vs absence of HBenβCD. The presence of HBenβCD results in an increase in the rate and extent of absorption, particularly during the initial 4 h following administration. Elimination appears to be enhanced in the presence of HBenβCD. Letrozole values are normalized to 1 mg kg^{-1} dosage and are expressed as mean ± s.e.m., n = 3.

Letrozole and letrozole–HBenβCD were administered intravenously to male and female rats via retro-orbital sinus. The two male intravenous dose groups (with and without HBenβCD) were significantly different, while the two female intravenous dose groups (0–24 h) were not statistically different from one another. However, there were clear gender differences (Figure 5A cf 5B). The differences between the male vs female letrozole intravenous doses ($P < 0.01$) and the differences between the male letrozole intravenous dose vs female letrozole–HBenβCD complex intravenous dose were significant ($P < 0.001$). None of the other Dunn's multiple comparisons were significant. In male rats, HBenβCD increased the peak blood levels (C_{max}) from 332 to 489 ng mL^{-1} (Figure 5A); in female rats, HBenβCD also increased C_{max} values (from 493 to 605 ng mL^{-1}) and delayed T_{max} (Figure 5B). The presence of HBenβCD did not produce any change in the apparent terminal elimination (data not shown). But a 13% increase in letrozole AUC_{0-24} (9809 ± 755 vs 8643 ± 542 ng h mL^{-1}) in female rats and a 44% increase in letrozole AUC_{0-24} (3669 ± 333 vs 2541 ± 58 ng h mL^{-1}; $P < 0.05$) in male rats was observed in these intravenous studies. Interestingly, a study of HBenβCD pharmacokinetics suggested a t½ of ~25 min (unpublished internal study); in the present study, the effects of HBenβCD on distribution of letrozole appear to be present up to 8 h following intravenous administration.

When administered orally as a capsule preparation to male rats (Figure 6A), HBenβCD increased the apparent absorption rate ($k_a = 0.50$ and 0.66 for letrozole and letrozole–HBenβCD, respectively) (Ritschel & Kearns 2004). The difference in the rates of absorption in male rats with and without HBenβCD was most profound during the first 4 h following oral administration (Figure 6A). Comparison of the two male oral capsule dose groups (with and without HBenβCD) failed to achieve significance ($P = 0.06$, Gaussian approximation). Nonetheless, the use of HBenβCD in male rats resulted in a 74% increase in AUC_{0-36} (1687 ± 83 vs 970 ± 95 ng h mL^{-1}), a 60% increase in C_{max} from 87 to 140 ng mL^{-1}, and a decrease in T_{max} from 8.4 h to 6.3 h. The effect of orally administering letrozole with and without HBenβCD was not as pronounced in female rats (Figure 6B). The two female oral capsule dose groups (with and without HBenβCD) were significantly different ($P = 0.0071$, Gaussian approximation). Due to the different time-points taken, these gender group data were evaluated using a one-way analysis of variance ($P < 0.0001$) followed by a Tukey–Kramer multiple comparisons test. Two comparisons were found to be extremely significant ($P < 0.001$), male letrozole vs female letrozole–HBenβCD and male letrozole HBenβCD vs female letrozole–HBenβCD; all other comparisons were not significant. In female rats (Figure 6B), the use of HBenβCD resulted in a 19% increase in AUC_{0-126} (27640 ± 1464 vs 23217 ± 821 ng h mL^{-1}), a 43% increase in C_{max} from 326 to 464 ng mL^{-1}, and a decrease in T_{max} from 16.4 ± 3.8 to 5.4 ± 1.7 h.

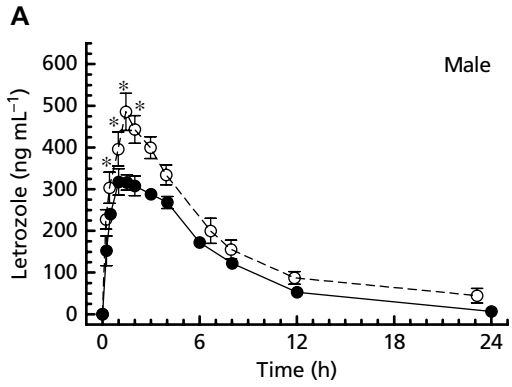

Figure 5 Pharmacokinetics of intravenous letrozole in the absence (filled symbols) and presence (open symbols) of HBenβCD in male (A) and female (B) rats. Samples were obtained at 0.25, 0.5, 1,0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24 h. *$P < 0.05$ vs absence of HBenβCD. Female rats had an additional sample obtained at 72 h; the letrozole levels of 15.7 ± 2.0 and 71.7 ± 4.8 ng mL^{-1} (*) were obtained in the absence and presence of HBenβCD, respectively. The presence of HBenβCD results in an increase in blood concentration of letrozole without significant changes in terminal elimination. Letrozole values are normalized to 1 mg kg^{-1} dosage and are expressed as mean ± s.e.m., n = 3 for each gender and formulation.

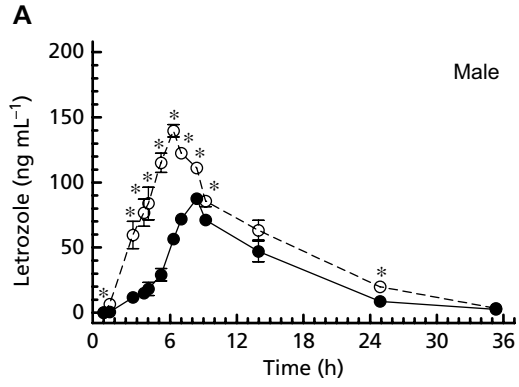

Figure 6 Pharmacokinetics of letrozole administered to rats as a capsule formulation in the presence of either carboxymethylcellulose (control; filled symbols) or HBenβCD (open symbols). Samples were obtained at 0.25, 0.5, 1,0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24 h. Additional samples were obtained at 36 h for males and 125 h for female rats. *$P < 0.05$ CMC vs HBenβCD. The presence of HBenβCD results in an increase in the rate and extent of absorption, particularly during the initial 6 h following administration. The effects of HBenβCD during the first 12 h appear more profound in female rats. Letrozole values are normalized to 1 mg kg^{-1} dosage and are expressed as mean ± s.e.m., n = 3 for each gender and formulation.

In both male and female rats, the increase in letrozole blood levels produced by HBenβCD was present for 12 h following administration. To help convey the profound differences in absorption engendered by the presence of HBenβCD, an 80% increase in the AUC_{0-12} for male rats (Figure 6A) and a 180% increase in the AUC_{0-12} for female rats (Figure 6B) was observed. Differences in letrozole blood levels between rats receiving letrozole/CMC and letrozole–HBenβCD capsules were resolved by 24 h.

The presence of HBenβCD improved the oral bioavailability of letrozole without altering apparent terminal half-life. The absolute oral bioavailability (Rowland & Tozer 1994) of letrozole in male rats obtained with the letrozole–HBenβCD capsule formulation was $46 \pm 2\%$ compared with $38 \pm 3\%$ with a letrozole/CMC capsule formulation. If AUC for the intravenous solution without HBenβCD is used, the absolute bioavailability in male rats becomes $66 \pm 2\%$; this illustrates the importance of conducting intravenous experiments with and without formulation additives. Although the presence of HBenβCD did increase absorption in female rats, the absolute bioavailability of letrozole was > 90%, regardless of the formulation (Table 2). The apparent terminal half-lives (t½s) in male rats (11.5 ± 1.8 h) and female rats (42.3 ± 2.9 h) from capsule oral administration were very similar to those reported by Liu et al (2000) and were unaffected by the presence of HBenβCD. These were analysed using the Mann–Whitney test and a two-tail P value test at the 95% confidence level; male and female were not significant ($P = 0.54$ and $P = 0.28$, respectively). Thus, letrozole has a smaller volume of distribution and a slower clearance in female rats compared with male rats. In fact, a recent biotransformation report demonstrates gender differences in the microsomal catalysed metabolic kinetics of letrozole in rats (Tao et al 2007) and helps to explain these in-vivo gender differences.

Conclusion

Letrozole, an inhibitor of CYP19 aromatase used clinically to treat women with breast cancer, has very poor solubility. The pharmacokinetics of letrozole capsule dosing are dramatically

different between male and female rats. In female rats, letrozole is well-absorbed (~95% bioavailability) and has a terminal half-life of ~42 h. In male rats, letrozole has a terminal half-life of ~12 h and a bioavailability of 38%.

Water-soluble hydroxybutenyl-β-cyclodextrin (HBen-βCD) was particularly effective at improving the solubility of letrozole. In-vitro dissolution studies have shown that the complex of letrozole with HBenβCD effectively releases letrozole, which forms a stable solution over many hours due to the presence of HBenβCD (both extent of release and stability are sharply higher than those of a letrozole/CMC control formulation).

With this background, it is interesting to study the effect of HBenβCD on the pharmacokinetics of letrozole. Complexation of letrozole with HBenβCD doubled absorption in male rats and maximized absorption in female rats. In male rats, therefore, letrozole oral absorption was limited by both solubility and uptake; when solubility is increased by complexation with HBenβCD, oral absorption is more rapid and bioavailability improves. In female rats, however, letrozole solubility limits the rate but not the extent of absorption. Complexation with HBenβCD produced a more rapid initial oral absorption, but it did not improve letrozole bioavailability. The data on oral absorption of letrozole and the effect of HBenβCD support the hypothesis that uptake of letrozole by female rats has higher affinity or greater capacity than in male rats. Following absorption, the effects of HBenβCD were similar in both sexes, with slightly higher blood concentrations achieved for 8–12 h following administration. When solubility is a limiting factor, HBenβCD can improve the oral absorption of letrozole.

References

Barone, J. A., Moskivitz, B. L., Guarnieri, J., Hassell, A. E., Colaizzi, J. L., Bierman, R. H., Jessen, L. (1998) Enhanced bioavailability of itraconazole in hydroxypropyl-β-cyclodextrin solution versus capsules in healthy volunteers. *Antimicrob. Agents Chemother*. **42**: 1862–1865

Breast International Group (BIG) 1–98 Collaborative Group (2006) A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *N. Engl. J. Med*. **353**: 2747–2757

Buchanan, C. M., Alderson, S. R., Cleven, C. D., Dixon, D. W., Ivanyi, R., Lambert, J. L., Lowman, D. W., Offerman, R. J., Szejtli, J., Szente, L. (2002) Synthesis and characterization of water-soluble hydroxybutenyl cyclodextrins. *Carbohydr. Res*. **327**: 493–507

Buchanan, C. M., Lambert, J. L., Large, S. E., Falling, S. N., Jicsinszky, L., Szejtli, J., Szente, L. (2003) Cyclodextrin sulfonates, guest inclusion complexes methods of making the same and related materials. US Patent 6,610,671

Buchanan, C. M, Buchanan, N. L., Edgar, K. J., Lambert, J. L., Posey-Dowty, J. D., Ramsey, M. G., Wempe, M. F. (2006) Solubilization and dissolution of tamoxifen-hydroxybutenyl complexes. *J. Pharm. Sci*. **95**: 2246–2255

Buchanan, C. M., Buchanan, N. L., Edgar, K. J., Little, J. L., Malcolm, M. O., Ruble, K. M., Wacher, V. J., Wempe, M. F. (2007) Pharmacokinetics of tamoxifen after intravenous and oral dosing of tamoxifen-hydroxybutenyl-β-cyclodextrin formulations. *J. Pharm. Sci*. **96**: 644–660

Buzdar, A. U., Robertson, J. F. R., Eiermann, W., Nabholtz, J.-M. (2002) An overview of the pharmacology and pharmacokinetics of the newer generation aromatase inhibitors anastrozole, letrozole, and exemestane. *Cancer* **95**: 2006–2016

Connors, K. A. (1997) The stability of cyclodextrin complexes in solution. *Chem. Rev*. **97**: 1325–1357

Dixon, J. M., Bundred, N. (2006) Aromatase inhibitors for early breast cancer therapy: a choice of effective treatment strategies. *Eur. J. Surg. Oncol*. **32**: 123–125

Haynes, B. P., Dowsett, M., Miller, W. R., Dixon, J. M., Bhatnagar, A. S. (2003) The pharmacology of letrozole. *J. Steroid Biochem. Mol. Biol*. **87**: 35–45

Hirayama, F., Uekama, K. (1999) Cyclodextrin-based controlled drug release system. *Adv. Drug Del. Rev*. **36**: 125–141

Howe, H. L., Wingo, P. A., Thun, M. J., Ries, L. A. G., Rosenberg, H. M., Feigal, E. G., Edwards, B. K. (2001) Annual report to the nation on the status of cancer (1973 through 1998), featuring cancers with recent increasing trends. *J. Natl Cancer. Inst*. **93**: 824–842

Liu, X.-D., Xie, L., Zhong, Y., Li, C.-X. (2000) Gender differences in letrozole pharmacokinetics in rats. *Acta Pharmacol. Sin*. **21**: 680–684

Little, J. L., Wempe, M. F., Buchanan, C. M. (2006) Liquid chromatography-mass spectrometry/mass spectrometry method development for drug metabolism studies: Examining lipid matrix ionization effects in plasma. *J. Chrom. B* **833**: 219–230

Loftsson, T., Fridriksdottir, H., Olafsdottir, B. J., Gudmundsson, O. (1991) Solubilization and stabilization of drugs through cyclodextrin complexation. *Acta Pharm. Nord*. **3**: 215–217

Loftsson, T., Sigurdsson, H. H., Masson, M., Schipper, N. (2004) Preparation of solid drug/cyclodextrin complexes of acidic and basic drugs. *Pharmazie* **59**: 25–29

Malmsten, M. (2002) *Surfactants and polymers in drug delivery*. Marcel Dekker, Inc., NY

Marfil, F., Pineau, V., Sioufi, A., Godbillon, J. (1996) High-performance liquid chromatrography of the aromatase inhibitor, letrozole, and its metabolite in biological fluids with automated liquid-solid extraction and fluorescence detection. *J Chrom. B* **683**: 251–258

Naseem, A., Olliff, C. J., Martini, L. G., Lloyd, A. W. (2004) Effects of plasma irradiation on the wettability and dissolution of compacts of griseofulvin. *Int. J. Pharm*. **269**: 443–450

Redenti, E., Szente, L., Szejtli, J. (2000) Drug/cyclodextrin/hydroxy acid multicomponent systems: properties and pharmaceutical applications. *J. Pharm. Sci*. **89**: 1–8

Rideout, D. C., Breslow, R. (1980) Hydrophobic acceleration of Diels-Alder reactions. *J. Am. Chem. Soc*. **102**: 7816–7817

Ritschel W. A., Kearns, G. L. (2004) *Handbook of basic pharmacokinetics . . . including clinical applications*. 6th edn, American Pharmacists Association, Washington, DC

Rowland, M., Tozer, T. N. (1994) Clinical pharmacokinetics concepts and applications. 3rd edn, Lippincott Williams & Wilkins, Baltimore

Stahl, P. H., Wermuth, C. G. (eds) (2002) Handbook of pharmaceutical salts properties, selection, and use. Wiley-VCH, NY

Sternbach, D. S., Rossana, D. M. (1982) Cyclodextrin catalysis in the intramolecular Diels-Alder reaction with the furan diene. *J. Am. Chem. Soc*. **104**: 5853–5854

Szejtli, J. (1991) Cyclodextrins in drug formulations: part II. *Pharm. Technol*. **15**: 24–38

Szejtli, J. (1995) Selectivity/structure correlation in cyclodextrin chemistry. *Supramolecular Chem*. **6**: 217–223

Tao, X., Piao, H., Canney, D. J., Borenstein, M. R., Nnane, I. P. (2007) Biotransformation of letrozole in rat liver microsomes: effects of gender and tamoxifen. *J. Pharm. Biomed. Anal*. **43**: 1078–1085

Uekama, K., Hirayama, F., Irie, T. (1998) Cyclodextrin drug carrier systems. *Chem. Rev*. **98**: 2045–2076

Wong, S. M., Kellaway, I. W., Murdan, S. (2006) Enhancement of the dissolution rate and oral absorption of a poorly water soluble drug by formation of surfactant-containing microparticles. *Int. J. Pharm*. **317**: 61–68