BIOPHARMACEUTICS & DRUG DISPOSITION, VOL. 18:9, 779–789 (1997)

ABSOLUTE BIOAVAILABILITY OF LETROZOLE IN HEALTHY POSTMENOPAUSAL WOMEN

A. SIOUFI*§, N. GAUDUCHEAU*, V. PINEAU*, F. MARFIL*, A. JAOUEN*, J. M. CARDOT*, J. GODBILLON*, C. CZENDLIK†, H. HOWALD†, CH. PFISTER† AND F. VREELAND‡

*Laboratoires Ciba–Geigy, Bioanalytics and Pharmacokinetics, B. P. 308, 92506 Rueil-Malmaison, France

†Ciba–Geigy Limited, Human Pharmacology and Bioanalytics and Pharmacokinetics, CH-4002, Basle, Switzerland

‡Ciba Pharmaceuticals, Clinical Development, Summit, NJ 07901-1398, U.S.A.

ABSTRACT

Letrozole is a new non-steroidal inhibitor of the aromatase enzyme system. It is currently under development for the treatment of postmenopausal women with advanced breast cancer. Absolute bioavailability of letrozole when given orally as one 2·5 mg film-coated tablet in comparison to the same dose given intravenously as a bolus injection was studied in 12 healthy postmenopausal women. Letrozole absolute systemic bioavailability after p.o. administration was $99{\cdot}9 \pm 16{\cdot}3\%$. Elimination of letrozole was slow. Total-body clearance of letrozole from plasma after i.v. administration was low ($2.21\ \mathrm{L\,h^{-1}}$). The calculated distribution volume at steady state ($1.87\ \mathrm{L\,kg^{-1}}$) suggests a rather high tissue distribution. Biotransformation of letrozole is the main elimination mechanism with the glucuronide conjugate of the secondary alcohol metabolite being the predominant species found in urine. The two study treatments were tolerated equally well. ©1997 John Wiley & Sons, Ltd.

KEY WORDS: letrozole; healthy postmenopausal women; single dose; pharmacokinetics; absolute bioavailability

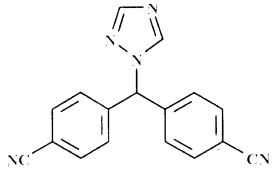
INTRODUCTION

Letrozole (4, 4′-[1H-1, 2, 4-triazol-1-ylmethylene]bis-benzonitrile) (Figure 1) is an orally active, non-steroidal and competitive inhibitor of the aromatase enzyme.[1,2] Single-dose phase I studies in healthy men and postmenopausal women[3,4] have shown that 0·1 mg was the minimal dose achieving a 50% estrogen suppression. Multiple-dose studies in postmenopausal women with advanced breast cancer[5,6] have shown that estradiol, estrone, and estrone sulfate are suppressed by more than 75–80% with doses from 0·1 up to 5 mg

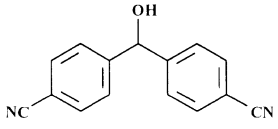
§Addressee for correspondence: Dr. A. Sioufi, Laboratoires Ciba–Geigy, Bioanalytics and Pharmacokinetics, B. P. 308, 2 et 4 rue Lionel Terray, 92506 Rueil-Malmaison Cédex, France.

CCC 0142–2782/97/090779–11$17.50

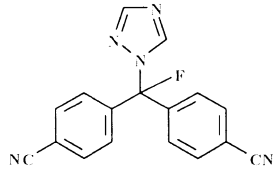
Received 7 May 1996
Accepted 29 November 1996



Letrozole



Secondary alcohol metabolite (SAM)



Internal standard (CGP 47645)

Figure 1. The chemical structures of letrozole, its metabolite, and the internal standard

daily. The tolerability of letrozole was very good at all doses tested. The dose of 2·5 mg daily is the maximum dose tested in the large phase III trials.

Pharmacokinetic studies with oral administration of letrozole have been performed in healthy men and postmenopausal women, and in postmenopausal breast cancer patients. Plasma kinetics of letrozole were characterized by a fast absorption (t_{max} = 1 h) and a rather slow elimination ($t_{1/2}$ = 2 d). The kinetics as expressed by AUC are dose proportional in the range of 0·1–10 mg.[7]

The aims of the present trial were to determine (i) the absolute bioavailability of letrozole, i.e. its bioavailability when given orally as a 2·5 mg tablet in comparison to the same dose given intravenously as a bolus injection, (ii) the pharmacokinetics after a single intravenous 2·5 mg dose, and (iii) the tolerability of single i.v. and p.o. doses of 2·5 mg letrozole.

SUBJECTS AND METHODS

Subjects

The study was performed in healthy postmenopausal women. They were 52–61 years old and their weight was between 51·8 and 78·7 kg. The postmenopausal status of the volunteers was confirmed from their case histories and by determination of estradiol ($<19{\cdot}1\,pg\,mL^{-1}$) and follicle stimulating hormone ($FSH > 40\,IU\,L^{-1}$) before inclusion in the study. A crossover design was chosen. A single dose of 2.5 mg letrozole was selected for intravenous and oral administrations (i.e. one ampoule or one tablet of letrozole).

The study was conducted in accordance with the World Medical Association's Declaration of Helsinki, Venice and Hong Kong amendments 1983 and 1989, and Good Clinical Practice (GCP). The study protocol and the subject informed consent forms had been approved by an ethical review board (ERB). Written informed consent for each subject was obtained prior to initiating any study procedures in accordance with the requirements as defined in the study protocol.

Dosage forms

Treatment M: film-coated tablets containing 2·5 mg letrozole.

Treatment N: ampoules containing 2·5 mg letrozole in 5 mL aqueous injectable solution for i.v. administration.

Study design

Both p.o. and i.v. single doses consisted of 2·5 mg letrozole. The study was an open, single-center, randomized, two-way crossover absolute bioavailability in 12 healthy postmenopausal female volunteers. A washout period of at least 4 weeks' duration was observed between the two treatments.

Both treatments were administered to the overnight fasted subjects between 7 and 8 o'clock in the morning. One tablet containing 2·5 mg letrozole was taken with 200 mL tap water at room temperature. One single i.v. dose of 2.5 mg letrozole was injected over 3 min via an indwelling venous cannula into a peripheral vein of the volunteer resting in supine position. Prior to injection, the contents of an ampoule were diluted with an equal volume of 0·9% NaCl solution, i.e. 2·5 mg letrozole were injected in a total volume of 10 mL. The clock-time at the end of injection was considered as the time of treatment administration. To ensure administration of the whole prescribed dose of letrozole, the cannula was flushed with 2 mL 0·9% NaCl after the injection of the syringe contents.

Potential study participants were included into the study after passing a pre-treatment screen, which was performed up to 2 weeks before administration of

the scheduled dose. Each administration was preceded and followed by blood sampling for analysis of letrozole plasma concentrations and blood and urine sampling for determination of clinical laboratory variables. Moreover, monitoring of blood pressure and of heart rate was performed. Subjects were asked to report on adverse experiences. A final check-up of clinical laboratory parameters was performed 1 week after dosing in the second period of the trial. If the results of this check were in the normal range, the study was concluded for the given subject. For an individual volunteer, the total duration of the study from the initial screen to the final post-treatment check was approximately 8 weeks.

The subjects reported to the trial site in the morning after an overnight fast. From 22:00 h of the preceding day and until 24 h after the dosing, the volunteers were not allowed to drink xanthine containing beverages. After recording the interim history and completion of preparatory measures (insertion of an indwelling cannula and obtaining baseline samples and measurements), the scheduled treatment was taken/administered. Subsequently, serial blood sampling and recording of vital parameters were performed for up to 336 h post-dosing (p.d.). The volunteers used appropriate forms to report all symptoms and/or clinical signs which might have appeared up to 336 h p.d.

For determination of letrozole concentrations in plasma, 5·5 mL venous blood were drawn into heparinized Monovette® tubes at the following periods: predose, 15 and 30 min, and 1, 1·5, 2, 3, 4, 6, 8, 24, 48, 72, 168, 240, and 336 h p.d. with treatment M (p.o. dose); predose, 0, 10, 20, and 30 min, and 1, 1·5, 2, 3, 4, 6, 8, 24, 48, 72, 168, 240, and 336 h p.d. with treatment N (i.v. dose). The blood was centrifuged at 2200 *g* for 5 min at room temperature. The plasma was removed and stored in polypropylene tubes at $-18\,^{\circ}C$ until analysis.

For determination of letrozole and its secondary alcohol metabolite (SAM) (Figure 1) in urine, samples were collected up to 336 h p.d. according to two schemes. During the first 3 d, i.e. up to 72 h p.d., all urine produced was collected in fractions at 0–8, 8–24, 24–48, and 48–72 h p.d. The weight of each fraction was recorded and a 20 mL aliquot retained for determination of letrozole. The remaining volume was rejected. During the sampling period, already voided urine was stored refrigerated at $+2$–$+8\,^{\circ}C$. The volume of the urine was calculated by considering urine density equal to $1\ kgL^{-1}$. On days 4–14, only one sample obtained between two subsequent complete bladder voidings was collected, whereby the urine from the first voiding was rejected and the whole quantity stemming from the second voiding was retained. Each sample was either delivered to the laboratory within 2–4 h after collection or stored deep frozen at $-18\,^{\circ}C$ until delivery.

Analytical method

Plasma and urine concentrations of letrozole were determined using a high-performance liquid chromatography method with fluorimetric detection and

an internal standard, CGP 47645 (Figure 1), which is a structural analog of letrozole.[8] Automated liquid–solid extraction of compounds from plasma and urine was performed on disposable 100 mg C_8 columns using the ASPEC system. The separation was achieved on an ODS Hypersil C_{18} column using acetonitrile–0·01 M phosphate buffer, pH 7, as the mobile phase at a flow rate of 1·5 mL min^{-1}. A fluorescence detector was used for the quantitation. The excitation and emission wavelengths were 230 and 295 nm, respectively. The limit of quantitation (LOQ) of letrozole in plasma and in urine was 1·40 nmol L^{-1} (0·4 ng mL^{-1}). The respective mean recoveries and coefficient of variation (CV) were 96·5% (9·8%) in plasma and 104% (7·7%) in urine. The compounds were well separated from co-extracted endogenous components and no interferences were observed at the retention times of compounds. SAM urine concentrations after enzymatic hydrolysis were determined using a modified high-performance liquid chromatography method with UV detection.[9] The LOQ was 107 nmol L^{-1}.

The concentrations were calculated from letrozole or SAM calibration curves which were obtained by plotting the peak height ratio (letrozole or SAM/IS, internal standard) versus the concentration of letrozole or SAM in the calibration sample. The equation was calculated by the least-squares method using weighted linear regression with a weighting factor of $1/conc^2$. The method was validated during the analysis by running series of drug-free (blank) human plasma or urine samples spiked with known amounts of letrozole and/or SAM. For letrozole in plasma, the validation range was between 1·40 and 70·1 nmol L^{-1} with respective mean recoveries and CV of 101% (16%) and 105% (5%). For letrozole in urine, it was between 1·40 and 140 nmol L^{-1} with respective mean recoveries and CV of 117% (16%) and 101% (6%). For SAM in urine, it was between 107 and 10 700 nmol L^{-1} with respective mean recoveries and CV of 91% (20%) and 96% (8%).

Pharmacokinetic data evaluation and statistical methodology

Plasma concentration–time profiles were generated for letrozole. Concentrations below the LOQ were considered as non-detectable (ND) and were taken as zero in mean values and kinetics calculation. The following letrozole pharmacokinetic parameters were determined from plasma data: C_{max}, highest observed plasma concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, plasma terminal elimination half-life, calculated from the slope of the linear least-squares regression line through the last log-transformed concentration points different from zero; $AUC(t_{last})$, area under the plasma concentration–time curve of letrozole obtained by the linear trapezoidal method, t_{last} being the last time point with a concentration different from zero; AUC, area under the plasma concentration–time curve of letrozole from zero to infinity calculated as $AUC(t_{last}) + C(t_{last})t_{1/2}/0{\cdot}693$; f, representing the fraction of the administered dose systemically available, calculated as $dose_{i.v.}\,AUC_{p.o.}/dose_{p.o.}\,AUC_{i.v.}$;

MRT, mean residence time representing the average duration of persistence of the drug in the body, calculated as AUMC/AUC where AUMC is the area under the first moment curve calculated by the trapezoidal rule up to time t_{last} and then extrapolated from t_{last} to infinity using the formula $C(t_{last})t_{1/2}/0{\cdot}693\,(t_{last}+0.693/t_{1/2})$; total-body clearance (Cl) following i.v. administration and apparent total body clearance (Cl/f) following oral administration of letrozole were calculated as dose/AUC; Cl/BW, total body clearance divided by body weight; V_{ss}, apparent volume of distribution per kilogram body weight at steady state after i.v. administration, calculated as: Cl MRT/body weight = $(AUMC\,dose_{i.v.}/AUC^2_{i.v.})$/body weight.

The parameters determined from urine data for letrozole or total SAM were the following: UE, urinary excretion calculated as the sum of the amount excreted during the first three days and the area under the curve of urinary excretion rate against mid-time-point ($\Delta U/\Delta t$ against t_{mid}) of collection for the fractions ΔU of days 4–14 p.d.; the curve $\Delta U/\Delta t$ against t_{mid} from days 1 to 14 p.d. was also established and excretion half-life was calculated as for $t_{1/2}$ in plasma; UE%, urinary excretion as a percentage of letrozole dose = (UE/dose) × 100; overall excretion = sum of letrozole + total SAM excretion (%); Cl_r, renal clearance of letrozole, Cl_r = UE of letrozole up to infinity/AUC; Cl_{nr}, non-renal clearance of letrozole: $Cl_{nr} = Cl - Cl_r$; $Cl_{nr} = Cl_m$ (metabolic clearance) if other non-renal elimination pathways are negligible (i.e., biliary/fecal).

The log-transformed AUCs for p.o. and i.v. treatments were statistically compared only.[10–15] This was analysed to examine the absolute bioavailability of letrozole by means of a general linear model (GLM procedure of SAS, PC version 6·08). The statistical model included sequence, subject within sequence, period, and treatment effect. Mean difference in log AUC was obtained using the ESTIMATE statement of the GLM procedure. As the ratio of oral to i.v. AUC falls within the 0.8–1·25 90% confidence limits, the two formulations are 'equivalent' in terms of AUC although 'equivalency' testing is not strictly relevant to i.v. formulations. A number of model checking criteria were examined using analysis of residuals before results were interpreted. The checks were performed specifically to test for constant variance in relation to mean and for normality. Other pharmacokinetic parameters were not analysed statistically, but were reported and examined descriptively.

RESULTS

Letrozole and secondary-alcohol-metabolite biological fluid concentrations, pharmacokinetic variables, and statistical results

Letrozole mean concentrations in plasma after both treatments are presented in Figure 2. Within 10 min after i.v. administration, plasma concentration

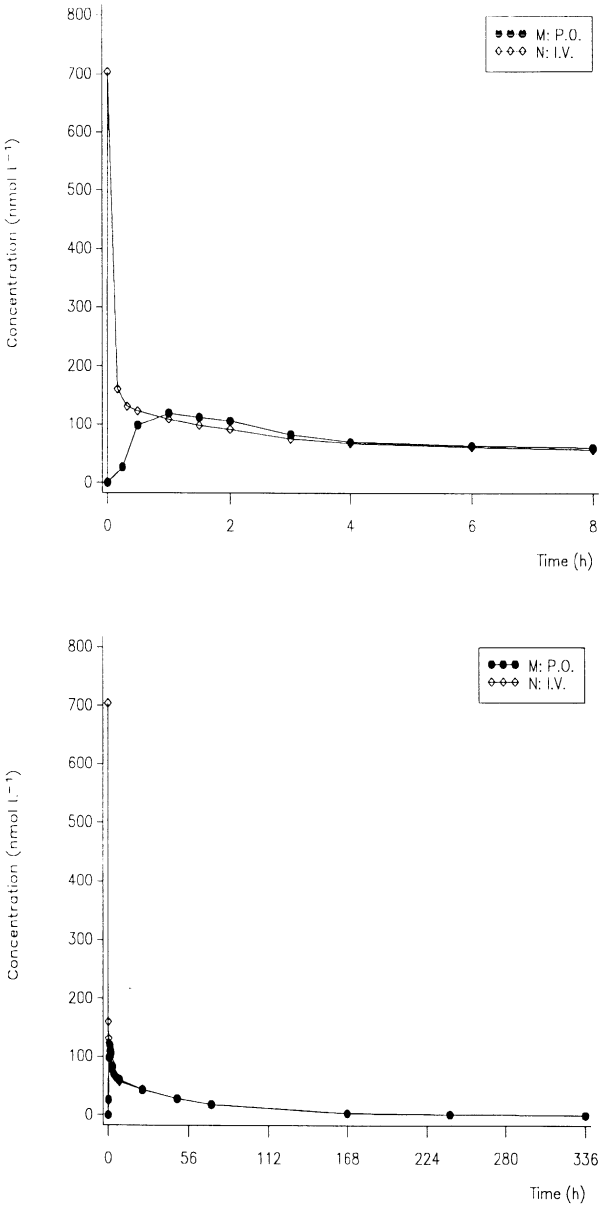


Figure 2. Letrozole mean plasma concentrations (upper graph, time 0–8 h; lower graph, time 0–336 h)

decreased about fourfold. Absorption of letrozole after p.o. administration seems to be rapid as plasma profiles after p.o. and i.v. administration were almost superimposable at 2–4 h after dosing. Maximum plasma concentrations were reached 1 h after p.o. dosing (median).

Mean AUC and $t_{1/2}$ values for letrozole in plasma are summarized in Table 1. They were similar for the two treatments. The inter-subject variability, as reflected by the CV, was approximately 34%.

The fraction, f, of the administered oral dose systemically available was $0{\cdot}999 \pm 0{\cdot}16$, indicating an absolute systemic bioavailability of $99{\cdot}9 \pm 16{\cdot}3\%$ (Table 1). Based on five previous pharmacokinetic studies, it was estimated that 12 subjects will provide more than 80% power and that the 90% confidence interval for the ratio of the two treatments will have limits within $\pm 25\%$ of the true ratio. The estimated ratio was 0·99 for AUC, indicating no substantial difference between the two treatments. The 90% CI of ratio (Table 1), being tight and containing unity, confirms the statistically non-significant difference between p.o. and i.v. as measured by AUC. Therefore a single 2·5mg oral dose of letrozole was completely absorbed from the gastrointestinal tract. The absorption process did not increase the variability of the letrozole plasma kinetics as similar variation coefficients for AUC were observed after p.o. and i.v. administration. The variability in the plasma kinetics seems, therefore, to lie in the variability of the disposition of letrozole. Letrozole kinetics after i.v. bolus administration are summarized in Table 1. The MRTs were similar after p.o. and i.v. administrations indicating a rapid absorption.

The UE from zero to infinity was the sum of UE up to 72h + UE between days 4 and 14 p.d. + UE beyond the last measured fraction on day 14 until infinity. The UE of this last period was considered as negligible. Due to the differences in LOQs, letrozole could be detected in some late urine fractions

Table 1. Mean ($n = 12$) letrozole pharmacokinetic parameters

Parameter	Treatment	Mean	CV (%)	Range
AUC[a] (nmol h L^{-1})	p.o.	4290	34·3	2320–7740
	i.v.	4330	33·6	2540–7960
$t_{1/2}$	p.o.	42·0	36·4	24·3–70·2
	i.v.	45·4	34·9	24·1–75·8
f	i.v.	0·999	16·3	0·739–1·299
Cl/BW (mL $h^{-1}kg^{-1}$)	i.v.	35·0	37·0	16–58
Cl (L h^{-1})	i.v.	2·21	29·5	1·10–3·45
Cl_r (L h^{-1})	i.v.	0·08	24·4	0·04–0·11
Cl_{nr} (L h^{-1})	i.v.	2·13	30·4	1·02–3·37
MRT (h)	i.v.	58·7	35·7	32·6–102
V_{ss} (L kg^{-1})	i.v.	1·87	24·9	1·47–3·24
Letrozole UE %	p.o.	3·69	41·3	1·80–7·21
	i.v.	3·94	36·6	1·99–6·76
Total SAM UE %	p.o.	66·4	17·5	48·4–83·7
	i.v.	60·7	17·6	30·9–74·8
Sum	p.o.	70·1	16·7	53·8–90·9
	i.v.	64·6	14·8	37·7–77·2

[a]DIFF = −0·014; SE = 0·050; Ratio = 0·99; 90% CI of Ratio = 0·90–1·08.

whereas SAM after enzymatic hydrolysis could not. The mean UE% of letrozole and SAM after p.o. and i.v. treatments is summarized in Table 1. Means and ranges for letrozole and SAM were similar in both treatments. The CV (%) was higher with letrozole due to the low amounts excreted.

DISCUSSION

The rapid decrease of letrozole plasma concentration shortly after i.v. bolus administration suggests a fast distribution of the compound. In many subjects, letrozole concentration–time curves showed a plateau to the period 4–8 h (Figure 2) after both p.o. and i.v. administration. In some subjects, multiple peaks were observed up to 8 h after administration. These two particularities might indicate that enterohepatic re-circulation and/or drug distribution/redistribution play a role in the early phase of letrozole disposition. These observations were made also after i.v. administration, thus they seem not to be a consequence of the disintegration of the tablet in the gastrointestinal tract.

Elimination of letrozole from plasma after 4 or 8 h was slow and characterized by a dominant phase with mean half-lives of $42{\cdot}0 \pm 15{\cdot}3$ and $45{\cdot}4 \pm 15{\cdot}9$ h for p.o. and i.v. administration, respectively. The $t_{1/2}$ inter-individual variability was high. This variability is comparable to that of letrozole plasma AUC.

V_{ss} was calculated to be between 1·47 and 3·24 L kg^{-1} in the 12 postmenopausal women. Knowing that letrozole is about equally distributed between erythrocytes and plasma,[16] this result suggests a rather high tissue distribution of the compound.

Total body clearance (Cl) was rather low ($2{\cdot}21 \pm 0{\cdot}65$ L h^{-1}) and not influenced by body weight. The renal clearance, Cl_r, was 0·08 L h^{-1} and contributed only to a small proportion of Cl. The non-renal clearance, Cl_{nr}, was assumed to be mainly metabolic clearance as biliary and fecal excretion of unchanged letrozole represent only insignificant elimination pathways (about 2%).[17]

The estimated metabolic clearance of letrozole (about 2·1 L h^{-1}) was low compared to hepatic blood flow (90 L h^{-1}). *In vitro*, the human plasma protein binding has been shown to be about 60%, determined at protein and drug concentrations that are in the same range as the concentrations in this study.[16] Therefore, letrozole metabolic clearance is predominantly determined by hepatic enzyme activity and not hepatic blood flow. Thus, the relatively slow elimination of letrozole ($t_{1/2} = 2$ d) is mainly due to a low intrinsic metabolic clearance.

The free SAM (Figure 1) was not determined in plasma and urine in this study. Based on previous pharmacokinetic data on file, free SAM was not detected in plasma and urine. Therefore, it is assumed to rapidly undergo glucuronidation.

SAM after hydrolysis was detectable in many subjects only for 144–168 h after dosing and in a few subjects up to 264 h. The urinary excretion of total SAM by these time points amounted to $66{\cdot}4 \pm 11{\cdot}6\%$ of the dose with p.o. and $60{\cdot}7 \pm 10{\cdot}7\%$ of the dose with i.v. administration. The sum of UE% for letrozole and total SAM was comparable for both treatments: around 70% for p.o. and 65% for i.v.

The mean excretion half-life of letrozole ($41{\cdot}5 \pm 18{\cdot}8$ h after p.o. and $39{\cdot}5 \pm 20{\cdot}2$ h after i.v.) correlated well with plasma $t_{1/2}$ ($42{\cdot}0 \pm 15{\cdot}3$ h after p.o. and $45{\cdot}4 \pm 15{\cdot}9$ h after i.v.), as did also the absolute bioavailability calculated from urinary data which was similar to that derived from plasma data, thus denoting coherent pharmacokinetic findings.

Both study treatments were tolerated equally well. Neither of the treatments was associated with changes in clinical laboratory values or vital sign measurements.

REFERENCES

1. A. M. H. Brodie, W. M. Garret, J. R. Hendrickson, C. H. Tsai-Morris and J. G. Williams, Aromatase inhibitors, their pharmacology and applications. *J. Steroid. Biochem.*, **19**, 53–58 (1983).
2. A. M. H. Brodie, Aromatase inhibition and its pharmacologic implications. *Biochem. Pharmacol.*, **34**, 3213–3219 (1985).
3. T. J. Iveson, I. E. Smith, J. Ahern, D. A. Smithers, P. F. Trunet and M. Dowsett, Phase I study of the oral non-steroidal aromatase inhibitor CGS 20267 in healthy postmenopausal women. *J. Clin. Endocrinol. Metab.*, **77**, 324–331 (1993).
4. P. F. Trunet, Ph. Müller, A. S. Bhatnagar, I. Dickes, G. Monnet and G. White, Open dose-finding study of a new potent and selective nonsteroidal aromatase inhibitor, CGS 20267, in healthy male subjects. *J. Clin. Endocrinol. Metab.*, **77**, 319–323 (1993).
5. T. J. Iveson, I. E. Smith, J. Ahern, D. A. Smithers, P. F. Trunet and M. Dowsett, Phase I study of the oral nonsteroidal aromatase inhibitor CGS 20267 in postmenopausal patients with advanced breast cancer. *Cancer Res.*, **53**, 266–270 (1993).
6. A. Lipton, L. M. Demers, H. A. Harvey, K. B. Kambic, H. Grossberg, C. Brady, H. Adlercruetz, P. F. Trunet and R. J. Santen, Letrozole (CGS 20267): a phase I study of new potent oral aromatase inhibitor of breast cancer. *Cancer*, **75**, 2132–2138 (1995).
7. Ch. Pfister, M. Dowsett, T. Iveson, P. Mueller, A. Sioufi and P. F. Trunet, Pharmacokinetics of new aromatase inhibitor CGS 20267. *Eur. J. Drug Metab. Pharmacokinet.*, **18** (suppl.), 117 (1993).
8. F. Marfil, V. Pineau, A. Sioufi and J. Godbillon, High-performance liquid chromatography of the aromatase inhibitor letrozole and its metabolite in biological fluids with automated liquid–solid extraction and fluorescence detection. *J. Chromatogr. Biomed. Appl.*, **683**, 251–258 (1996).
9. Ch. Pfister, M. Duval, J. Godbillon, G. Gosset, D. Gygax, F. Marfil, A. Sioufi and B. Winkler, Development, application and comparison of an enzyme immunoassay and a high-performance liquid chromatography method for the determination of the aromatase inhibitor CGS 20267 in biological fluids. *J. Pharm. Sci.*, **83**, 520–524 (1994).
10. S. R. Searle, *General Linear Models*, Wiley, New York, 1971.
11. D. Mandallaz and J. Mau, Comparison of different methods for decision-making in bioequivalence assessment. *Biometrics*, **37**, 213–222 (1981).
12. H. Fluehler, A. P. Grieve, D. Mandallaz, J. Mau and H. A. Moser, Bayesian approach to bioequivalence assessment: an example. *J. Pharm. Sci.*, **72**, 1178–1181 (1983).
13. Commission of the European Communities (C.E.C.), *Note for Guidance: Investigation of Bioavailability and Bioequivalence*, III/54/89-EN, 1991.

14. D. J. Shuirman, A comparison of the two one sided test procedure and the power approach for assessing the equivalence of average bioavailability. *J. Pharmacokinet. Biopharm.*, **15**, 657–680 (1987).
15. W. J. Westlake, The design and analysis of comparative blood-level trials. In *Current Concepts in the Pharmaceutical Sciences*, J. Swarbrick (Ed.), Lea and Febiger, Philadelphia, PA, 1973.
16. D. Colussi, Personal communication, Laboratoires Ciba–Geigy, F-92506 Rueil-Malmaison, France.
17. F. Waldmeier, Personal communication, Ciba Pharma, CH-4002 Basle, Switzerland.