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A bioavailability study of two preparations of tamoxifen after single doses

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Abstract: The bioavailability of two different tablet formulations of tamoxifen was studied in twelve healthy male volunteers. Two tablets, each of 10 mg, of both preparations were administered orally at intervals of two weeks in a randomized cross-over design. Samples of blood for tamoxifen measurement were taken for up to 48 h following administration. Tamoxifen was measured by an HPLC method sensitive to 2.0 ng ml^{-1}. The area under the concentration–time curve was similar for both preparations. The study, therefore, did not demonstrate any differences between the bioavailability of the two preparations of tamoxifen.

Keywords: *Tamoxifen; pharmacokinetics; bioavailability.*

Introduction

It has only been appreciated relatively recently that differences in the formulation of the same drug can lead to large differences in speed of onset, intensity and duration of drug response [1]. It is therefore important to be aware of these differences when comparing different formulations, both in regard to their pharmacokinetics and to their therapeutic effectiveness. This awareness may be achieved by measuring the comparative bioavailability of the different preparations of the drug under investigation.

Recently a new preparation of tamoxifen has been introduced and, since tamoxifen is widely used in cancer chemotherapy [2], it is important to establish bioavailability. In addition, little is known concerning the pharmacokinetics of tamoxifen. Although values for the elimination half-life have been quoted [3, 4], there are considerable differences between the values, and in no case have the methods of calculation been presented. Since the persistence of tamoxifen in the body is a vital consideration for its therapeutic action, it is important to evaluate the pharmacokinetics of the drug.

The analysis of tamoxifen has been difficult and only a few techniques are available. Recent methods include gas chromatography–mass spectrometry [5], thin layer chromatography with densitometry [6] and high-performance liquid chromatography (HPLC) [7, 8]. All these methods are sensitive to low concentrations of tamoxifen, but because they are time-consuming they are inappropriate for the measurement of large numbers of samples and therefore, the study of the pharmacokinetics of tamoxifen, in

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any great detail. We have modified the HPLC method of Golander and Sternson [7] in order to speed up assay time. The modified procedure has proved eminently suitable for measuring the plasma concentration of tamoxifen in healthy volunteers when assessing the bioavailability of a new preparation of tamoxifen, following single oral doses of 20 mg.

Experimental

Subjects

Twelve healthy male subjects of 18–43 years old were entered into the study and all gave written informed consent. The volunteers were clinically fit and with haematological and biochemical profiles within the normal limits. No subject had a history of evidence of cardiac, renal or hepatic disease, or drug allergy. Pulse rate, blood pressure and ECG showed no abnormality. No medication was taken for one week prior to, or during, the study. Two out of the twelve were smokers but none was graded as a heavy smoker (>15 cigarettes a day).

The protocol for this study was approved by the Ethical Committee of St Luke's Hospital, Guildford.

Study design

The twelve volunteers were allocated at random to a treatment sequence determined by a Latin Square. The two preparations of tamoxifen were Nolvadex® (ICI) and tamoxifen citrate BP (Berk) 10 mg. Two tablets of each preparation were administered two weeks apart.

Procedure

On the night preceeding each study day, subjects fasted for 12 h before drug administration. They were allowed to take water or squash freely up to 2 h post administration. On the study day, the volunteers took the allocated tablet preparation together with 150 ml of water. All preparations were administered at 08.30 h or shortly after. A light lunch was taken 5 h after drug administration.

Before each dose and at 1, 2, 3, 4, 5, 6, 8, 10, 24, 32 and 48 h after taking the drug, blood samples (10 ml) were collected into lithium-heparin tubes from venepunctures from the antecubital vein. The plasma was separated immediately and stored at −20°C until assayed.

Assay

Plasma samples were assayed using a modification of the fluorimetric HPLC method of Golander and Sternson [7].

Portions (1 ml) of all samples were mixed with 0.5 ml of 1 M ammonium hydroxide and extracted with 10 ml diethyl ether. The ether layer was evaporated to dryness and the residue reconstituted in 300 μl mobile phase, which was then irradiated at 254 nm for exactly 5 min using a bactericidal ultraviolet lamp (Original Hanau). A 100 μl sample of the irradiated samples was then chromatographed using a 10 cm × 5 mm 5 μm spherisorb ODS column with a mobile phase of methanol–water–glacial acetic acid (80:20:0.5, v/v/v) containing 25 mM sodium pentane sulphonate. Drug-free plasma, spiked with known concentrations of tamoxifen, was used to calibrate each batch of analyses.

Calculation of pharmacokinetic parameters

The observed maximum plasma concentrations following each dose for each subject were used as peak plasma concentrations. The observed time at which the tamoxifen concentration curve for each subject reached its maximum was taken as time-to-peak. The area under the plasma concentration–time curve to 48 h (AUC_{48}) was calculated by the trapezoidal rule [9] and for each subject the ratios of the AUC_{48} for both drugs were calculated. The average values of the ratios were taken as a measure of the relative bioavailability [10]. Additional pharmacokinetic parameters, absorption rate, elimination rate and AUC to infinity (AUC_{∞}) were calculated by a modification of the NONLIN program [11].

Statistical analysis

Drug concentrations at each sampling time, peak concentrations, rate of absorption and AUC_{48} were compared for both formulations using analysis of variance for a crossover design. Wilcoxon matched-pairs signed-ranks test [12] was used to test for significant differences between pairs of treatment mean values.

Results

The exact duration of UV irradiation required to produce a quantitative yield of phenanthrene depends upon lamp intensity, distance of sample from source and solvent used. It was found that the conditions we used gave a quantitative yield within 5 min. Standard solutions of tamoxifen in mobile phase were used to check the precision of the derivatization step. The irradiation step was found to have a coefficient of variation (CV) of 2.7% within batch and 3.7% between batches.

Using the HPLC conditions described earlier, tamoxifen chromatographs with a retention time of 8 min. The major metabolite, *N*-desmethyl tamoxifen, has a retention time of 3 min and therefore is well resolved. No peaks that would interfere with the tamoxifen assay were found in any predose samples. Specimen chromatograms are shown in Fig. 1.

Calibration standards were assayed with each batch of unknown samples. The correlation coefficients of standard curves were always >0.990. Recovery of added drug to drug-free plasma over the concentration range 25–150 ng ml^{-1} was 108.7 with a CV of 9.4%. As a check of assay variation from day to day, approximately 30% of the samples were assayed on two different days. These duplicate assays gave a mean percentage difference of 17.9%. Samples and duplicates were all assayed at random.

The mean tamoxifen plasma concentrations and the standard error of the mean (S.E.M.) for both preparations are illustrated in Table 1. Comparison of the concentrations at each sampling point revealed no significant difference between treatments. A pharmacokinetic analysis of the data revealed that the concentration–time profile of tamoxifen could be described by an open two-compartment model, where the mean distribution and elimination half-lives for the Berk preparation were 2.3 and 49.2 h and for the ICI preparation 2.4 and 46.9 h respectively. The mean time-to-peak was 5.8 and 5.3 h for the ICI and Berk preparation respectively, although the range of values (3–10 h) was large. There was no difference in the rates of absorption of the two preparations (Table 2).

Mean estimates of AUC_{48} are shown in Table 3. Analysis of AUC_{48} data did not indicate any significant difference between the two treatments. Table 3 also shows the

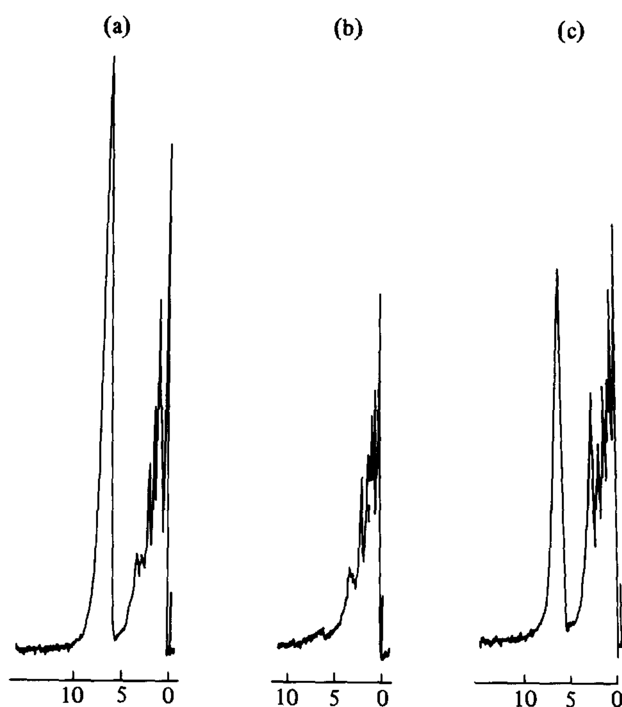

Figure 1
Specimen HPLC chromatograms for tamoxifen. (A) Tamoxifen spiked into drug free plasma (60 ng ml^{-1}); (B) drug-free plasma; (C) plasma from volunteer after tamoxifen dose. Flow rate 3.0 ml min^{-1}, detection fluorescence, excitation 256 nm, with a 320 nm cut-off filter. Other conditions as in text.

Table 1
Mean tamoxifen plasma concentrations (ng ml^{-1} ± S.E.) in 12 healthy volunteers receiving 20 mg tamoxifen in two different formulations

Time (h)	Berk (ng ml^{-1})	ICI (ng ml^{-1})
0	1.1 ± 0.4	1.2 ± 0.5
1	9.8 ± 1.5	8.8 ± 2.4
2	16.2 ± 1.3	15.0 ± 2.5
3	29.3 ± 2.7	24.0 ± 3.3
4	30.3 ± 2.8	28.1 ± 3.4
5	29.9 ± 2.1	30.9 ± 2.8
6	30.2 ± 2.6	27.1 ± 2.1
8	25.9 ± 2.2	25.1 ± 2.5
10	23.7 ± 2.0	22.0 ± 1.4
24	14.5 ± 1.4	14.2 ± 1.1
32	12.9 ± 1.5	12.7 ± 2.0
48	9.9 ± 1.2	10.5 ± 1.2

Table 2
Pharmacokinetic parameters of tamoxifen following oral administration of 20 mg preparation A (Nolvadex®) and preparation B (Berk) in 12 healthy volunteers

Tablet preparation	C_{max} (ng ml^{-1})	t_{max} (h)	Half-life: Absorption (h)	Half-life: Distribution (h)	Half-life: Elimination (h)	Oral* clearance (ml min^{-1})
A	34.2 ± 3.4	5.3	1.1 ± 0.2	2.3 ± 0.6	49.2 ± 17.5	88.6 ± 7.1
B	34.8 ± 2.2	5.8	1.1 ± 0.2	2.4 ± 0.8	46.9 ± 11.4	88.1 ± 7.2

*Clearance calculated assuming that oral bioavailability is 100% in the absence of any comparative intravenous data.

Table 3
Area under the curve (AUC) from zero to 48 h and relative bioavailability of two preparations of tamoxifen following oral administration

Subjects	AUC_{48} (Berk) (ng ml^{-1} h^{-1})	AUC_{48} (ICI) (ng ml^{-1} h^{-1})	Relative bioavailability
A	817.1	645.2	126.2
B	763.7	758.9	100.6
C	700.1	766.2	91.4
D	854.9	960.6	90.0
E	1103.8	908.9	121.4
F	596.1	629.7	94.7
M	966.3	731.7	132.1
J	915.4	724.3	114.5
H	1145.8	1267.8	90.6
G	496.4	569.6	88.0
L	668.3	839.0	80.0
K	598.5	454.8	124.6
Mean	792.8	771.1	104.5
95% confidence limits of the mean	—	—	93.0–115.0

relative bioavailability of the two formulations, calculated from the ratio of the AUC_{48} for each subject. Ratio values (×100) for each subject, together with the mean, and 95% confidence limits are shown.

Discussion

The tamoxifen plasma concentration profile over 48 h following an oral dose of 20 mg was described by an open two-compartment model. The absorption half-life was reasonably slow with an overall mean of 1.1 h. Peak concentrations occurred between 3 and 10 h. A distribution phase was detected, and the mean elimination half-life was calculated to be about 48 h. Other values for the elimination half-life of tamoxifen quoted in the literature vary. Thus, Adams *et al.* [3] have quoted three different values; namely, 90, 96 and 156 h for each of three studies carried out successively at 4-week intervals, indicating an increase in half-life with successive doses, although no details of calculations were presented. Fabian *et al.* [4] have quoted the elimination half-life to be between 9 and 12 h following single oral doses. However, following multiple dose

therapy, the elimination half-life was calculated to be between 3 and 21 days after stopping the drug. Again, no details of calculation were presented. In the present study measurements of tamoxifen were made for only 48 h. This may have been an inadequate time to obtain a true estimate of the half-life, although in most cases the difference between the observed values and the expected values during the elimination phase was small ($<10\%$). This would indicate a good fit of the data.

It was noted that the elimination half-life varied considerably, presumably due to variations in the hepatic clearance of tamoxifen. A number of metabolites have been identified which are mostly observed during chronic treatment or with large doses [13, 14]. Some of these have antioestrogenic activity and this may be important during long-term therapy, but would be unlikely to influence the conclusions of this single dose study. The fact that there is a wide variation in clearance would suggest the need to monitor tamoxifen concentrations in patients who do not seem to respond to treatment.

The study compared bioavailability by comparing the AUCs of the two preparations over 48 h. There was no significant difference between the two preparations. When the values for the AUC_∞ were calculated, the results were 1186.1 ± 94.7 and 1426.3 ± 209.0 ng ml^{-1} h^{-1} for the ICI and Berk preparations respectively, again with no statistically significant difference between the two preparations.

The analysis of tamoxifen has been difficult. Recent methods have employed different techniques [5, 6, 8] but most require that tamoxifen be converted, prior to chromatography, to a fluorescent phenanthrene derivative which then can be measured spectrofluorometrically. Our modification of the methodology which involves a single extraction of drug from plasma, a short irradiation time, and more ion pair reagent has enabled a sensitivity of 2 ng ml^{-1} to be reached, with good recoveries. It has also resulted in a considerable speeding up in the assay, making the assay suitable for pharmacokinetic analyses.

Tamoxifen is a potent drug used in the management of breast cancer. In such cases, it is important to ensure accuracy of dosage between different preparations. This study has not demonstrated any difference in bioavailability between the two preparations studied.

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