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Comparison of bioavailability in man of tamoxifen after oral and rectal administration

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The bioavailability of tamoxifen from 40 mg suppositories was tested in six male volunteers and compared with that of tamoxifen (Nolvadex) tablets. Plasma concentrations of tamoxifen and its major metabolites, 4-hydroxytamoxifen and *N*-desmethyltamoxifen, were measured by extraction from plasma obtained at different times after administration, separated by HPLC, converted on-line to fluorescent phenanthrene derivatives and quantified with a fluorescence detector. The mean relative bioavailability from the suppositories was 28%; the addition of a surfactive agent diminished the bioavailability to 13%. Simulation of repeated administration of 40 mg suppositories suggests a mean steady state plasma concentration for tamoxifen of approximately 70 ng ml^{-1}, i.e. 30% of the steady state value after simulated oral administration. Rectal administration of tamoxifen leads to a lower bioavailability than that by oral administration and therefore cannot be recommended when used in equivalent doses.

Tamoxifen (ICI 46,474; Nolvadex) is widely used in the management of breast cancer, both for the advanced disease and as an adjuvant to traditional locoregional therapies of stage I and II disease. Recently it was decided that tamoxifen could be considered as a treatment of choice in the group of postmenopausal women with positive lymph nodes and positive hormone receptor levels (Anonymous 1985).

In general, tamoxifen has been a safe and well-tolerated drug, particularly when considered in the context of other available therapeutic alternatives. Of 2232 patients on whom toxicity information was available, 96% were able to tolerate their entire course of therapy at the original dose (Lippman 1983). The most common side-effects are mild nausea and vomiting occurring in approximately 10% of patients (Heel et al 1978) and hot flushes. A possible alternative for patients, who do not tolerate tamoxifen administered by the oral route would be a rectal suppository containing tamoxifen.

The present study was designed to establish the bioavailability of tamoxifen after administration as a single dose of 40 mg in fatty rectal suppositories, in order to develop an alternative dosage form for this drug. Since tamoxifen rapidly converts in aqueous solutions to its *cis*-isomer, which contains oestrogenic properties, no aqueous enema can be developed.

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MATERIALS AND METHODS

Solvents and reagents

Solvents were of Lichrosolv or Uvasol grade (Merck Darmstadt, FRG).

Tamoxifen, *N*-desmethyltamoxifen (ICI 55,548), and 4′-monohydroxytamoxifen were gifts from ICI (Pharmaceutical Division Rotterdam, The Netherlands); [^{3}H]tamoxifen (3 TBq $mmol^{-1}$) was purchased from New England Nuclear, Dreieich, FRG. Stock standard solutions were prepared in methanol, and kept refrigerated in the dark. Working standards were prepared by dilution of the stock solution in the chromatographic solvent.

Dosage forms

Tablets, containing 20 mg tamoxifen as tamoxifen citrate (Nolvadex-20, batch 83119), were kindly supplied by ICI (Pharmaceutical Division Rotterdam, The Netherlands).

Suppositories were prepared with the fatty bases Witepsol H15 (Dynamit Nobel Troisdorf, FRG) and Suppocire AML (Gattefossé Saint-Priest, France). Tamoxifen tablets were ground in a mortar and sieved through gauze 160 μm. Suppositories were prepared by mixing the sieved powder with molten suppository base at approximately 40 °C, and adding sufficient base to give 40 mg tamoxifen per suppository poured into moulds of 2·8 ml. The suppositories were kept cool and in the dark. They contained 40 mg tamoxifen (CV < 2%; n = 5); the amount of *cis*-tamoxifen was not increased compared with the tablets.

In-vitro release profile

The release profile of tamoxifen in-vitro was determined for suppositories prepared with Witepsol H15. A sedimentation release apparatus was used (de Blaey & Rutten-Kingma 1977) with 1000 ml demineralized water as release medium. At regular intervals (see Fig. 2) a 5 ml sample of the release medium was removed and measured spectrophotometrically at λ = 237 nm. The release from Suppocire AML suppositories could not be determined spectrophotometrically, since lecithin interfered with the assay.

Solubility of tamoxifen citrate

The solubility of tamoxifen citrate in increasing amounts of NaCl (0–0·9% m/v) was determined by shaking an excess amount of the compound with varying concentrations of sodium chloride at 37 °C in the dark. After dilution of the supernatant the amount of tamoxifen dissolved was calculated.

Bioavailability trial

Six healthy, male volunteers (age 23–30 years) had normal haematological and biochemical parameters. The experiment was approved by the local ethical committee. All volunteers gave written informed consent.

The three dosage forms, tablets and two suppositories, were administered to the volunteers in a randomized sequence with intervals of 4 weeks. In each session the equivalence of 40 mg tamoxifen (2 tablets or 1 suppository) was administered.

The tablets were swallowed with 250 ml of water on an empty stomach; the suppositories were administered after evacuation of the bowel. No food or drink was allowed until 3 h after ingestion (t = 3), when drink was freely allowed with the exception of coffee. No defaecation occurred within 6 h after administration of the suppository.

Blood samples were drawn on every first day of drug administration from a Teflon cannula (Abbocath-T 18G, Abbott Ireland Ltd Sligo, Ireland), inserted in a forearm vein and collected in heparinized polycarbonate tubes. On the following days the samples were taken by venepuncture. The samples were centrifuged within 2 h of sampling, and the plasma separated and stored at −20 °C until analysis. The sampling schedule was as follows: 0 (or just before administration of the dosage form), 1, 2, 3, 4, 5, 6, 8, 12, 24, 72, 168, 336 and 672 h after administration.

The relative bioavailability of the rectal dosage forms compared with the oral tablets was calculated as the ratio of the area under the plasma concentration-time curves (AUC; units: nmol h ml^{-1}) for tamoxifen after rectal and oral administration. The AUCs were calculated using the logarithmic trapezoidal method (Chiou 1978) until 672 h after administration. The major metabolite, *N*-desmethyltamoxifen, has similar binding affinity to the oestrogen receptor in human mammary cancer tissue (Furr & Jordan 1984). However, in the calculation of the bioavailability this compound was not taken into account. Theoretical steady state levels after repeated dosing were estimated according to:

$$Css = AUC/\tau \quad (1)$$

in which τ is the dosing interval in hours.

For statistical testing the 95% confidence intervals of the relative bioavailabilities were calculated.

Sample preparation

The complete procedure of extraction was performed at 0 °C in an icebath. All manipulations were carried out in subdued light to avoid degradation of the analytes. To 1·0 ml plasma samples, 50 µl of a solution of [^{3}H]tamoxifen containing 4000 Bq/50 µl was added, mixed and allowed to stand for at least 10 min before extraction; the added amount of [^{3}H]tamoxifen was undetectable by the fluorescence detector. The samples were mixed on a vortex mixer three times with 10 ml of a mixture of hexane–butanol (98 : 2) for approximately 3 s and centrifuged for 15 min. After freezing the aqueous phase, the supernatant was decanted into 12 ml glass tubes and evaporated at ≈40 °C under a stream of nitrogen. The dried samples were redissolved in 100 µl of HPLC solvent; 20 µl were used for injection and the radioactivity in another 20 µl was used to compensate the extraction efficacy.

When the procedure was not at 0 °C or was interrupted for one or more days after the extraction and evaporation step, a second peak appeared in the chromatogram close to tamoxifen; the nature of this degradation product is not known.

Apparatus and chromatographic conditions

The procedure for the assay of tamoxifen and its metabolites was based on that published by Brown et al (1983) which in our hands did not give a sufficient separation of tamoxifen and *N*-desmethyltamoxifen.

A Waters 6000A Solvent Delivery System and a Waters U6K Injection System (Waters-Millipore Etten-Leur, The Netherlands) were used. The solvent was a mixture of acetonitrile and water (35 : 65) with 0·01 M perchloric acid; the flow rate was

2·0 ml min^{-1}. The stationary phase was a reversed phase resin (PRP-1, Hamilton Reno, USA; 150 × 4·1 mm); the column was kept at a constant temperature of 50 °C. From the column the flow passed through a fluorescence activator, to convert the triphenylethylene nucleus to fluorescent phenanthrenes. Teflon tubing (approximately 80 cm × 0·3 mm i.d.; Rubber Hilversum, The Netherlands) was interposed at approximately 4 cm between four mercury UV lamps (Sylvania G8T5). The outflowing stream from this activator was passed through a filter fluorescence detector (LS-1, Perkin Elmer, Beaconsfield, UK) fitted with a 260 nm excitation filter and an emission wavelength set at 390 nm. The fluorimeter signal was passed through a recording integrator (3390 A, Hewlett-Packard, Palo Alto, USA) which recorded peak heights and retention times.

Concentrations of the samples were calculated after comparison of the peak heights with similarly treated standards. The results were corrected for the extraction efficacy after counting the disintegrations per minute in the samples as described before under Sample preparation.

RESULTS

Chromatographic analysis

With the analytical method described, a separation of tamoxifen and its two major metabolites, *N*-desmethyltamoxifen and 4′-monohydroxytamoxifen was achieved, which was superior to that of Brown et al (1983). The detection limit for the three compounds was 2 ng ml^{-1}. An intra-assay variance of 6·1% for tamoxifen was obtained (n = 10); the recovery of tamoxifen was calculated as 79 ± 4% (s.d.; n = 10).

Based on the low variance it can be concluded that [^{3}H]tamoxifen as an internal standard might be omitted without problems.

Release in-vitro

The suppositories released their active content in-vitro quickly but incompletely: (⩾65%) after approximately 1 h (Fig. 1). The reason for this incomplete solubility might be the lipophilic character of the drug. The solubility of tamoxifen citrate is such to allow complete dissolution (≈300 mg litre^{-1} in water at 20 °C). The release of tamoxifen from suppositories was sufficient to warrant the bioavailability study.

Solubility of tamoxifen citrate

The solubility of tamoxifen-citrate decreased dramatically with increasing amounts of sodium chloride

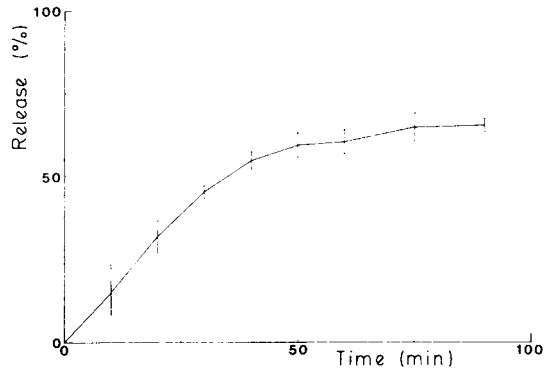

FIG. 1. In-vitro release to demineralized water of tamoxifen from fatty suppositories, prepared with Witepsol H15. The results are means of 4 determinations (± s.d.).

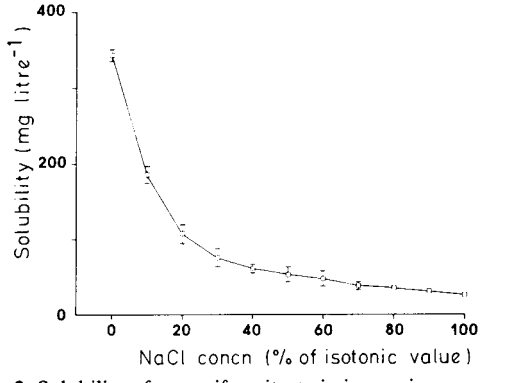

FIG. 2. Solubility of tamoxifen citrate in increasing concentrations of sodium chloride; 100% = isotonic solution of 0·9% m/v NaCl (n = 3; ± s.d.).

(Fig. 2). The solubility in 0·9% NaCl is 26 ± 2 mg litre^{-1} compared with 344 ± 8 mg litre^{-1} in demineralized water at 37 °C.

Bioavailability

After oral administration of tamoxifen, measurable concentrations of drug and *N*-desmethyltamoxifen were detected immediately. No other metabolites were found in this single dose study. For tamoxifen in the six volunteers a mean level of 82 ng ml^{-1} was achieved (95% confidence interval between 64 and 100 ng ml^{-1}), in a mean peak-concentration time of 4·5 h (intervals: 3·5–5·5 h). The elimination of tamoxifen appeared to be biphasic (Fig. 3a), with an initial elimination or distribution rate whose apparent half life in approximately 10 h–only the data points in the first 80 h after ingestion are shown in this graph to make the absorption and redistribution

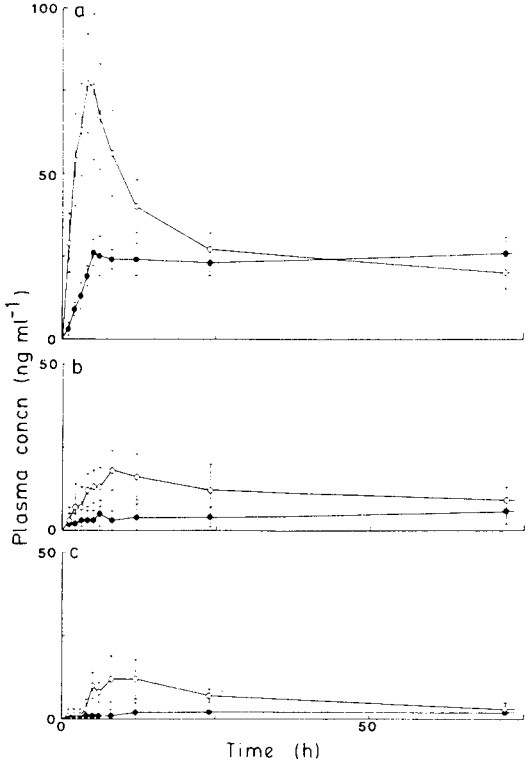

FIG. 3. Mean plasma curves of six volunteers. a = tablet, b = Witepsol H15 suppository, c = Suppocire AML suppository (n = 6, ± s.d.); ○ = tamoxifen, ● = *N*-desmethyltamoxifen.

phase discernable. The terminal elimination phase showed an elimination half-life of approximately (130 h).

After rectal dosing, no distinct maximum concentration of tamoxifen in the plasma was reached, but in most cases a mean plateau of approximately 15 ng ml^{-1} during many hours was achieved after administration of a Witepsol suppository (Fig. 3b). None of the volunteers defaecated within 6 h after suppository insertion.

The relative bioavailability of the rectal dosage form is expressed as the ratio of the AUCs after rectal and oral administration, and is given in Table 1. For Witepsol, the 95% confidence interval of the relative bioavailability is between 0·17 and 0·39, indicating that compared with oral tablets 20–40% is absorbed and delivered to the systemic circulation as the parent compound.

The powdered tablets were also incorporated in Suppocire AML, a suppository base which contains 2% lecithin, added to improve viscosity properties of the mixture and the spreading of the molten suppository. The plasma concentrations were even lower than after Witepsol suppositories (Fig. 3c), resulting in a calculated relative bioavailability of 13% (95% confidence interval between 9 and 17%; significantly different from Witepsol H15).

Table 1. Bioavailability parameters of tamoxifen and *N*-desmethyltamoxifen after oral and rectal administration to six male volunteers.

Volunteer		T	r	D
1	t	8·22		19·78
	w	2·69	0·33	4·67
	s	0·99	0·12	2·45
2	t	13·23		36·47
	w	2·82	0·21	0·91
	s	2·01	0·15	2·46
3	t	20·33		19·28
	w	5·30	0·26	3·21
	s	2·96	0·15	1·51
4	t	20·59		37·27
	w	8·11	0·39	12·53
	s	3·71	0·18	6·83
5	t	16·69		31·18
	w	1·64	0·10	3·47
	s	2·05	0·12	5·44
6	t	16·06		27·19
	w	6·02	0·37	7·30
	s	0·95	0·06	2·69
Mean (±s.d.)	t	15·85 (4·65)		28·53 (7·88)
	w	4·43 (2·46)	0·28 (0·11)	5·35 (4·09)
	s	2·11 (1·09)	0·13 (0·04)	3·56 (2·08)

t = tablet; w = suppository with Witepsol H15; s = suppository with Suppocire AML; T = AUC for tamoxifen (until 672 h after ingestion; nmol h ml^{-1}); D = AUC for *N*-desmethyltamoxifen (until 672 h after ingestion; nmol h ml^{-1}); r = relative bioavailability of tamoxifen (AUC supp/AUC tablet).

DISCUSSION

In this study an adaptation is proposed of a known HPLC method for the determination of tamoxifen and its major metabolites in human plasma. With a reversed phase resin PRP-1 an improvement in separation was achieved between tamoxifen and *N*-desmethyltamoxifen. The application of small bore Teflon tubing for post-column fluorescence activation is much less complicated than the use of an analytical bore quartz capillary column as proposed by Brown et al (1983), since such a quartz column is difficult to acquire. Moreover, the interconnection between HPLC-lining and a Teflon tubing is not complicated, in contrast with glass-steel connections.

Tamoxifen absorption from tablets was slow, despite fast dissolution of the tablets; the maximum plasma concentration was reached after a mean peak value time of 4·5 h. This slow rise of plasma tamoxifen suggests a slow overall process of tablet

disintegration, and dissolution and/or absorption. The solubility of tamoxifen in pure water is such that complete dissolution occurs in 250 ml of water. However, the solubility of tamoxifen in water decreases dramatically with increasing ion concentration from 340 in water to 26 mg litre^{-1} in isotonic salt solution. It might be possible that a lower solubility in gastric and intestinal mucus decreases the absorption rate of the drug substantially.

Another explanation for the retarded absorption might be a direct interaction between tamoxifen and the mucus of the intestine. Tamoxifen and the related anti-oestrogenic compound, clomiphene, have a direct effect on the cervical mucus, reducing its quantity and increasing its viscosity (Elstein & Fawcett 1984). Thus tamoxifen might be able to counteract its own absorption by increasing the viscosity of intestinal mucus.

The prepared suppositories released their active content in-vitro to an extent of 65% within 1 h. In-vivo however, the bioavailability was inferior to the tablets. The low relative bioavailability of the suppositories might be caused by a combination of factors: the small amount of rectal fluid ($\approx$3 ml), and consequently a small area for absorption, offers a poor condition for fast absorption. Moreover, the dramatically decreased solubility of tamoxifen in salt solutions, such as in an iso-osmotic environment like the rectal mucosa, might decrease the release rate of the drug from the suppository in a major way.

Despite the fact that no relation has been found between plasma values and clinical effect, the rectal administration of tamoxifen to breast cancer patients in fatty suppositories cannot be recommended without further investigating the cause of the discrepancy between the bioavailability after oral versus rectal administration. Since it is hypothesized that the low solubility of tamoxifen in the rectal mucus might be at least one cause for the low absorption rate, its incorporation in another delivery form is not likely to improve the absorption.

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