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Note

Simultaneous permeation of tamoxifen and γ linolenic acid across excised human skin. Further evidence of the permeation of solvated complexes

Clare Karia [a], John L. Harwood [b], Andy P. Morris [a], Charles M. Heard [a,*]

[a] *Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3XF, UK*
[b] *School of Biosciences, Cardiff University, Cardiff CF10 3SU, UK*

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Abstract

Tamoxifen is the hormonal treatment of choice in women who have hormone-dependent breast cancer and its efficacy in those women considered to have a high risk of developing breast cancer, has also been established. γ linolenic acid (GLA) has been shown to decrease the invasion of breast cancer and recent studies have demonstrated that GLA can enhance the oestrogen receptor down-regulation induced by tamoxifen. However, tamoxifen is associated with serious side-effects due mainly to systemic delivery, and targeted delivery of both tamoxifen and GLA would be highly beneficial. This work was a preliminary study for the development of a transcutaneous system to simultaneously deliver both tamoxifen and GLA directly to the breast. Full thickness human skin was dosed with 500 μl saturated solution of tamoxifen in borage oil (25% GLA) and the simultaneous permeation of the two actives determined. There was rapid flux with minimal lag time, the cumulative permeation at 24 h was $764.3 \pm 94.2\ \mu g\ cm^{-2}$ for GLA and $5.44 \pm 0.67\ \mu g\ cm^{-2}$ for tamoxifen: the latter being comparable to the amount of tamoxifen associated with cancerous breast tissue from a 20 mg oral dose. The ratio of GLA/tamoxifen permeated at different timepoints was quite consistent, both in terms of mass (mean 138, S.D. 15.1) and mols (mean 184, S.D. 20.3). It was determined that 2.5 molecules of GLA were associated with each molecule of tamoxifen in the permeation process, equating to a solvation cage of three molecules of triacylglycerol. This study has demonstrated the feasibility of administering simultaneously tamoxifen and GLA using borage oil as vehicle, which warrants further investigation as a novel topical two-component system in relation to or prophylaxis of those perceived at high risk of developing breast cancer. The study also provides further evidence of the permeation of solvated complexes across skin, rather than discrete penetrant molecules.
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1. Introduction

Tamoxifen is the hormonal treatment of choice in women who have hormone-dependent breast cancer. In addition, its efficacy in those women considered to have a high risk of developing breast cancer, has recently demonstrated its prophylactic value (Cuzick et al., 2003). The $n-6$ polyunsaturated fatty acid γ linolenic acid (GLA) has been shown to decrease the invasion of breast cancer tumours whilst in animal studies it has been shown to reduce tumour growth. It has also been shown that, unlike other essential

* Corresponding author. Tel.: +44-29-2087-5819; fax: +44-29-2087-5819.
E-mail address: heard1@cf.ac.uk (C.M. Heard).

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fatty acids GLA is selective in exerting its anti-cancer actions and, under comparable conditions, is more toxic to cancer cells than to normal cells (Jiang et al., 1998; Kenny et al., 2001). Two studies have demonstrated that GLA can enhance the oestrogen receptor (ER) down-regulation induced by tamoxifen and thereby provide a valuable addition to current treatment (Kenny et al., 2000, 2001). Orally administered tamoxifen is known to be associated with side-effects including hot flushes, and the more serious issues of endometrial cancer due to oestrogen effects and liver cancer due to the formation of DNA adducts. The wide distribution of tamoxifen and GLA also potentially decreases their therapeutic efficacies at the tumour site. Alternative, non-systemic delivery may reduce the incidence of these side-effects and deliver treatment more directly to the site of action. This work is concerned with development of a transcutaneous delivery system (i.e. across entire skin to the target underlying tissue) for the delivery of both tamoxifen and GLA directly to the breast.

2. Materials and methods

2.1. Materials

Tamoxifen (99%), borage oil and butylated hydroxyanisole (BHA) (99%) were obtained from Sigma Chemical Co., Poole, UK. Trifluoroacetic acid (TFA) was obtained from Aldrich, Gillingham, UK. HPLC grade chloroform, methanol and analytical grade diethyl ether were obtained from Fisher, Loughborough, UK. Myristyltrimethylammonium bromide (Cetrimide) (99%) was obtained from Acros Organics, Geel, Belgium. Female abdominal skin was obtained post-cosmetic surgery and was stored at −20 °C prior to use.

2.2. Skin permeation experiments

Using a scalpel, subcutaneous fat was removed from the thawed skin and then cut into 2 cm^2 sections, before being mounted in all-glass Franz-type diffusion cells. The receptor chambers were filled with degassed receptor phase (30 mg ml^{-1} Cetrimide + 0.05% BHA) (Maguire et al., 2002) and were constantly agitated by magnetic stirrer bars. Complete cells, maintained at 32 °C in a water bath, were then dosed with 500 μl of saturated solution of tamoxifen in borage oil prepared at 32 °C and the donor phases occluded. At appropriate timepoints, the receptor phase was removed using a tube-tipped syringe and transferred to a glass vial. The receptor chambers were immediately replenished with pre-warmed receptor solution. Replication was $n = 10$. A 400 μl aliquot of each sample was transferred to an autosampler vial for analysis of tamoxifen and a 1 ml aliquot was transferred to a second vial for the analysis of GLA. Samples were stored at −20 °C until required for analysis.

2.3. Determination of tamoxifen by high performance liquid chromatography

Samples were analysed on a Hewlett-Packard 1100 automated system fitted with a Kingsorb C18 250 mm × 4.6 mm 5μ column (Phenomenex, Macclesfield, UK). The mobile phase was 75:25:0.1 methanol:water:TFA, pumped at 0.6 ml min^{-1}. A 100 μl sample was injected and tamoxifen detected at 244 nm. The retention time for tamoxifen was 12.2 min, the peak being fully baseline resolved from BHA anti-oxidant. The calibration curve was linear ($R^2 = 1$) over the range 0.2–10 μg ml^{-1} using standards prepared in receptor phase.

2.4. Determination of GLA by gas chromatography

Receptor phase samples obtained from the skin permeation experiments were pooled in pairs to increase sensitivity, and the lipids extracted with diethyl ether before conversion to fatty acid methyl esters (FAMEs) by acid-catalysed methanolysis. This extraction was found to be quantitative for borage oil and for non-esterified fatty acids contained or liberated by hydrolysis. FAMEs were analysed using an Autosystem XL gas chromatograph (Perkin-Elmer Instruments, Beaconsfield, UK) using the general method described by Curtis et al. (2000). Quantitation was made with Autosystem software using a pentadecanoate internal standard.

3. Results and discussion

Permeation data for both tamoxifen and GLA provided in Table 1, cumulative permeation are plotted

Table 1
Cumulative permeation data for the simultaneous permeation of tamoxifen and GLA across human skin from saturated solution of tamoxifen in borage oil ($n = 10$, ±S.E.M.)

Time (h)	GLA ($\mu g\,cm^{-2}$)	Tamoxifen ($\mu g\,cm^{-2}$)	GLA/Tam ($\mu g\,\mu g^{-1}$)	GLA ($\mu mol\,cm^{-2}$)	Tamoxifen ($nmol\,cm^{-2}$)	GLA/Tam ($mol\,mol^{-1}$)
3	200.4 ± 26.4	1.57 ± 0.2	127.6	0.72 ± 0.01	4.22 ± 0.54	170.6
6	492.4 ± 60.6	3.11 ± 0.32	158.3	1.77 ± 0.22	8.37 ± 0.86	211.5
12	566.6 ± 62.3	4.52 ± 0.6	125.4	2.04 ± 0.22	12.2 ± 1.61	167.2
24	764.3 ± 94.2	5.44 ± 0.67	140.5	2.75 ± 0.34	14.6 ± 1.80	188.4

in Fig. 1. In both cases, there was a concomitant flux of the two actives with steady state occurring up to 6 h, after this time the permeation rate decreased. The probable explanation for this was saturation of the (predominantly aqueous) receptor phase with tamoxifen (log *P* 7.9) (El-Kattan et al., 2001), GLA and other components of the oil. Lag times were surprisingly short—so short as to make determination impossible.

The cumulative permeation (mean ± S.E.M.) at 24 h was 764.3 ± 94.2 $\mu g\,cm^{-2}$ for GLA and 5.44 ± 0.67 $\mu g\,cm^{-2}$ for tamoxifen. Despite the substantial amount of GLA permeating the skin, it was far short of being able to deliver passively the 2.8 g per day used effectively in the trial by Kenny et al. (2001). Furthermore, when used prophylactically, a 30–40% reduction in the incidence of breast cancer was achieved from a dose of 20 mg tamoxifen per day (Cuzick et al., 2003)—again some 3000× greater than achieved in the current work. However, two factors should be borne in mind. Firstly, it is not known if the reported doses were optimal in relation to pharmacokinetics/systemic effects. For example, it is known that 99% of tamoxifen is bound to serum albumin (Paterson et al., 2003), necessitating rel-

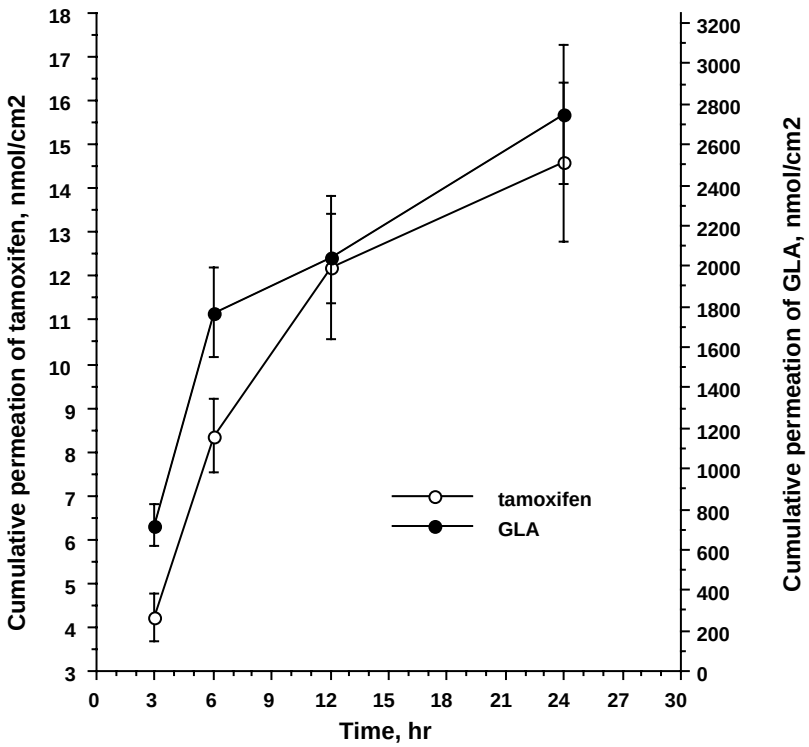

Fig. 1. Plot of the simultaneous permeation of tamoxifen and GLA across human skin from saturated solution of tamoxifen in borage oil.

atively high oral doses to achieve efficacious free plasma concentrations. Kisanga et al. (2003) reported that from a 20 mg oral dose of tamoxifen, the plasma level was 75.9 ng ml^{-1}, normal breast tissue level was 822.6 and 909.3 ng ml^{-1} in cancerous breast tissue. Secondly, a prophylactic system based upon transcutaneous delivery could, on the basis of the above figures, be efficacious by delivering such lower doses directly into breast tissue. For example, assuming zero systemic uptake, $\sim$5 μg cm^{-2} from a topical dose compares quite favourably to $\sim$1 μg ml^{-1}, especially as the latter accounts for some 1/20,000th of the oral dose—the balance being in circulation and potentially giving rise to side-effects. To provide an effective transcutaneous system, therapeutic doses would need to be established and account taken of the proportion of actives cleared into the system by the dermal vasculature.

Transdermal/transcutaneous delivery of tamoxifen has received scant attention. Gao and Singh (1998) described the use of ethanolic vehicles to study the permeation of tamoxifen across porcine skin. It can be determined that 23 nmol cm^{-2} were obtained after 12 h from 10% ethanolic oleic acid/propylene glycol vehicle, 3 nmol cm^{-2} from propylene glycol and 10 nmol cm^{-2} from the control (ethanol). In the current work 12 nmol cm^{-2} permeated after 12 h, which was achieved using human skin and without high alcohol content, which may have deleterious effects on the skin barrier. Similarly, Zhao et al. (2001) investigated vehicles of high alcohol content plus menthone to enhance the permeation of tamoxifen across porcine skin. No reports were found from other workers on the skin permeation of essential fatty acid-bearing oils. The enhancement potential of linolenic acid has previously been studied in the transdermal delivery of melatonin across rat and porcine skin (Kandimalla et al., 1999) and for luteinising hormone release hormone across human epidermis (Bhatia and Singh, 1999). However, the behaviour of amphiphilic free fatty acids would be expected to be substantially different to lipophilic triacylglycerols.

In the current work, transcutaneous delivery was modelled using full thickness human skin, complete with dermis. Initial uptake would be a consequence of the miscibility of the vehicle and the intercellular lipids of the stratum corneum, although it was recently found that stratum corneum could accommodate some 600$\times$ mols of primaquine relative to the amount of extractable lipid (Heard et al., 2003), much being bound to keratin. The dermis is polar in nature relative to the lipids of the stratum corneum and is generally perceived as a barrier to the ingress of highly lipophilic molecules (Guy and Hadgraft, 1989). From the relatively high amounts of both tamoxifen and GLA that permeated the skin, it appears that the driving force of the vehicle (combination of liquid oil, infinite dose) was sufficient to overcome this barrier.

When NSAIDs were applied to pig ear skin in a fish oil vehicle, there were similar large fluxes of ibuprofen or ketoprofen concomitant with eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. In the same study it was found that the rate of permeation of NSAID was linked to the rate of permeation of EPA and DHA in the fish oil, suggesting permeation involved fixed NSAID/triacylglycerol complexes (Heard et al., 2003). Table 1 shows that the ratio of GLA/tamoxifen permeated at different timepoints was fairly consistent, both in terms of mass (mean 138, S.D. 15.1) and mols (mean 184, S.D. 20.3). It may be argued that this reflects the relative permeabilities of tamoxifen and borage oil. However, if this were so, the ratio would change over the duration of the experiment with an increasing excess of the faster permeating specie. Thus, it appears once again that the rate of permeation of the oil was linked to that of solute via a fixed solvation cage, rather than discrete molecules.

The solubility of tamoxifen (MW 563.7) in borage oil, as determined by standard methods, at 32 °C was 4.27 $\pm$ 0.10 mg ml^{-1} (1.15 $\times$ 10^{5} mol ml^{-1}). The density of borage oil was determined to be 935 mg ml^{-1} and the average content of GLA (MW 278.4) within borage oil was 25% (233.8 mg ml^{-1}; 8.40 $\times$ 10^{-4} mol ml^{-1}). Therefore, the ratio of GLA to tamoxifen in the vehicle was 8.4 $\times$ 10^{-4} mol ml^{-1}/1.15 $\times$ 10^{-5} mol ml^{-1} = 73. To maintain a fixed relationship between permeated GLA and tamoxifen (mean 184), the number of molecules of GLA molecules associated with each molecule of tamoxifen in the permeation process must have been 184/73 $\sim$ 2.5, i.e. the solvation cage surrounding tamoxifen comprised 2.5 molecules of GLA. As GLA comprised 25% of the total oil, each molecule of tamoxifen was thus associated with 2.5 $\times$ 4 = 10 free fatty acid equivalents, or 10/3 = 3.33 triacylglycerols. Thus, the solvation cage of borage oil co-permeating

with one molecule of tamoxifen comprised 3–4 triacylglycerol units.

It has yet to be determined if GLA permeated human skin as part of a triacylglycerol unit or whether the free fatty acid had been liberated by the action of skin-based enzymes. Whereas the dermal metabolism of essential fatty acids has been studied (Ziboh et al., 2000), the dermal hydrolysis of triacylglycerols has yet to be determined. Breakdown of the borage oil either in the skin or within breast tissue (currently under investigation), would yield relatively high local concentrations of GLA in the target breast tissue available immediately (along with co-permeated tamoxifen) for uptake into cancerous cells.

In summary, this study has demonstrated the feasibility of administering simultaneously tamoxifen and GLA using borage oil as vehicle across human skin. Further development of this system could yield a novel topical two-component system for early intervention therapy or prophylaxis for those perceived at high risk of developing breast cancer. It is envisaged that a device can be fabricated for attachment directly to the breast. The wider implications of the permeation of solvated complexes warrants further investigation.

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