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In-vitro transcutaneous delivery of tamoxifen and γ-linolenic acid from borage oil containing ethanol and 1,8-cineole

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Abstract

The objective of this study was to examine the effects of ethanol and 1,8-cineole on the transcutaneous delivery of tamoxifen and γ-linolenic acid (GLA) as a two-pronged anti-breast cancer therapy. Formulations containing tamoxifen and varying concentrations of borage oil (~25% GLA), 1,8-cineole and ethanol were prepared and the simultaneous permeation of tamoxifen and GLA determined across full-thickness pig skin using Franz-type diffusion cells over 48 h. Analysis of tamoxifen and GLA (as methyl ester) were by reverse-phase HPLC. The highest flux of tamoxifen of $488.2 \pm 191 \times 10^{-3}$ μg cm^{-2} h^{-1} was observed with a formulation containing 20% 1,8-cineole and 20% ethanol. The same formulation also provided the greatest flux of GLA, 830.6×10^{-3} μg cm^{-2} h^{-1}. The findings from this work demonstrate the ability of 1,8-cineole and ethanol to enhance the in-vitro permeation of tamoxifen and GLA across the skin and support the plausibility of simultaneously delivering tamoxifen and GLA transcutaneously as a two-pronged anti-breast cancer system.

Introduction

Tamoxifen is a commonly used orally administered non-steroidal oestrogen-receptor (ER) antagonist and agonist that has recently been shown to be effective in breast cancer prophylaxis (Cuzick et al 2003). However, the drug has been linked with numerous serious side-effects including primary endometrial cancer, venous thrombosis and secondary cancer in uterine endometrium. Tamoxifen has also been shown to be associated with the formation of covalent DNA adducts in rodents (Han & Liehr 1992) and the development of liver cancer via a genotoxic mechanism (Davies et al 1997). Despite these and other undesirable effects, tamoxifen remains the drug of choice in the management of ER-positive breast cancer.

Polyunsaturated fatty acids (PUFAs) and their metabolites have a variety of physiological roles (Johnson et al 1997; Tapiero et al 2002) and studies have revealed that dietary fat intake is linked to the incidence of breast cancer (Wynder et al 1986). The PUFA γ-linolenic acid (GLA) has been consistently shown to possess anti-cancer and chemopreventive activity with negligible systemic toxicity (Begin et al 1988; Rose & Connolly 1999). In a study conducted by Cai et al (1999), GLA was shown to inhibit angiogenesis, a process of forming new blood vessels that is essential for malignant tumour development. Other mechanisms for the anti-tumour activity of GLA have been reported (Naidu et al 1992; Jiang et al 1998a, b).

Following oral administration, GLA has been found to enhance the effects of tamoxifen, eliciting a faster clinical response than tamoxifen alone. Results from a phase II trial where 38 breast cancer patients took oral GLA 2.8 g per day with 20 mg tamoxifen revealed that these patients achieved faster clinical responses compared with the 47 patients who took tamoxifen alone. Responses were evident at only 6 weeks of treatment and were maintained at 3 months (Kenny et al 2000). Working on this concept, a study investigating the effect of dietary GLA alone on the growth rate and ER expression of ER-positive breast cancer demonstrated GLA to have a modulatory effect on receptor expression. The author suggested that this might be the mechanism by which GLA inhibits ER-positive breast cancer growth and it is believed

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GLA also has a synergistic action with tamoxifen via down-regulation of ER (Kenny et al 2001). Thus a combination of GLA and tamoxifen could be a valuable addition to the current treatment of ER-positive breast cancer.

A major drawback is that oral administration of tamoxifen and GLA leads to wide distribution throughout the body, with potentially life-threatening consequences regarding tamoxifen. Delivering tamoxifen and GLA simultaneously directly across breast skin could provide a better delivery system, particularly by reducing the systemic distribution of tamoxifen. Transcutaneous delivery (i.e. delivery across entire skin to the underlying tissues) is also attractive since the first-pass hepatic metabolism can also be avoided, thus potentially enabling a much-decreased daily drug dosage. It is envisaged that such a system would be particularly useful as a prophylactic regimen.

Pujol et al (1995) reported disappointing results for the percutaneous delivery of 4-hydroxy tamoxifen, which may have been due to its more polar nature or the delivery vehicle used. We recently found that tamoxifen and GLA permeated excised human skin from a saturated solution of tamoxifen in borage oil (Karia et al 2004), which contains 20–25% GLA. The objective of this study was to probe the effect of the penetration enhancers (Williams 2003) ethanol and 1,8-cineole on the simultaneous permeation of tamoxifen and GLA across skin. Ethanol is a commonly used excipient in many topical preparations. Ethanol and terpene (limonene) were used to enhance the permeation of luteinizing hormone release hormone across human skin, passively and in conjunction with iontophoresis (Bhatia & Singh 1999). 1,8-Cineole (eucalyptol), is the major terpenoid of eucalyptus oil and has been shown to be a highly effective enhancer e.g. for the flux of 5-fluorouracil (Yamane et al 1995). Its use is favoured due to low cutaneous irritancy and good toxicological profile therefore providing a relatively safe, effective and clinically acceptable accelerant for drug permeation through the skin with a pleasant medicinal odour. In this work, full-thickness pig ear skin was used as a model for transcutaneous delivery across human skin (Simon & Maibach 2000; Meyer et al 2001).

Materials and Methods

Materials

Tamoxifen, borage oil, 1,8-cineole, methyl γ-linolenate, Cetrimide USP and butylated hydroxyanisole (BHA) were all obtained from Sigma Chemicals (Poole, UK). HPLC-grade acetic acid, methanol and acetonitrile and triethanolamine, petroleum ether BP 60–80°C and ethanol were obtained from Fisher Scientific (Loughborough, UK). Pig ears were obtained from a local abattoir (before steam cleaning) and stored for < 7 days at -20°C until required.

Preparation of formulations

The control formulation was prepared by adding tamoxifen, at increments of 1 mg, to a known amount of borage oil, until saturation was obtained at 32°C. This was achieved with 15 mg of tamoxifen per gram of borage oil (1.5% w/w). The same level was maintained in the test formulations to provide data in an acceptable form for commercial purposes. The required amounts of tamoxifen, borage oil, ethanol and 1,8-cineole were weighed on an electronic measuring balance and vortex mixed to give formulations of the required composition (Table 1).

Table 1 Composition of formulations

Formulation	Tamoxifen (%)	Borage oil (%)	Ethanol (%)	1,8-Cineole (%)
I	1.5	88.5	5	5
II	1.5	83.5	10	5
III	1.5	73.5	20	5
IV	1.5	68.5	20	10
V	1.5	58.5	20	20

Preparation of pig skin membranes

Four pig ears, previously frozen for ~1 week post excision (Babu et al 2003), were allowed to thaw to room temperature and washed thoroughly under cold water. Hairs were removed using electric clippers and the skin was carefully removed from the underlying cartilage using a scalpel, before being cut into 2-cm^2 sections and stored at 2–4°C until required (< 24 h).

Preparation of receptor solution

Cetrimide solution (30 mg mL^{-1}) was used as receptor phase to provide a sink for both tamoxifen and borage oil. A preliminary study indicated that this receptor phase performed in a similar manner to the more ubiquitous alcohol-in-water system, giving rise to no perceptible deleterious effects on the skin (Heard et al 2002). Antioxidant, BHA, was included to inhibit decomposition of the PUFAs. Fifteen grams of Cetrimide USP and 250 mg of BHA were added to a 500-mL volumetric flask and deionised water added to make the solution up to 500 mL, producing 30 mg mL^{-1} cetrimide solution. The receptor solution was degassed before use by vacuum filtration using a 0.45-μm cellulose membrane filter (Whatman, UK).

In-vitro skin permeation experiments

Skin permeation was determined using all glass Franz-type diffusion cells (nominally 0.78 cm^2 diffusional area; 3 mL receptor volume). The skin sections from the four donors, equally distributed between each treatment, were placed between the two pre-greased halves of cells, stratum corneum uppermost, and held in place by metal clamps. The receptor chambers were filled with receptor solution using a 10-mL tube-tipped syringe ensuring no air was in contact with the underside of the skin. A magnetic stirrer bar was placed in each receptor compartment

and diffusion cells were placed on a magnetic stirrer plate (Variomag, Daytona Beach, USA), immersed in a Clifton unstirred water bath to maintain skin temperature at a constant 32°C. After equilibration for 10 min, cells were dosed with 200 μL of the formulations that had been maintained at 32°C for 20 min before use, and the donor compartment occluded. The sampling ports were also occluded using glass caps. Replication was n = 7, split over 2 permeation experiments. Two blank cells, where no formulation was applied to the skin, were also used as controls. At 3, 6, 12, 24, 36 and 48 h, the entire contents of the receptor compartment were taken and refilled with fresh temperature-equilibrated receptor solution. A sample (600 μL) was transferred to a 2-mL autosampler vial and the rest was transferred to a 1.5-mL Eppendorf vial. Samples were sealed and stored at −20°C until required for analysis.

Reverse-phase HPLC analysis of tamoxifen

Tamoxifen was determined by HPLC using a Hewlett Packard 1100 automated system (HP, Germany) equipped with a UV-detector. Separation was carried out on a reverse-phase Phenomenex Kingsorb 5 μm C18 (250 × 4.60 mm) column, using a mobile phase of methanol–acetonitrile–water (350:350:300), 2 mL acetic acid and 1 mL triethanolamine. The mobile phase was filtered through a 0.45-μm pore-size nylon membrane filter (Whatman, UK) and pumped isocratically at a flow rate of 1.8 mL min^{-1} with UV detection at 237 nm. The injection volume was 50 μL and the run time was 10 min. Data acquisition and integration of peaks were performed using JCL 6000 software (Jones Chromatography, Hengoed, UK). Calibration curves were prepared in the range 1–120 μg mL^{-1} for tamoxifen prepared in receptor phase and provided regressions of 0.9999. GLA was baseline resolved and had a limit of detection of 0.45 μg mL^{-1}.

Analysis of GLA

Preparation of fatty acid methyl esters

Permeated GLA was determined by HPLC following conversion to fatty acid methyl esters (FAMEs), generally following a previously published method (Curtis et al 2000). Pooled in pairs so that each time point was represented by four 3-mL samples, the samples were placed in snap-top glass vials and stored at −20°C, before being placed into liquid nitrogen and transferred into a freeze-dryer to be dried overnight. The freeze-dried residues were transferred to Pyrex methylating tubes and fatty acids present were transmethylated by heating under reflux in 10 mL methylating solution (2.5% sulphuric acid in methanol) for 2 h in an oil bath maintained at 70°C. The solution was cooled to room temperature and the reaction was stopped by the addition of 2.5 mL aqueous sodium chloride (5% w/v). FAMEs were then extracted after shaking, with 3 × 20 mL petroleum ether (60–80°C bp). The upper layers (ether phase) were retained and transferred to a Pyrex round-bottom flask before being evaporated to dryness using the rotary evaporator. The remaining yellow-coloured product was diluted with 2 mL HPLC-grade acetonitrile, transferred into a 2-mL autosampler vial, bubbled with nitrogen, sealed and stored in the dark at room temperature. The process was validated using a known amount of GLA. Mass spectrometry (Thermoquest LCQ-Deca) yielded a base peak at m/z 293, confirming the presence of GLA-methyl ester by the high conversion from GLA (no peak at m/z 278.4).

Analysis of GLA methyl ester

GLA methyl ester was determined by HPLC with an isocratic mobile phase adapted from a previously described gradient method (Lin et al 1995). Complete baseline resolution of methyl GLA from other FAMEs (confirmed by spiking with standard) was achieved with an isocratic mobile phase of methanol–water (95:5) at a flow rate of 1 mL min^{-1} and detection wavelength of 210 nm. An injection volume of 20 μL was used. The retention time of GLA methyl ester was approximately 17 min and the run-time was 20 min. Calibration curves were prepared from 1, 5, 10, 20, 40, 80, 100 and 120 μg mL^{-1} GLA methyl ester prepared in receptor phase. Peak areas of GLA methyl ester versus the corresponding drug concentration were plotted to create a linear calibration curve. As permeation of tamoxifen was much greater in the presence of 1,8-cineole, determination of GLA was restricted to formulations III–V.

Data processing

Concentrations of tamoxifen and GLA were corrected for dilution effects and plotted as cumulative permeation per unit area vs time. Because each set of replicate data was accrued over two individual permeation experiments the error associated with mean values is provided as standard error of the mean (s.e.m.). Steady-state flux was taken from the linear portions of these plots between 24 and 48 h and lag time was obtained by extrapolation to the time axis. Permeability coefficient (k_p, cm h^{-1}) was determined by dividing the steady-state flux (μg cm^{-2} h^{-1}) with the donor concentration (μg cm^{-3}). However, as GLA is randomly incorporated into the triacylglycerol units that comprise borage oil, the dose applied can only be provided as an average value (i.e. 25%), hence apparent $k_{p(app)}$ of GLA was reported. Q_{48} data are the cumulative amounts permeated after 48 h. Data are generally presented as mean ± s.e.m. as a consequence of the multiple skin membranes employed. Enhancement ratios were determined by dividing the fluxes of tamoxifen and GLA from the test formulations by that of the control.

Statistical analysis

Statistical comparisons were performed by analysis of variance. Kruskall Wallis test was used for comparing data from more than two sets. Mann Whitney U test was used to compare data from two groups. The statistical package, SPSS for Windows, Version 11 was used. The level of significance was taken as $P < 0.05$.

Results

Transcutaneous delivery of tamoxifen

Control

Permeation data are summarised in Table 2. For the control solution, the flux of tamoxifen was $69.5 \pm 12.0 \times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$. The k_p of tamoxifen was 4.63×10^{-6} cm h^{-1} and the lag time approximately 12 h. Q_{48} was $2.17 \pm 0.38\,\mu g\,cm^{-2}$.

Formulations with varying amounts of ethanol

The greatest flux of tamoxifen, $89.7 \pm 13.0 \times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$, and shortest lag time (10 h) were observed with formulation III, which contained 20% ethanol (Table 2). After 48 h the cumulative permeation was $2.98 \pm 0.39\,\mu g\,cm^{-2}$. The lowest flux, $56.4 \pm 15.0 \times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$, was observed with formulation containing 5% ethanol. After 48 h only $1.68 \pm 0.36\,\mu g\,cm^{-2}$ permeated. This was lower than that observed from the control formulation. However, no statistically significant difference between the rates of release of tamoxifen in the three formulations and control samples was found ($P = 0.454$). The lag times of tamoxifen from formulations I and II appeared to be no different from that observed from the control, approx 12 h. Enhancement ratios are illustrated in Figure 1.

Formulations with varying amounts of 1,8-cineole

Typical permeation profiles were obtained for tamoxifen with differing amounts of 1,8 cineole, and the data are summarised in Table 2. There was a concentration-dependent increase in the flux of tamoxifen as the proportion of 1,8-cineole in the formulations increased. The fluxes of tamoxifen from formulations III, IV and V were all found to be higher than that observed from the control. The highest flux of tamoxifen, $488.2 \pm 191 \times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$, was observed with formulation V, containing 20% 1,8-cineole. After 48 h, $5.420 \pm 2.222\,\mu g\,cm^{-2}$ had permeated. The lowest flux of $89.7 \pm 13.0 \times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$ was observed from formulation III, which contained the lowest amount of 1,8-cineole (5%). The lag times produced from formulations III–V did not show any obvious differences, although for formulation V it was slightly shorter, 9 h. There was a significant difference between the flux of tamoxifen from formulations IV and V (enhancement ratios: 3 and 7, respectively; Figure 1) compared with the control ($P = 0.003$ and $P = 0.037$, respectively). However, no significant difference was observed between formulation III and the control ($P = 0.391$).

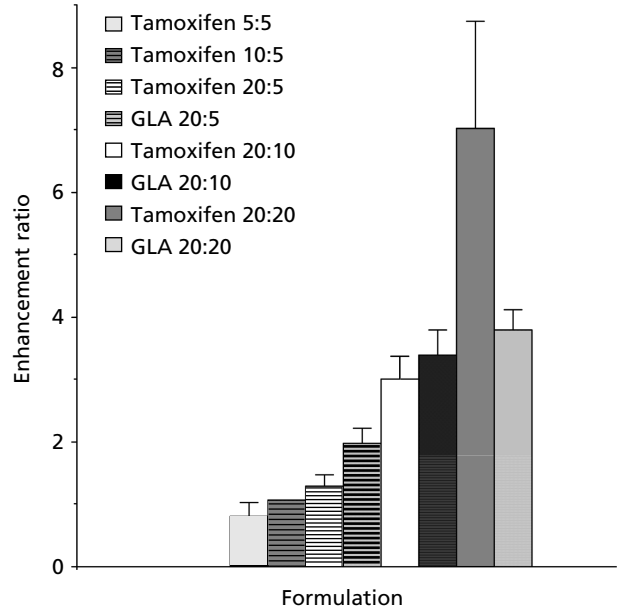

Figure 1 Enhancement ratios for the flux of tamoxifen and GLA in response to different proportions of 1,8-cineole and ethanol. Data are means ± s.e.m., n = 7. For key refer to columns 2 and 3 of Table 2.

Transcutaneous delivery of GLA

Control

Permeation data are summarised in Table 3. Despite the high lipophilicity of the borage oil, a typical permeation profile was observed. The steady-state flux of GLA was $219.7 \pm 10.0 \times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$ and the $k_{p(app)}$ was 1.10×10^{-6} cm h^{-1}. The lag time was approximately 2.8 h and the Q_{48} was $9.41 \pm 0.51\,\mu g\,cm^{-2}$. GLA was not found in any of further control cells (those with no borage oil

Table 2 Summary of permeation data for transcutaneous delivery of tamoxifen across pig ear skin

Formulation	EtOH (%)	Cineole (%)	Lag time (h)	Steady-state flux ($\times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$)	Enhancement ratio	Mean k_p ($\times 10^{-6}$ cm h^{-1})	Q_{48} ($\mu g\,cm^{-2}$)
Control	0	0	12	69.5 ± 12.0	—	4.63	2.170 ± 0.380
I	5	5	12	56.4 ± 15.0	0.81 ± 0.22	3.76	1.680 ± 0.359
II	10	5	12	74.2 ± 5.0	1.07 ± 0.08	4.95	2.300 ± 0.190
III	20	5	10	89.7 ± 13.0	1.29 ± 0.18	5.98	2.980 ± 0.391
IV	20	10	9	200.3 ± 24.0	3.00 ± 0.37	13.35	5.230 ± 0.909
V	20	20	9	488.2 ± 191.0	7.03 ± 2.70	32.55	5.420 ± 2.222

Data are means ± s.e.m., n = 7.

Table 3 Summary of permeation data for transcutaneous delivery of GLA across pig ear skin

Formulation	EtOH (%)	Cineole (%)	Lag time (h)	Steady-state flux ($\times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$)	Enhancement ratio	Mean $k_{p(app)}$ ($\times 10^{-6}$ cm h^{-1})	Q_{48} ($\mu g\,cm^{-2}$)
Control	0	0	2.8	219.7 ± 10.0	—	1.10	9.41 ± 0.51
III	20	5	6.0	432.7 ± 53.0	1.97 ± 0.24	2.94	17.82 ± 2.19
IV	20	10	~1	747.8 ± 88.0	3.40 ± 0.40	5.46	33.59 ± 4.10
V	20	20	~1	830.6 ± 67.0	3.80 ± 0.31	7.10	34.38 ± 3.68

Data are means ± s.e.m., n = 7.

applied), demonstrating that detectable quantities of GLA did not leach from the skin.

Formulations with varying amounts of 1,8-cineole

Typical permeation profiles were obtained and the data is summarised in Table 3. The greatest flux of GLA, $830.6 \pm 67.0 \times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$, was achieved with formulation V; after 48 h $34.38 \pm 3.68\,\mu g\,cm^{-2}$ had permeated. The lowest flux, $432.7 \pm 53.0 \times 10^{-3}\,\mu g\,cm^{-2}\,h^{-1}$ was obtained with formulation III: after 48 h $17.82 \pm 2.19\,\mu g\,cm^{-2}$ had permeated. The mean cumulative permeation of GLA after 48 h from formulations III, IV and V was higher than that observed from the control formulation. Based on these observations, the flux of GLA increases as the amount of 1,8-cineole in the formulations increases. There was a significant difference in the flux of GLA permeation from formulations III–IV and the control ($P = 0.02$). However, there was no significant difference between formulation IV and V ($P = 0.819$). With regards to the enhancement ratios of 1,8-cineole and ethanol on GLA, the enhancer activity was almost doubled when 1,8-cineole was increased from 5 to 10%. However, enhancement was not further increased with a rise from 10 to 20% 1,8-cineole, indicating saturability of the enhancing effect.

Discussion

Transcutaneous delivery of tamoxifen

It is generally accepted that in-vitro skin permeation experiments are useful predictors of in-vivo topical delivery (Valiveti et al 2004). However, the permeation data clearly need to be confirmed in an in-vivo investigation. Lag times in all cases were relatively long with poorly defined lag phase/steady-state flux transitions. It is probable that long lag time behaviour of tamoxifen was due to a combination of a low subsaturated concentration gradient (1.5% w/w) and high lipophilicity (logP 7.87 ± 0.75). For drugs with a log P greater than 2, the formation of a reservoir in the stratum corneum is not unusual.

Being highly lipophilic, tamoxifen would be expected to be highly miscible with the lipophilic intercellular domains of the stratum corneum, which has been shown capable of accommodating high amounts of permeant (Banning & Heard 2002; Heard et al 2003a). This also seems reasonable given the significant and simultaneous absorption of the borage oil. Only when the tissues have become saturated can steady state commence although at that stage the dermis, being substantially more polar in nature, may have become a rate-limiting barrier. It has previously been shown that the presence of 1,8-cineole and ethanol would reduce lag time and enable steady-state flux to be established more quickly (Yamane et al 1995). However, the lag time was only reduced by approximately 1 h in formulation IV (20% ethanol, 10% 1,8-cineole) compared with the control.

Although, the lag time was not substantially reduced by an increase in the concentration of both permeation enhancers, an increase in the flux of tamoxifen was observed. This was illustrated by the rise in enhancement ratio of flux as the concentration of 1,8-cineole and ethanol increased. The greatest enhancement ratio, achieved with formulation V, was 7, which is greater than observed in previous studies. The permeation of tamoxifen across full thickness hairless mouse skin from a hydroxypropyl cellulose gel was increased by approximately 2-fold with inclusion of the terpenoid nerolidol in the formulation (El-Kattan 2001). Permeation of low-molecular-weight heparin through human skin exhibited a 3.5-fold increase in flux with 10% 1,8-cineole (Xiong et al 1996). Also, studies have shown that the ability of 1,8-cineole to improve the partitioning of a drug increases when the pre-treatment time was increased to 12 h (Bhatia & Singh 1999), indicating the possibility that enhancement activity was not reached until this time, explaining the long lag time.

Other than the control, each applied dose used in this work contained a fixed subsaturated 1.5% of tamoxifen. According to Fick's first law, the flux of a drug at a given concentration will increase as its solubility in the vehicle decreases. Therefore, a vehicle in which a drug is poorly soluble will give a higher flux than one in which it is highly soluble. This means that the flux is proportional to a gradient of thermodynamic activity rather than concentration, which may explain why the flux of tamoxifen from the control, which contained an amount of tamoxifen that was close to saturation, was higher than that from formulation I. It was observed during the preparation of the formulations that the solubility of tamoxifen increased as the concentration of ethanol or 1,8-cineole

increased so, theoretically, the flux of the drug should decrease (if the permeation enhancing activity was ignored). Because formulation I contained the least amount of both permeation enhancers, the lower observed flux may indicate that the concentration of either or both enhancers was insufficient to compensate for the low thermodynamic activity of the drug in the vehicle. This also explains the statistically insignificant difference in the steady-state flux of tamoxifen from formulations I–III compared with the control. However, when the concentration of 1,8-cineole was increased from 5 to 10 and 20%, the flux of tamoxifen was increased significantly compared with the control. This clearly indicates that 1,8-cineole was able to enhance the flux of tamoxifen, perhaps because of its greater lipophilicity (Hori et al 1991). It also suggests that the use of penetration enhancers provides a higher degree of permeation enhancement than that offered by a saturated solution. Ethanol was reported to have a synergistic effect in permeation enhancement with some terpenes like menthol and 1,8-cineole (Levison et al 1994), hence it can be a good co-solvent for topical formulations. In this work the fact that permeation of tamoxifen was not substantially increased by increasing amounts of ethanol whereas there was a dose-dependent increase relative to the proportion of 1,8-cineole suggests that they operate by different mechanisms. Of approximately 3 mg of tamoxifen present in each 200 μL donor solution of formulation V, only 6.36 $\mu g\,cm^{-2}$ (some 0.21% of the applied dose) permeated the skin over 48 h and this was the highest mean cumulative amount observed.

Permeation of GLA

It is clear from Table 3 that substantial amounts of GLA permeated the skin. Whether this occurred as complete triacylglycerol units or as liberated free fatty acid cleaved from the glycerol backbone through the action of dermal enzymes remains to be elucidated. Although it is known that some formulation excipients can be absorbed into the stratum corneum, study of the transcutaneous permeation of a bioactive vehicle has hitherto received little attention, especially simultaneously with a further active solute. These data support our other work involving tamoxifen and GLA (Karia et al 2004) in addition to NSAIDs and PUFAs of fish oil (Heard et al 2003b).

Unlike tamoxifen, lag times were very short for GLA (unmeasurably short for formulations IV and V). GLA was present in much higher concentrations, producing a high concentration gradient and a high driving force for maximum initial diffusion. The highest mean cumulative permeation of GLA after 48 h was observed from formulation V. Of approximately 23 mg GLA present in each 200 μL donor solution of formulation V, only 34 $\mu g\,cm^{-2}$ (some 0.15%) permeated the skin over 48 h. This was even lower than the amount of tamoxifen permeated. A possible explanation for this is that GLA may have been incorporated into the lipid bilayer or its permeation retarded by the polar viable dermis and epidermis. Alternatively, this may reflect tissue saturation.

Clinical considerations

The findings from this study support the plausibility of simultaneously delivering tamoxifen and GLA transcutaneously as a two-pronged anti-breast cancer system. The highest Q_{48} values for tamoxifen and GLA of 5.42 and 34.38 $\mu g\,cm^{-2}$, respectively, were both obtained with formulation V employing both ethanol and 1,8-cineole at 20%. Steady-state fluxes of tamoxifen and GLA were maintained for 48 h or longer, which is appropriate as this is the time period that a transcutaneous delivery device is likely to be applied before being replaced.

Two scenarios are envisaged for a working system. Firstly, this system may be of use in early intervention. Unfortunately, despite several attempts, to date no clear correlations between serum levels and minimally effective dose have been established (Patterson et al 1980; Fabian et al 1981; Decensi et al 2003). Tamoxifen administered in a dose of 10 mg twice daily would provide a steady-state plasma concentration of 77–274 $ng\,mL^{-1}$ (Furr & Jordan 1984). However, studies have shown that 99% of tamoxifen is bound to serum albumin (Patterson et al 1980) and relatively high oral doses of tamoxifen are therefore required to achieve minimum effective free plasma concentrations to gain efficacy at its receptor. The serious adverse effects of tamoxifen may be concentration dependent. If all the tamoxifen were cleared from the skin into the systemic circulation then the achievable dose from this work is 5.4 $\mu g\,cm^{-2}$ at 48 h — a 4×4 cm patch could deliver $\sim$86 μg direct to the breast tissue in 2 days. Furthermore, enhancement of the activity of tamoxifen by the co-permeating GLA could reduce levels necessary to attain efficacy. The total daily dose of GLA in combination with tamoxifen used by Kenny et al (2001) was 2.8 g, a level clearly unobtainable based upon the current data. However, as with tamoxifen, efficacious doses remain unknown and transcutaneous delivery would provide a more preferable option, and because it would be localised at the target tissue it is likely that substantially lower doses would provide a beneficial effect.

Secondly, the system is perhaps more appropriate as a prophylactic for those deemed to be at high risk of developing breast cancer. Although much developmental work would be required to determine effective transcutaneous prophylactic doses, localised transcutaneous delivery of tamoxifen would be a more feasible approach than systemic delivery since the doses of tamoxifen required at the site of the tissue at risk will be much lower than those required to produce steady-state plasma concentrations. Application of a patch-based system, at a rate less frequent than systemic dosing, could also facilitate increased compliance.

Conclusions

The findings from this work support the plausibility of simultaneously delivering tamoxifen and GLA transcutaneously as a two-pronged anti-breast cancer system that could allow the delivery of tamoxifen and GLA directly to the breast, without major systemic delivery. Such delivery

could be extremely beneficial as it could eliminate or reduce undesirable side effects of tamoxifen on the CNS, uterus and the liver. It also offers the potential to administer lower doses of tamoxifen and GLA than those currently used. In addition to providing the benefits outlined above, a system to deliver tamoxifen and GLA across the skin would permit the controlled release of the two drugs with kinetics that approach zero order. The preparation or device may also require less frequent dosing than oral doses and could be more convenient for patients to use, which can potentially increase patient compliance. A device could be fabricated that could be attached to the breast in a discreet and comfortable manner to minimise patient discomfort.

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