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Transdermal Flux Predictions for Selected Selective Estrogen Receptor Modulators (SERMs): Comparison with Experimental Results

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Graphical abstract
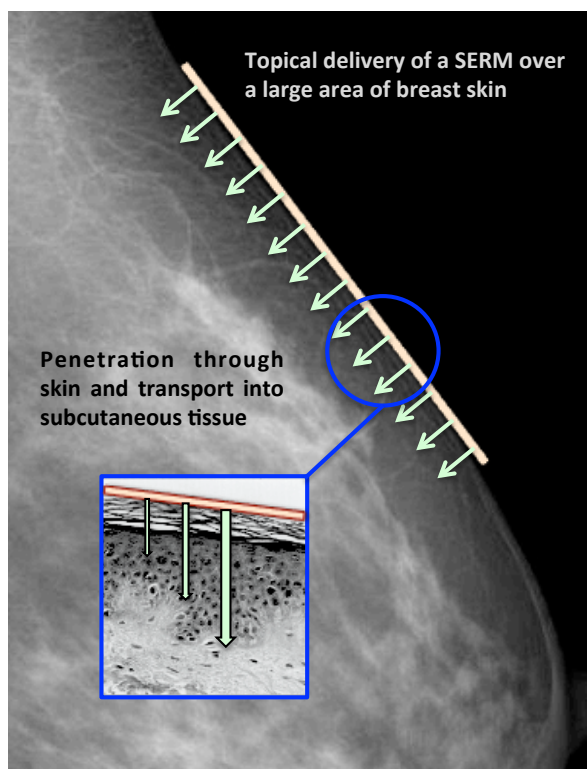
Schematic view of SERM delivery to cancerous breast tissue following application of a topical formualtion over a relatively large area of skin.

Abstract

The aim of this work was to evaluate the feasibility of delivering transdermally a series of highly lipophilic compounds (log P ~4-7), comprising several selective estrogen receptor modulators and a modified testosterone (danazol). The maximum fluxes of the drugs were predicted theoretically using the modified Potts & Guy algorithm (to determine the permeability coefficient (k_p) from water) and the calculated aqueous solubilities. The correction provided by Cleek & Bunge took into account the contribution of the viable epidermal barrier to the skin permeation of highly lipophilic compounds. Experimental measurements of drug fluxes from saturated hydroalcoholic solutions were determined *in vitro* through excised pig skin. Overall, the predicted fluxes were in good general agreement (within a factor of 10) with the experimental results. Most of experimental fluxes were greater than those predicted theoretically suggesting that the 70:30 v/v ethanol-water vehicle employed may have had a modest skin penetration enhancement effect. This investigation shows that the transdermal fluxes of highly lipophilic compounds can be reasonably predicted from first principles provided that the viable epidermis, underlying the stratum corneum, is included as a potentially important contributor to the skin's overall barrier function. Furthermore, the absolute values of the measured fluxes, when considered in parallel with previous clinical studies, indicate that it might be feasible to *topically* deliver a therapeutically useful amount of some of the compounds considered to cancerous breast tissue.

Keywords: selective estrogen receptor modulators; transdermal drug delivery; topical drug delivery; skin flux prediction; breast cancer

1. Introduction

Selective estrogen receptor modulators (SERMs) are synthetic hormones that act as agonists or antagonists of estradiol depending on the tissue. For example, SERMs are agonists in bone, liver, and the cardiovascular system, antagonists in breast tissue, and mixed agonist/antagonists in the uterus [1,2]. Several SERMs are marketed or in clinical development, including tamoxifen, 4-hydroxytamoxifen (4-OH tamoxifen), toremifene, clomiphene, endoxifen and droloxifene [1-3]. Tamoxifen has been shown to be the most important therapeutic agent for the treatment of estrogen receptor positive breast cancer for the past three decades. It is widely used in adjuvant endocrine therapy of early breast cancer, in the palliative treatment of advanced disease, and for prevention of breast cancer in high-risk subjects [2,4]. It has also been evaluated in the treatment of mastalgia [5]. In addition to its anti-estrogenic activity in the breast, tamoxifen also acts as an estrogen in the uterus [1,2]. Thus, the most important side effects of tamoxifen treatment are development of endometrial polyps, endometriosis and increased risk of endometrial cancer [6]. Tamoxifen also has other dose dependent side effects including hot flushes, deep vein thrombosis, retinopathy, cataract, and corneal opacities [3,7].

Tamoxifen is extensively biotransformed to metabolites that have different potencies and anti-estrogenic activities [8-10]. High inter-individual variability in tamoxifen response has been attributed to the different degrees of metabolism [10]. Tamoxifen has anti-estrogenic activity but it is also a pro-drug. Its efficacy also depends on its major metabolites, 4-hydroxy-tamoxifen (4-OH tamoxifen, afimoxifene) and N-desmethyl-4-hydroxytamoxifen (endoxifen). The binding affinities of 4-OH tamoxifen and endoxifen are both 25 times greater for ERα and 56 times greater for ERβ than that of tamoxifen [9,10]. 4-OH tamoxifen (afimoxifene), the most active metabolite of tamoxifen has higher anti-estrogenic potency than the parent drug [11]. Endoxifen, another major metabolite of tamoxifen (N-desmethyl-4-hydroxytamoxifen), is found in higher concentration than that of 4-OH tamoxifen [9,10]. Although its pharmacological activity has not been explained in detail, the potency of endoxifen has been shown to be similar to 4-OH tamoxifen in terms of both estrogen binding and inhibition of estradiol-induced cell proliferation [9,10]. In recent years, it has been shown to be a new potent anti-estrogen agent for the treatment of breast cancer [12-14]. The clinical trial studies have been carried out on oral administration of endoxifen hydrochloride [15,16].

Danazol has also been evaluated for the treatment of pain and nodularity related to cyclical mastalgia. Although significant decrease in pain and nodularity was observed compared to placebo, oral danazol treatment caused significant side effects that restrict its use for long-term treatment [17].

To circumvent the variability and patient compliance problems (including adverse effects) of oral SERMs, their direct transdermal administration to breast for example, to treat benign diseases, and mastalgia, and for prophylactic protection of healthy, but at risk women has been proposed and considered. For

example, the skin penetration of tamoxifen in vitro in the presence of different chemical enhancers [18-21] has been assessed, as has the liposomal delivery of the drug [22-24]. Clinically, the transdermal delivery of 4-OH tamoxifen has been evaluated [25-28], and promising results have been obtained. In post-menopausal women with estrogen receptor positive breast cancer, direct topical application to the breast of 4-OH tamoxifen gel resulted in anti-estrogenic activity in the tissue, and systemic concentrations of the drug that were much less than those measured after oral administration [27]. Topical delivery was shown to inhibit tumour proliferation equivalent to that achieved with an oral dose of 20 mg/day. In a phase II clinical trial of 4-OH tamoxifen, the efficacy of a topical gel in the treatment of cyclical mastalgia was demonstrated with high tolerability and safety [28]. This gel has also been investigated in another phase II trial for the treatment of women with newly diagnosed ductal breast carcinoma *in situ* [29]. Furthermore, given the potential of oral endoxifen to treat breast cancer [12-14], the transdermal delivery of this tamoxifen metabolite has been assessed [30,31]; the skin penetration enhancer, oleic acid, increased drug delivery sufficiently that this approach may be feasible for the direct input of the SERM to breast tissue.

However, the skin is a complex membrane the outermost layer of which, the stratum corneum (SC), has been bioengineered to be a very efficient barrier to the ingress of xenobiotics, including drugs. Indeed, skin penetration is very sensitive to the physicochemical properties of the permeant, and is most favourable for low molecular weight compounds (< 500 Daltons being most typically cited) and those of moderate lipophilicity (log (octanol-water partition coefficient), log P ~ 1-3) [32]. SERMs, though, are typically more lipophilic with log P values of 5 or more, suggesting that their uptake into the skin may well be controlled by the rate at which they are able to transfer out of the SC and into the underlying, much more aqueous, viable layers of the skin. These factors have been successfully modelled [33,34] such that a drug's skin permeability, and its maximum flux through the barrier, may be predicted from a simple set of algorithms [35].

In this paper, therefore, this theoretical approach has been used to estimate the transdermal fluxes of a series of highly lipophilic SERMs, as well as that of danazol, and to compare these results with experimental measurements made *in vitro* across porcine skin (a widely accepted model for the human counterpart [36]). Finally, the data obtained are considered in light of the anticipated drug levels required for local efficacy in breast tissue with a view to evaluating the potential feasibility and optimisation of a topical delivery formulation.

2. Materials and Methods

2.1. Chemicals and reagents

Tamoxifen citrate, clomiphene citrate, and cetrimide were purchased from Sigma (Poole, UK). Droloxifene citrate and endoxifen, 4-OH tamoxifen were supplied by Besins Healthcare SA, Brussels, Belgium. Toremifene and danazol were obtained from Axxora (Nottingham, UK). HPLC grade acetonitrile and methanol, absolute ethanol, triethanolamine and trifluoroacetic were from Fischer Scientific (Loughborough, UK).

2.2. Calculation of lipophilicity and aqueous solubility of SERMs

Log (octanol-water partition coefficient) (log P) values and aqueous solubilities ($C_{w,sat}$) were estimated for the unionised forms of the drugs using the ALOGPS 2.1 algorithm from the Virtual Computational Chemistry Laboratory (http://www.vcclab.org), and from ChemSpider (http://www.chemspider.com). The former provided 8 calculated values of log P and 2 for $C_{w,sat}$; the latter furnished one log P estimate (which was identical to one of the 8 from the VCCL) and 2 additional estimates of $C_{w,sat}$, one based on the calculated log P, the other from a molecular fragment approach. The mean and standard deviation of the log P (n=8) and $C_{w,sat}$ (n=4) values were subsequently determined.

2.3. Predictive model of drug flux across skin

The steady-state diffusion of a drug across skin may be described by Fick's first law:

$$J_{max} = (D/h) \bullet K_{SC/v} \bullet C_{v,sat} = k_p \bullet C_{v,sat} \tag{Eq. 1}$$

where J_{max} is the maximum drug flux (amount per unit area per unit time); D is its diffusion coefficient in the stratum corneum (SC); $K_{SC/v}$ is the drug's SC-vehicle partition coefficient, h is the path length taken by the penetrant through the SC, and $C_{v,sat}$ is saturated solubility of the drug in the vehicle. The permeability coefficient of the drug through the SC (k_p) following its application in an aqueous vehicle may be predicted from Eq. 2 [33]:

$$\log k_p \text{ (cm/h)} = -2.7(\pm0.8) + 0.71(\pm0.06) \bullet \log P - 0.0061(\pm0.0006) \bullet MW \tag{Eq. 2}$$

where P is the compound's octanol-water partition coefficient and MW is its molecular weight. For very lipophilic compounds, the contribution of the viable skin layers to the skin's barrier must be taken into account through a correction to the permeability coefficient proposed by Cleek and Bunge [34]:

$$k_{p,corr} = k_p / \{1 + (k_p \bullet MW^{\frac{1}{2}}/2.6)\} \tag{Eq. 3}$$

It follows, therefore, that an estimate of J_{max} can be obtained via Eq. 1 by multiplying the value of $k_{p,corr}$ (Eq. 3) by the aqueous solubility of the drug.

It should be pointed out that the derivation of Eq. 3 assumed a nominal viable tissue thickness of 0.1 mm; i.e., a value approximately equivalent to the thickness of the epidermis, rather than that of a skin sample typically used in an *in vitro* Franz cell experiment – see below. While this might be expected to undermine a comparison of experimental and theoretically predicted flux, for the very lipophilic compounds considered here, the rate-determining step in their penetration is effectively mass transfer at the stratum corneum-viable tissue interface; subsequent diffusion through the viable tissue is likely to be relatively fast and the impact of the absolute thickness of this layer will be modest at most.

2.4. Solubility determination

The saturated solubilities of danazol and the six SERMs considered were determined in a series of ethanol-water mixtures (40:60, 50:50, 60:40 and 70:30, v/v) and in the aqueous cetrimide solution (3%, w/v) used as the receptor fluid in the skin permeation experiments. An excess of each compound was added to 1 mL of the solvent and the suspensions were shaken for at least 24 hours at room temperature to ensure saturation. The samples then were filtered through a 0.45 µm membrane filter (Millex® Syringe Filter, Milipore), and saturated concentrations were determined by HPLC analysis after appropriate dilution (see below).

2.5. Skin permeation experiments

Porcine abdominal skin was obtained from a local slaughterhouse and cleaned with cold water. The fat and subcutaneous layers were separated carefully with a scalpel. The tissue was then dermatomed to ~750 µm thickness (Zimmer™, Dover, OH) and cut into small pieces, which were wrapped individually in Parafilm™ and stored at -20°C until required. Five to ten replicates were performed for each compound using skin from at least two different animals.

The permeation studies were carried out using opaque side-by-side diffusion cells with an effective diffusion area of 0.71 cm^2. The receptor compartment had a volume of 3.2 ml and was maintained at 37 (±0.5)°C by circulating warmed water through a jacket surrounding the cell. The receptor solution was 3% w/v cetrimide and ensured that sink conditions [21] were maintained. The receptor was stirred continuously with a Teflon-coated magnetic bar at 100 rpm. The skin was allowed to defrost and re-hydrate in isotonic saline solution for 1 h before the permeation experiment began. 3 ml of a saturated solution of each compound in 70:30 v/v ethanol-water was introduced into the donor chamber ensuring maximum thermodynamic activity. Experiments were performed under complete occlusion. At 1, 9, 20 and 24 hours post-application, 1 ml samples were taken from the receiver compartment and replaced with the same volume of fresh, temperature-equilibrated receptor fluid. Samples were filtered through a 0.45 µm membrane filter, and the amount of penetrant was determined by HPLC using the method described below.

2.6. Analytical method

Drug concentrations were determined by HPLC (Jasco model PU-2080 Plus pump; model AS-2051 plus auto-sampler; UV detector (Jasco, Japan, Model UV-2975 Plus)). Chromatographic resolution of 4-OH tamoxifen, tamoxifen citrate, clomifene citrate, endoxifen, droloxifene citrate and toremifene was achieved on a C_{18} reverse-phase column (5 μm, 250 mm x 4.6 mm, HiQ sil, KYA Tec, Japan) with a guard column (ODS Hypersil 5 μm, 10 mm x 4 mm, Thermo Electron Corporation, UK). Separation of danazol used a C_{18} reverse-phase column (5 μm, 150 mm x 4.6 mm, Acclaim) with the same guard column as above. The analysis details for each compound are summarized in Table 1. The mobile phases were filtered through a 0.45 μm membrane. The injection volume was 50 μL. Calibrations employed the external standard method, and standard solutions were prepared in methanol. Dilutions were made with 3% w/v cetrimide solution. The calibration curve was established between 0.25 and 50 μg.mL^{-1} for 4-OH tamoxifen, and in the range 0.1 - 20 μg.mL^{-1} for all other compounds ($r^2 > 0.999$). Each sample was assayed in triplicate, and all HPLC methods were fully validated. Accuracy, expressed as a percentage of the mean recovery, ranged from 98% to 104%; precision, expressed as a relative standard deviation (RSD), was less than 2%.

Table 1. Chromatographic conditions for HPLC analysis.

Compound	Mobile phase	Flow rate (ml.min^{-1})	UV (nm)
Clomiphene citrate	Methanol: water: TFA[a] (75: 25:0.1)	0.6	235
Danazol	Acetonitrile : water (65:35)	1.0	287
Droloxifen citrate	Methanol : acetonitrile: water: acetic acid : TEA[b] (350 : 350 : 300 : 2 : 1)	1.0	235
Endoxifen	Methanol : acetonitrile: water: acetic acid: TEA[b] (350 : 350 : 300 : 2 : 1)	0.7	244
4-OH Tamoxifen	Methanol : acetonitrile : water : acetic acid: TEA[b] (350 : 350 : 300: 2: 1)	1.0	243
Tamoxifen citrate	Methanol : water : TFA[a] (75 : 25 : 0.1)	0.6	237
Toremifene	Methanol : acetonitrile : water : TFA[a] (500 : 310 : 190 : 0.35)	0.5	237

[a] TFA: Trifluoroacetic acid

[b] TEA: Triethanolamine

3. Results & Discussion

The first step in the research described here was to predict the maximum skin fluxes (J_{max}) of the lipophilic drugs considered. To do so, as explained above, required knowledge of MW, log P and aqueous solubility ($C_{w,sat}$). The former were easily looked up, while estimation of log P and $C_{w,sat}$ were calculated for the unionised forms of the drugs using freely accessible algorithms. The results obtained are in Table 2. There were 8 separate calculations of log P and 4 of $C_{w,sat}$; while the former were quite consistent with a coefficient of variation between 10 and 15% of the mean, the latter differed far more and, for all drugs except danazol, one of the estimates was a clear statistical outlier (at $p<0.05$, www.graphpad.com/quickcalcs/Grabsbs1.cfm). This value was not used, therefore, to determine the average $C_{w,sat}$ required for the calculation of J_{max}.

Table 2.   Estimated log P (n = 8) and $C_{w,sat}$ (n = 3, except for danazol for which n = 4) values (mean ± standard deviation) for the compounds studied.

| Compound	MW (Da)	log P	$C_{w,sat}$ (µg/mL)
Clomiphene	405.97	6.65 ± 0.76	0.16 ± 0.23
Danazol	337.46	4.14 ± 0.57	10.3 ± 8.29
Droloxifene	387.52	5.95 ± 0.84	1.77 ± 1.20
Endoxifen	373.50	5.45 ± 0.64	1.97 ± 0.96
4-OH Tamoxifen	387.52	5.96 ± 0.85	1.77 ± 1.20
Tamoxifen	371.52	6.39 ± 0.84	0.41 ± 0.53
Toremifene	405.97	6.06 ± 0.75	0.38 ± 0.36

The corrected skin permeability coefficients (determined from Eqs. 2 and 3) [33,34], and the calculated J_{max} values for seven drugs are in Table 3. The final estimates of the permeability coefficients of these very lipophilic compounds are in the range of 0.01 to 0.1 cm.hr^{-1}, consistent with the rate-limiting role of the viable, aqueous skin tissue in their percutaneous transport [37]. Nevertheless, the predicted J_{max} values are no more than 150 ng.cm^{-2}.hr^{-1}, reflecting the very low aqueous solubilities of these drugs. It should be emphasized that all calculations are based on the properties of the unionised compounds, even for those formulated as salts in the experimental method.

Table 3. The predicted skin permeability parameters of the compounds studied.

Compound	$k_{p,corr}$ (cm.h^{-1})	J_{max} (µg.cm^{-2}.h^{-1})
Clomiphene	0.097	0.015
Danazol	0.015	0.15
Droloxifene	0.072	0.13
Endoxifen	0.052	0.10
4-OH Tamoxifen	0.073	0.13
Tamoxifen	0.101	0.042
Toremifene	0.069	0.026

Figure 1. Solubility of the compounds studied in different mixtures of ethanol (the %v/v of which is indicated on the graph) and water. Each value shown represents the mean of 3 replicates (standard deviations are too small to be visible on the y-axis scale employed).
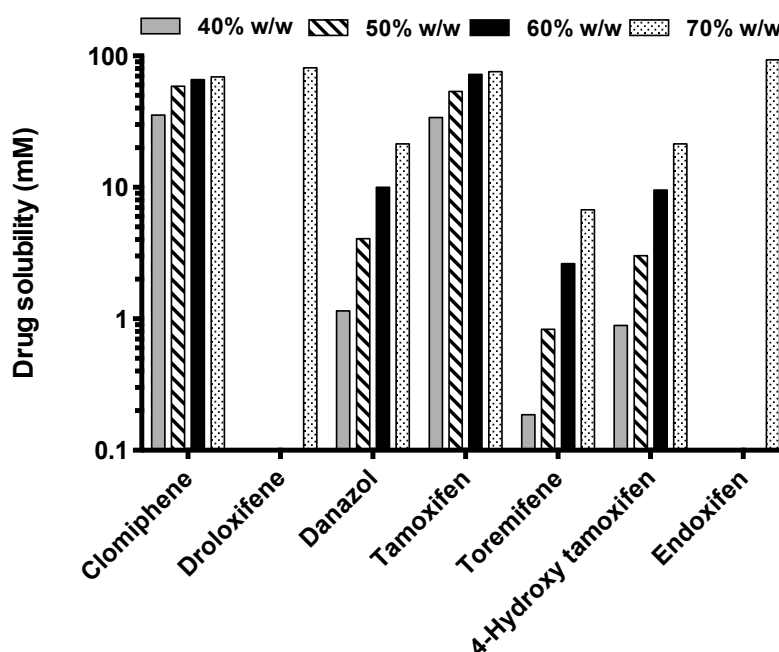
The second component of the work was to measure J_{max} for the SERMs and danazol experimentally. Because of the extremely poor water solubilities of these compounds, their delivery from saturated ethanol-water mixtures was selected so that drug transport would not be affected by significant depletion of the active moiety in the vehicle. Of course, according to Eq. 1, J_{max} should not be affected by the nature of the vehicle when the drug is applied as a saturated solution (i.e., at its maximum thermodynamic activity), provided that the vehicle itself does not alter skin barrier function in some way

[33]. The measured solubilities of the drug in various ethanol-water mixtures are in Figure 1 and these results informed the choice of 70:30 v/v ethanol-water vehicle for the skin penetration studies: at this co-solvent ratio, it was considered that all the compounds under study had sufficient solubility to ensure that quantification of their absorption would be unambiguously measurable.

The cumulative amounts of the 7 drugs absorbed across the skin in 24 hours are in Table 4. Because of the low fluxes observed, it was not possible to sample the receptor solution with sufficient frequency to obtain a complete permeation profile or to determine a classic lag-time. In any case, for the drug delivery application envisaged, the treatment would obviously be chronic and would involve repetitive administration to the same area(s) of skin. Presumably, this would ultimately permit establishment of something close to steady-state conditions and the duration of the initial lag time becomes irrelevant. An approximation to the experimental J_{max} was therefore derived by dividing the cumulative delivery during a 1-day exposure by 24 hours - these values are also in Table 4. Comparison of the predicted maximum fluxes in Table 3 with those in Table 4 is illustrated in Figure 2. While all the ratios are within an order of magnitude of the ideal value of 1 (in other words, in perfectly acceptable agreement given the error associated with the prediction – see Eqn 2), most of experimental fluxes were higher than those predicted theoretically. This may indicate that the use of the 70:30 v/v ethanol-water vehicle under occlusion had a modest skin penetration enhancement effect.

Table 4. Cumulative transdermal delivery in 24 hours (Q_{24}) and estimated, experimental skin flux (J_{expt} = Q_{24}/24) of the compounds studied.

Compound	Q_{24} ($\mu g.cm^{-2}$)	J_{expt} ($\mu g.cm^{-2}.h^{-1}$)	Area (cm^2) required to deliver 1 mg in 24 hrs[a]
Clomiphene citrate	2.4 ± 2.3	0.10 ± 0.09	417 [2749]
Danazol	7.2 ± 9.1	0.30 ± 0.08	139 [28]
Droloxifene citrate	1.3 ± 0.8	0.06 ± 0.03	694 [326]
Endoxifen	23 ± 12	0.94 ± 0.50	44 [407]
4-OH Tamoxifen	13 ± 6.3	0.52 ± 0.30	80 [324]
Tamoxifen citrate	3.3 ± 1.9	0.14 ± 0.08	297 [995]
Toremifene	5.1 ± 3.5	0.21 ± 0.10	198 [1604]

[a]The first value is determined from the experimental skin permeation data (i.e., area = 1000/Q_{24}); the second number (in square parentheses) is calculated from the theoretically predicted fluxes in Table 3 (i.e., area = 1000/(J_{max} x 24)).

From the predicted and experimental fluxes, it is possible to evaluate the skin area across which J_{max} would have to be sustained to deliver 1 mg of drug over a 24 hours period. These values are also in

Table 4 and suggest that the conventional transdermal delivery approach for the SERMs (whereby the effective plasma levels of a drug following either oral or sublingual or intravenous delivery are matched by those achieved post-application of a patch) is simply not feasible as typical daily doses of the SERMs are closer to 20 mg rather than 1 mg; that is, the areas in Table 4 would, in reality, have to be 20-fold larger.
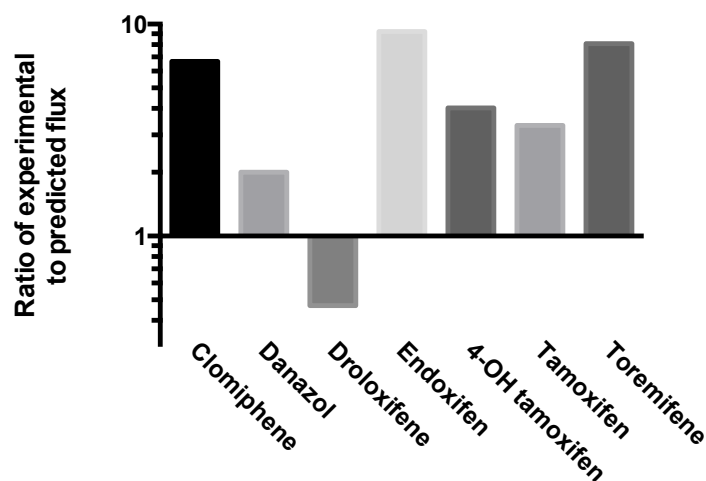

Figure 2.   Ratios of experimental to theoretical fluxes of the compounds studied.

On the other hand, instead of addressing the drug delivery challenge to cancerous breast tissue from the "inside-out" (i.e., deliver the drug to the central compartment and wait for it to distribute to peripheral tissues), consider topical administration from the "outside-in" to achieve the target site levels required *via* transport through the skin, but with significantly reduced systemic exposure. If one assumes the application of a formulation that can sustain J_{max} over the dosing period when administered to the entire surface of an "average" breast (surface area ~200 cm^2, mass ~750 g) [38], then the resulting delivery rates, in nanograms of drug per gram of tissue per hour, based on predicted and experimental J_{max} determinations are reported in Table 5. These tissue delivery rates cannot be readily converted into steady-state concentrations because the local clearance of drug from breast tissue (or, indeed, from different compartments within breast tissue) is not known. While it may ultimately be possible to use the increasingly sophisticated models being developed to characterise skin clearance [39 and references therein], the calculated delivery rates can be compared for now with tissue levels that have been measured and reported in other studies.

For example, tamoxifen and its metabolite concentrations have been measured in serum, normal breast tissue and in breast tumour after repeated daily oral doses of 1, 5 and 20 mg [8]. The resulting serum and breast tumour levels of tamoxifen were 7.5, 25.2 and 83.6 ng.mL^{-1}, and 78.2, 272 and 744 ng.g^{-1}, respectively. Given the range of tissue delivery rates calculated in Table 5 for this drug, it would appear plausible that its topical application to the breast in a suitable formulation may, at steady-state, sustain

tissue concentrations within the range achieved by oral administration. In the same study, the same compartments were also analysed for 4-OH tamoxifen, which is ~25-50 times more potent than the parent drug due to its higher affinity for estrogen receptors [11]. Following the oral administration of 1, 5 and 20 mg of tamoxifen, the serum and breast cancer tissue levels of the 4-OH metabolite were 0.6, 1.3 and 3.1 $ng.mL^{-1}$, and 0.4, 4.4 and 28.5 $ng.g^{-1}$, respectively [8].

Table 5.   Calculated delivery rates of the compounds studied into breast tissue when applied topically in a vehicle capable of sustaining drug delivery at, or close to, the maximum percutaneous flux possible (estimates based upon an application area of 200 cm^2 over a tissue mass of 750 g).

Compound	Calculated delivery rate based on experimentally measured flux ($ng.hr^{-1}$ per gram of tissue)	Calculated delivery rate based on maximum theoretical flux ($ng.hr^{-1}$ per gram of tissue)
Clomiphene citrate	27	4
Danazol	80	40
Droloxifene citrate	16	34
Endoxifen	251	27
4-OH Tamoxifen	139	34
Tamoxifen citrate	37	11
Toremifene	56	7

In a different investigation [27], the plasma and breast tumour levels of 4-OH tamoxifen were determined after (i) oral dosing (20 mg/day), and (ii) topical application of a gel (2 mg/day), both formulations containing the metabolite, not tamoxifen. The median plasma concentration following oral administration was 1.50 $ng.mL^{-1}$, approximately an order of magnitude greater than that detected post-application to the skin (0.16 $ng.mL^{-1}$). In contrast, the respective levels in breast tumour differed by only 2-3 fold: oral, 4.2 $ng.g^{-1}$, *versus* topical, 1.7 $ng.g^{-1}$. It was pointed out that these concentrations would both be effectively anti-estrogenic and that topical administration, relative to oral dosing, had the obvious advantage of delivering a useful quantity of drug to the target tissue while minimising systemic exposure.

It is also worth noting that endoxifen, another metabolite of tamoxifen, is similar, in terms of estrogen binding and estradiol-induced proliferation activities, to 4-OH tamoxifen [9,10,14]. After a 20 mg oral dose of tamoxifen, in fact, endoxifen plasma levels have been reported to be as high as 6 times those of the 4-OH metabolite [9]. Given that the percutaneous delivery of endoxifen has been established in this study and previously, and taking into account its relative pharmacological potency, it is again reasonable

to hypothesise that the topical application of this compound directly to the breast may permit attainment of sufficient local tissue concentrations to produce a useful anti-cancer outcome.

4. Conclusions

The maximum skin fluxes of a series of highly lipophilic compounds with known activity against breast cancer have been predicted theoretically and measured experimentally in a well-accepted model system. Agreement between prediction and measurement was good. The calculated rates of uptake of the drugs, when applied to the entire breast surface from an optimised formulation, suggest that effective, local tissue and tumour levels should be achievable, with the additional benefit that systemic adverse effects may be substantially reduced or avoided completely. Such an "outside-in" approach across the skin to the treatment of breast cancer is considered worthy of further investigation, therefore.

5. Acknowledgements

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7. Figure legends

<u>Figure 1</u>.   Solubility of the compounds studied in different mixtures of ethanol (the %v/v of which is indicated on the graph) and water. Each value shown represents the mean of 3 replicates (standard deviations are too small to be visible on the y-axis scale employed).

<u>Figure 2</u>.   Ratios of experimental to theoretical fluxes of the compounds studied.