JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 787–794

Received December 20, 2006
Accepted February 26, 2007
DOI 10.1211/jpp.59.6.0005
ISSN 0022-3573

Microemulsions as topical delivery vehicles for the anti-melanoma prodrug, temozolomide hexyl ester (TMZA-HE)

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Abstract

A prodrug, temozolomide acid hexyl ester (TMZA-HE), was identified as a skin-deliverable congener for temozolomide (TMZ) to treat skin cancers. Poor solubility and instability of TMZA-HE rendered a serious challenge for formulation of a topical preparation. Microemulsions (ME) were chosen as a potential vehicle for TMZA-HE topical preparations. ME systems were constructed with either oleic acid (OA) or isopropyl myristate (IPM) as the oil phase and tocopheryl (vitamin E) polyethylene glycol 1000 succinate (VE-TPGS) as a surfactant. Topical formulations of OA and IPM ME systems demonstrated beneficial solubilising ability and provided a stable environment for the prodrug, TMZA-HE. Significant differences between the microstructures of OA and IPM ME systems were revealed by freeze fracture electron microscopy (FFEM) and different loading abilities and permeation potencies between the two systems were also identified. In permeation studies, IPM ME systems, with inclusion of isopropyl alcohol (IPA) as a co-surfactant, significantly increased TMZA-HE permeation through silicon membranes and rat skin resulting in less drug retention within the skin, while OA ME systems demonstrated higher solubilising ability and a higher concentration of TMZA-HE retained within the skin. Therefore IPM ME systems are promising for transdermal delivery of TMZA-HE and OA ME systems may be a suitable choice for a topical formulation of TMZA-HE.

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Acknowledgements and funding: The authors would like to thank Tasly Group and Tianjin North Pharma Sci-Tech CO. Ltd China for the provision of TMZ, TMZA and TMZA-HE and the provision of a research grant.

Introduction

During investigation of skin-deliverable congeners for temozolomide for the treatment of skin cancers, temozolomide acid hexyl ester (TMZA-HE) was identified as a potential candidate for further development. In-vitro and in-vivo studies demonstrated that TMZA-HE is readily converted into the parental temozolomide acid (TMZA) and has a promising flux value (J_{ss}) and permeability coefficient (K_p) through rat and human skin (Suppasansatorn et al 2006). In addition, in-vitro cytotoxicity assays showed that TMZA-HE has equal activity to temozolomide and TMZA against glioma and melanoma cancer cell lines; and in a preliminary in-vivo anti-cancer study, TMZA-HE significantly inhibited tumour growth in mice inoculated with melanoma via topical administration (Suppasansatorn et al 2006).

The difficulties associated with formulation development of temozolomide and related compounds are solubility and stability. Temozolomide is virtually insoluble in both organic and aqueous solvents. Its solubility in water is 0.3% (w/w) and in dimethyl sulfoxide (DMSO) is 5% (w/w) (Du 2005). Temozolomide, in solid form, is relatively stable to light and moisture; however, in solution it is instable, and a solution of temozolomide in DMSO develops a red colour within minutes (Du 2005). The instability arises from nucleophilic attack of the triazine ring in temozolomide from all sources of nucleophilic atoms, such as the oxygen in water and DMSO (Wheelhouse et al 1993). Consequently, the number of dosage forms investigated for temozolomide delivery is limited, with only a temozolomide injection (Middleton et al 2000) and micro-crystals (Sampson et al 1999; Heimberger et al 2000) reported, although only oral administration of capsules of temozolomide has been approved.

Although temozolomide has been demonstrated to be effective in treatment of skin cancer, Phase III trials failed to show a significant improvement over dacarbazine (Middleton et al 2000). TMZA-HE has demonstrated a good balance between skin penetration and retention,

an equivalent anti-cancer activity in-vitro to temozolomide and effectiveness at treating tumours in in-vivo studies. Development of a clinically acceptable topical formulation for TMZA-HE could increase local levels of drug, resulting in improved anti-melanoma activity.

In comparison with temozolomide, the solubility of TMZA-HE in aprotic solvents is significantly higher (e.g., solubility of TMZA-HE in DMSO and ethyl acetate is 50% (w/w) and 17% (w/w), respectively, while in water it is 0.04% (w/w)). However, development of a clinically acceptable topical formulation for TMZA-HE remains a challenge when considering limited available excipients. In addition, stability of TMZA-HE in solution towards a nucleophilic species remains a problem, with decomposition in DMSO having been observed (Du 2005).

In recent years, there has been a considerable amount of research into the development of microemulsions (ME) for cutaneous drug delivery (Kreilgaard 2002) due to their improved drug solubilisation, long shelf life and ease of preparation. Since ME systems are thermodynamically stable and are formed spontaneously by simple mixing of the various components (Sintov & Shapiro 2004), an ME system may thus be a suitable formulation for the topical administration of TMZA-HE.

In this study, we investigated the suitability of TMZA-HE for topical delivery and explored ME systems as a vehicle to deliver TMZA-HE. Oleic acid (OA) and isopropyl myristate (IPM) were selected as the oil phase and tocopheryl (vitamin E) polyethylene glycol 1000 succinate (VE-TPGS) as the surfactant. Isopropyl alcohol (IPA) was included as a co-surfactant for formulations with IPM as the oil phase. Drug release and skin penetration and retention were measured for a selected group of promising formulations. The aim of this study was to characterise and evaluate feasibility of a TMZA-HE topical ME formulation for local delivery of the prodrug designed to treat skin cancers.

Materials and Methods

Materials

Temozolomide, TMZA and TMZA-HE were provided by Tasly Group (China). Isopropyl myristate (IPM), oleic acid (OA) and isopropyl alcohol (IPA) were purchased from Sigma-Aldrich Inc. VE-TPGS and sodium dioctyl sulfosuccinate, Aerosol OT NF grade were purchased from Eastman Chemical Company (UK). Acetonitrile and methanol were HPLC grade (Fisher Scientific).

Stability studies of TMZA-HE

Stability of TMZA-HE under high humidity conditions

TMZA-HE powder samples were accurately weighed and widely spread on glass dishes (n = 3). The glass dishes were kept in a chamber with a relative humidity of 92.5% maintained using a saturated solution of potassium nitrate at 25°C. The sample weights were accurately measured and stability was assessed on days 0, 5 and 10 using HPLC.

Photostability of TMZA-HE

TMZA-HE powder samples were accurately weighed and widely spread on glass dishes (n = 3). The samples were kept in a thermostat chamber (25°C) with relative illumination 4500 ± 500 lx. The sample weights were accurately measured and stability was assessed on days 0, 5 and 10 using HPLC.

Solubility study

To determine the solubility of TMZA-HE in water, neat IPM and oleic acid, an excess amount of the drug (0.05 g) was added to 2 mL of these solvents. The mixtures were kept stirring at room temperature for 2 days, then the contents were centrifuged at 10 000 rev min^{-1} for 15 min (Schmalfusz et al 1997; Narishetty & Panchagnula 2004). One-hundred microlitre volumes of supernatant containing the saturated solutions were diluted with 20% v/v acetonitrile in water to 100 mL and the solutions were assayed by HPLC.

Preparation of microemulsions

VE-TPGS was melted at 45 ± 0.2°C. The oil phase, OA or IPM, was then added and kept stirring using a magnetic stirrer until homogeneously mixed. Finally, the mixture was titrated with water and stirred until it became clear. To prepare drug-loaded micoemulsions, a defined amount (1.5, 2.0, 2.5, 3.0% w/w) of TMZA-HE was first dissolved in the oil phase and then molten VE-TPGS was added. The mixture was finally titrated with water to form ME systems.

Construction of ternary phase diagram

Pseudo-ternary phase diagrams of the oil (OA or IPM), water and the surfactant (VE-TPGS) or combination of the co-surfactant (IPA)/surfactant were preliminarily constructed by preparing various combinations of those constituents corresponding to the whole range of the ternary phase diagram. The boundary was subsequently determined by titration of a small amount of water with the desired oil–surfactant ratio obtained from the previous preliminary studies.

Visual inspection after stirring confirmed the formation of a ME and the boundary ratios were then marked as points on the phase diagram. The phase state was classified into three categories: a clear single-phase of low viscosity; a non-birefringent, high viscosity, gel-like phase; and a multiple phase (Kawakami et al 2002; Agatonovic-Kustrin et al 2003). The areas covered by these phases were termed the microemulsion, microemulsion gel and non-microemulsion regions of existence respectively.

Characterisation of microemulsions

Polarisation microscopy

A Polyvar-Met 66 microscope was used to visualise the microemulsions. Formulations that appeared dark when viewed between crossed-polarised light exhibited non-birefringent (isotropic) properties, while the samples not showing non-birefringent (anisotropic) properties were classified as liquid crystals (Baroli et al 2000; Alany et al 2001).

Freeze fracture electron microscopy (FFEM)

Samples were frozen from 26°C as a thin film between two copper double-sided replica specimen holders (Bal-tec AG, BU0 12 055-T) using slushed nitrogen (−210°C). Samples were fractured at −150°C, shadowed with platinum/carbon and coated with carbon in a Polaron E7500 freeze fracture unit mounted on a Polaron E6000 series high vacuum station (Quorum Technologies). The replicas were recovered by submerging the specimen holder and replica in chloroform–methanol (2:1 v/v) (Analar grade) for 2–3 min and then floating the replica off in distilled water. Replicas were transferred with a platinum loop to copper mesh grids (460 lines/inch hexagonal) (Agar Scientific Ltd G2750C) and examined and photographed at 100 KV in a JEOL JEM-1200EX transmission electron microscope.

In-vitro release and permeation studies

TMZA-HE release rates from different ME formulations were measured through a silicone membrane (Advance Bio-Technology, Inc., Silverdale, USA; 0.005" thick) using jacketed Franz-type diffusion cells with a diffusion area of 2.83 cm^2. The membranes were mounted between two chambers and the receptor compartment filled with degassed double-distilled water. Water at 37°C was circulated through the diffusion cells. The system was allowed to equilibrate for 30 min before the addition of samples. The formulations (3 g) were placed on the membrane sheet in the donor compartment. During the experiment, the receptor phase was stirred at 400 rev min^{-1} and at pre-designated time intervals, 1-mL volumes were removed from the receptor phase and replaced with the same volume of water. TMZA-HE was assayed using HPLC.

The permeation experiments were carried out on hairless mouse skin (HsdO1a:MF1; Harlan UK Limited, Bicester, Oxon, UK). Hairless mice, 30–40 g, aged 9–10 weeks, were sacrificed by cervical dislocation and the abdominal skin was then carefully removed using scissors. The underlying fatty tissue subsequently was removed, and then the skin was cut into pieces and used immediately. The experiments were carried out under the same conditions as those used in the release studies. Drug in the receptor chamber was also determined using HPLC. The steady-state flux (J_{ss}) was estimated from the slope of the linear portion of the cumulative amount of drug absorbed against time profiles.

The permeability coefficient was also calculated using the following equation:

$$k_p = J_{ss}/C_v \quad (1)$$

where C_v is the concentration of drug in microemulsion (Gupta et al 2005).

In-vitro skin retention studies

After 24 h of skin permeation studies as described above, the skin was carefully washed with distilled water. The stratum corneum was removed by skin stripping (De Rosa et al 2003). The skins were stretched and fixed in a Petri dish. The stratum corneum was removed by stripping the skin ten times with adhesive tape (3M Microspore). Ten tape strips were incubated in 20 mL of acetronitrile–water (80:20), left overnight, sonicated for 1 min, and then the solution was filtered. The amount of drug in the filtrate was determined by HPLC.

HPLC analysis of TMZA and TMZA-HE

All HPLC studies were carried out under isocratic conditions using a Shimadzu manual injector, LC-10AD pump and SPD-10A UV-Vis detector at 330 nm. All separations were carried out using a flow rate of 1.0 mL min^{-1}, a 20-μL injection volume on a Hypersil ODS 5 μm column (250 × 4.6 mm). TMZA was quantified using a mixture of 0.5% acetic acid–methanol (90:10) as mobile phase and mitozolomide as the internal standard (Suppasansatorn et al 2006). The ester was quantified using acetonitrile–water (80:20) as mobile phase and anthracene as the internal standard (Suppasansatorn et al 2006). A calibration curve of peak area ratio against drug concentration was constructed for each compound giving good linearity ($r \geq 0.999$) and used to quantify the unknown samples.

Statistical interpretation of results

Statistical analysis of the effect of formulations (ME1, 2, 3, 4 and 5) and time on amount of the drug permeated through silicone membrane ($n = 3$), the effect of formulations (ME3 and 5) and time, as well as oil phase (OA and IPM), on amount of the drug permeated through hairless mouse skin ($n = 4–6$), and the effect of formulation (ME3 and ME5) and oil phase (OA and IPM) on amount of the drug retained in the stratum corneum ($n = 4–6$) was performed using a one-way analysis of variance using SPSS. In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's Honestly Significant Difference test and Dunnett's *t*-test; $P < 0.05$ denoted significant difference in all cases.

Results and Discussion

Since instability is a common drawback of temozolomide-type compounds, stability of TMZA-HE is a major concern in formulation development. When applied to skin, the drug in a formulation will come into contact with moisture within the skin and in the surrounding air, and the drug will be exposed to light for a prolonged period until absorption is completed. Therefore stability of TMZA-HE was investigated before formulation.

When TMZA-HE was exposed to a high-humidity environment, there was no change in its appearance; it remained as white scale-like crystals and there was no increase in sample weight, and no evidence of degradation using HPLC. When TMZA-HE was exposed to a strong illumination (4500 ± 500 lx) at 25°C for 10 days, no change in appearance was observed and there was no evidence of degradation.

A formulation for topical delivery of TMZA-HE must demonstrate a high loading and stable environment for the drug. ME systems (Lawrence & Rees 2000; Kawakami et al 2002; Peltola et al 2003) were chosen as they have been

recognised as promising vehicles for cutaneous delivery of drugs, demonstrating high drug loading capacity combined with a penetration-enhancer effect. These systems may increase topical or transdermal delivery of a compound by different mechanisms. Firstly, a large amount of drug can be introduced in the system due to their high solubilisation power. Secondly, an increase in the transdermal flux can be expected because the thermodynamic activity of the drug in the system can be modified to favour partitioning into the stratum corneum, which is composed of keratin-rich dead cells embedded in a lipid matrix and forms the primary barrier of the skin. Thirdly, the surfactants in ME systems may reduce the diffusional barrier of the stratum corneum (Delgado-Charro et al 1997). In some cases, the oil phase itself can act as a penetration enhancer to facilitate transdermal drug delivery (Lawrence & Rees 2000; Kreilgaard 2002).

The choice of components for an ME system is often a balance between drug and excipients. They must be nontoxic, able to form ME systems and to fulfill the requirements of a good vehicle for optimal absorption (i.e., high solubility of drug of interest). After a series of comparative tests with a number of available surfactants, such as lecithin, Tween 80 and sodium dioctyl sulfosuccinate Aerosol OT, VE-TPGS was chosen as a surfactant in the ME systems. VE-TPGS NF is a relatively new excipient and a monograph was recently adopted by the United States Pharmacopoeia (Wu & Hopkins 1999). It is amphipathic and hydrophilic, exhibiting the characteristics of typical surface-active agents and it can also be used as an emulsifier, solubiliser, absorption enhancer and a vehicle for lipid-based drug delivery formulations (Ke et al 2005). In addition, it is considered safe in skin and eye irritation studies (Wu & Hopkins 1999). Several oil like-substances in ME systems have been reported to function as skin enhancers (Peltola et al 2003). In this study, OA and IPM were used as the oil phase. It is known that OA can interact with stratum corneum lipids and disrupt their structures, increasing their fluidity and consequently increasing the flux (Larrucea et al 2001). IPM has been used in several transdermal formulations as a skin penetration enhancer, though its mechanism of action is poorly understood (Peltola et al 2003).

First, the solubility of the drug in the oil phase, OA, IPM and water was measured to inform design of ME systems. OA showed the highest solubilising power, with a drug solubility of $17.22 \pm 0.23\,mg\,mL^{-1}$. TMZA-HE had a solubility of $13.62 \pm 0.67\,mg\,mL^{-1}$ in IPM and, as expected, aqueous solubility was lowest at $0.050 \pm 0.004\,mg\,mL^{-1}$. This implies that OA ME systems may be capable of higher drug loading than corresponding IPM-containing formulations.

Pseudo-ternary phase diagrams of the oil (OA or IPM), water and the surfactant (VE-TPGS) or combinations of the co-surfactant (IPA)/surfactant were preliminarily constructed by preparing various combinations of those constituents corresponding to the whole range of the ternary phase diagram (oil–water–surfactant, 90:10:0, 80:10:10 to 0:10:90). The boundary was subsequently determined by titration of a small amount of water with the desired oil–surfactant ratio obtained from the previous preliminary studies. Phase diagrams for both OA and IPM systems were constructed accordingly (Figure1 A, B, C). The area of phase behaviour was mapped on phase diagrams with the top apex representing water and the other apices showing the oil-like substance (OA or IPM) and surfactant (VE-TPGS) or the combination of surfactant and co-surfactant (VE-TPGS and IPA). The transparent,

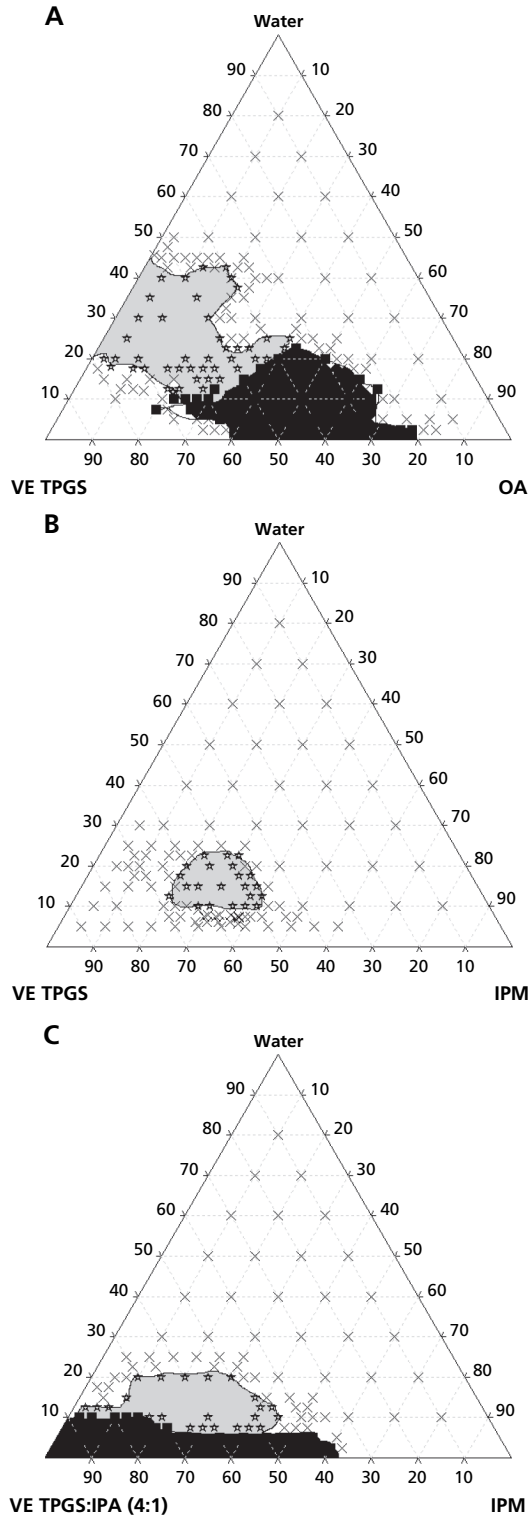

Figure 1 Pseudo-ternary phase diagrams. A. ME systems consisting of VE-TPGS, water and OA. B, ME systems consisting VE-TPGS, water and IPM. C, ME systems consisting VE-TPGS / IPA and IPM.

low-viscosity ME domains are shown in the dark areas, while high-viscosity ME gel domains are represented by the grey regions. The rest of the regions on the phase diagrams represent turbid and conventional emulsions based on visual observations. As shown in Figure 1A, OA used as oil phase can form an ME system without the requirement for any co-surfactant. However, when IPM was used, only a narrow ME gel domain resulted (Figure 1B). The short chain alcohol (IPA) was thus employed as a co-surfactant. The surfactant and co-surfactant ratio was fixed at 4:1. As illustrated in Figure 1C, the ME gel region was broadened, and a liquid ME was formed. This is probably due to lowering of the interfacial tension of the surfactant film resulting in a more flexible and dynamic layer (Kreilgaard 2002; Sintov & Shapiro 2004).

Selected formulations of ME systems, with drug loadings above 1.5% w/v and incorporating a range of excipients, were prepared (Table 1). Since VE-TPGS is a solid waxy substance with a melting point of ~37–41°C (Wu & Hopkins 1999), the key step in preparation of ME systems using VE-TPGS as the surfactant is to melt VE-TPGS first before mixing with other ingredients. To avoid the precipitation of VE-TPGS during formation of an ME, the preparation process was carried out at temperatures higher than the VE-TPGS melting point. In this study, the temperature was maintained at 45°C throughout ME preparation.

Electron microscopy was used to identify cross polarisation of the ME systems (Table 1) and their isotropic properties were confirmed. For ME 1, ME 2 and ME 3, completely isotropic behaviour was observed (the formulations appeared dark between cross polarisers), while gel-like preparations of ME and ME 5 also showed isotropic behaviour.

Freeze fracture electron microscopy (FFEM) is a well-established technique for investigation of the microstructure of ME systems. In FFEM the specimens are rapidly frozen, fractured, shadowed and replicated with a thin metal film. The metal replica of the fracture surface, the morphology of which is controlled by the sample's microstructure, is then viewed using transmission electron microscopy (TEM) (Burauer et al 2003). Microstructures of an ME are defined by its components and their ratios, such as the structures vary systematically from oil droplets in a continuous water phase at high water content to water droplets in a continuous oil phase at high oil content via a bicontinuous network at comparable contents of water and oil. The ME systems in these studies are primarily dominated by an oil phase and the surfactant, while water comprises only a minor component (8–20%). FFEM showed that ME3 with OA as the oil phase appeared to be a water-in-oil globular system (Figure 2A) (Bodet et al 1988), while ME4 with IPM as the oil phase appeared to be a stacked flat bilayer system (Figure 2B) (Jahn & Strey 1988; Bolzinger et al 1998).

Table 1 Composition of MS systems

Formulation	% Drug loading (w/w)	Ingredient (w/w, %)				
		Water	OA	IPM	Vitamin E-TPGS	IPA
ME 1[a]	1.5	20	50	—	30	—
ME 2[a]	2.0	10	50	—	40	—
ME 3[a]	3.0	11	33	—	56	—
ME 4[b]	2.0	8	—	35	57	—
ME 5[c]	2.0	10	—	40	40	10

[a]Chosen from ME system diagram shown in Figure 1A; [b]chosen from ME system diagram shown in Figure 1B; [c]chosen from ME system diagram shown in Figure 1C.

In comparison with TMZA-HE solubility in aqueous solutions (0.04% w/w), the ME systems effectively solubilised the drug, with increases in solubility of up to 75 fold. When OA was used as the oil phase, the loading of the drug in the systems increased with increasing concentration of VE-TPGS (ME 1, ME 2, ME 3 in Table 1). However, for the ME systems with IPM as the oil phase, this phenomenon was not observed and it seemed that increasing VE-TPGS decreased the solubility power of the systems (ME 3 and ME 4 in Table 1). This may because of the higher solubility of TMZA-HE in OA than IPM and the differences in the microstructures of two systems.

In-vitro release of TMZA-HE from the ME formulations was studied using a silicone membrane for 8 h and permeation was studied using full-thickness hairless mice skin for 24 h with Franz diffusion cells. Using a silicone membrane allowed study of the drug flux from the formulations, without the effect of hydrolysis, and only the ester was detected. The cumulative permeated TMZA-HE was assayed using HPLC. The flux value (J_{ss}) and permeability coefficient (K_p) were calculated. The release profiles of TMZA-HE from different ME formulations were first examined with synthetic silicone membrane with TMZA-HE aqueous solution (10% w/w propylene glycol in water) as a control. The flux of TMZA-HE from ME preparations across the membrane continued to increase for the study period over 8 h, while the flux of the drug from the aqueous solution plateaued after 4 h (Table 2). In all cases, ME formulations increased the release rate of TMZA-HE up to 7 fold compared with the control solution. As Table 2 shows, ME 5 is a more effective vehicle in increasing TMZA-HE release and permeation through silicone membrane than ME 4 with the same amount of drug loading. This may due to introduction of IPA into the ME system. Although TMZA-HE loading in ME 3 is higher than in ME 4 and ME 5, the latter resulted in faster release rates. This seems the dermal enhancement effect of IPM used in ME 4 and ME 5 is more effective than OA employed in ME 3. However, only ME 5 has a greater K_p value than the control (Table 2). Among ME 1, ME 2 and ME 3, it was found that an increase of the drug loading in the ME systems subsequently leads to an increase in permeation rate of the drug through silicon membrane. This is in line with the literature report that an increase in drug loading is likely to be an effective method to improve the skin permeation rate of various compounds (Chen et al 2004).

In OA ME systems and in IPM ME systems, ME 3 and ME 5 exhibited a significant increase in TMZA-HE permeation rate through silicone membrane and were selected to test on hairless mouse skin in comparison with the neat oil phases, OA and IPM, as they are expected to act as permeation enhancers in this study. Similar to a previous report

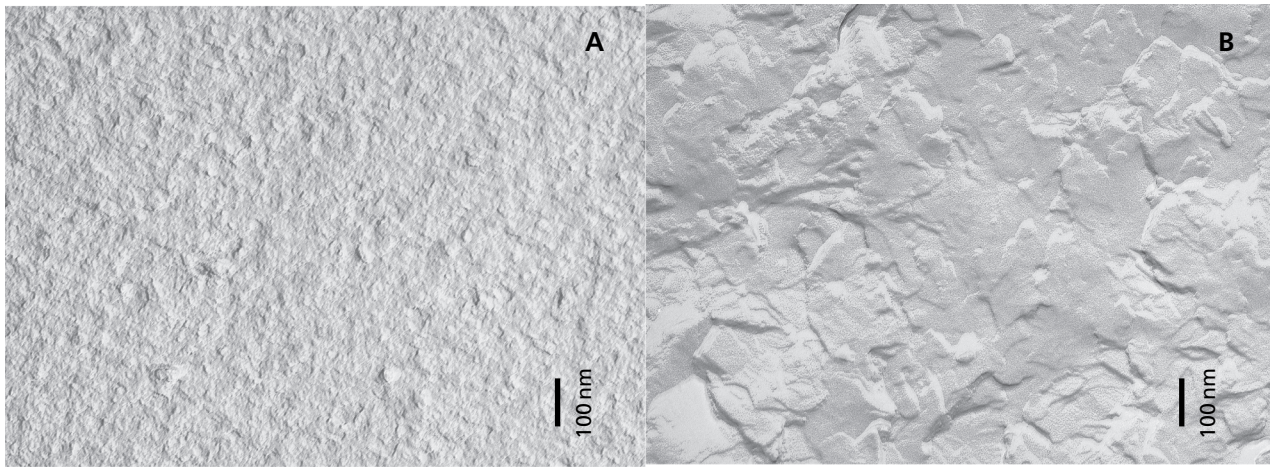

Figure 2 Images of freeze fractured ME 3 with OA as oil phase (A) and freeze fractured ME 5 with IPM as oil phase (B).

(Suppasansatorn et al 2006), TMZA-HE was hydrolysed by esterases within the skin generating TMZA and a small amount of TMZA-HE was also detected (up to 10% of total amount permeated; data not shown). Both TMZA-HE and TMZA were detected in the receptor fluid using their individual HPLC conditions. The concentration of each compound was calculated in nanomoles and the result expressed as the sum of both concentrations.

Figure 3 shows the summed permeation profile through full-thickness hairless mice skin. As can be seen in Table 3, TMZA-HE fluxes from ME formulations were considerably higher (up to 7 fold) than those from their neat oil constituents. The drug flux from neat IPM is higher than that from neat OA. This implies IPM is more effective than OA in increasing TMZA-HE permeation rate through the skin and provides some explanation as to why ME 4 and ME 5 showed higher flux than ME 1, ME 2 and ME 3 in the experiments with silicon membranes. As expected, ME 5 preparation showed the highest permeation rate through full-thickness hairless mice skin.

Table 2 In-vitro TMZA-HE fluxes through silicone membrane

Formulation	Drug loading ($mg mL^{-1}$)	Flux (J_{ss}) ($nmol\ cm^{-2}h^{-1}$)	Permeability coefficients (K_p) ($cm\ h^{-1} \times 10^3$)
ME 1[a]	15.3	67.94 ± 2.23	12.39 ± 0.41
ME 2[a]	20.8	79.01 ± 5.04	10.60 ± 0.68
ME 3[a]	31.8	101.00 ± 1.83	8.86 ± 0.16
ME 4[b]	22.4	162.86 ± 19.26	20.29 ± 2.40
ME 5[c]	21.6	263.58 ± 4.90	34.05 ± 0.63
Control (aqueous solution[d])	0.4[e]	35.14 ± 5.95[e]	22.64 ± 3.83

Data are presented as mean ± s.d., n = 3. [a]Chosen from ME system diagram shown in Figure 1A; [b]chosen from ME system diagram shown in Figure 1B; [c]chosen from ME system diagram shown in Figure 1C; [d]10% w/w propylene glycol in water; [e]data from previous report (Suppasansatorn et al 2006).

Skin stripping was used to determine TMZA-HE retention in the skin following skin permeation experiments. Drug retained in the skin was extracted and determined by HPLC. Both TMZA-HE and TMZA were detected in the skin extraction studies; however, in comparison with the permeation experiments, only a relatively small amount of TMZA was detected, less than 20% of total amount drug retained (data

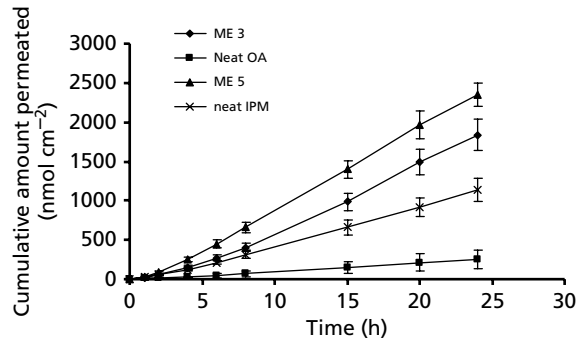

Figure 3 Permeation profiles for TMZA-HE (combination amounts of TMZA-HE and TEMA) from ME 3, ME 5, OA and IPM though hairless mouse skin (mean ± s.d., n = 4–6).

Table 3 In-vitro TMZA-HE flux through hairless mice skin

Formulation	% Drug loading ($mg mL^{-1}$)	Flux ($nmol\ cm^{-2}h^{-1}$)	Permeability coefficients (K_p) ($cm\ h^{-1} \times 10^3$)
1 ME 3	31.8	78.70 ± 8.26	6.90 ± 0.73
2 Neat OA^{Sat}	17	10.80 ± 5.38	1.77 ± 0.88
3 ME 5	21.6	101.56 ± 7.08	13.12 ± 0.91
4 Neat IPM^{Sat}	13	47.97 ± 6.62	10.30 ± 1.42

Data are presented as mean ± s.d., n = 4–6. OA^{Sat}, saturated TMZA-HE in OA; IPM^{Sat}, saturated TMZA-HE in IPM.

Table 4 Drug retention in stratum corneum and permeation through hairless mouse skin over 24 h

Formulation	Cumulative amount of drug permeated (nmol cm^{-2})	Amount of drug retained (nmol cm^{-2})	Retention of permeated dose (%)
ME 3	1839.47 ± 148.67	5.66 ± 2.18	0.31 ± 0.12
Neat OA	245.67 ± 151.37	7.29 ± 0.62	3.31 ± 0.99
ME 5	2353.76 ± 195.25	4.83 ± 2.01	0.21 ± 0.10
Neat IPM	1135.41 ± 119.79	2.01 ± 0.81	0.18 ± 0.06

Data are presented as mean ± s.d., n = 4–6.

not shown). This was expected as the esterase enzyme is not active in the stratum corneum layer. The combination of retained TMZA-HE and TMZA was calculated in nanomoles. Neat OA shows the greatest drug retention in stratum corneum (3.31 ± 0.99% of drugs permeated, Table 4), while the least retention was found with neat IPM (0.18 ± 0.06%). The amount of retained drugs in stratum corneum for these two systems was determined by the oil phase used. IPM systems showed lower retained drug than OA systems (0.31 ± 0.12% and 0.21 ± 0.10% for ME 3 and ME 5, respectively).

This experiment demonstrated that both ME 5 and ME 3 systems were able to deliver the drug to the skin and retention of the drug in the skin was dependent on the oil phase used (Table 3, 4). The significance of the results from this experiment is that the ME formulations can behave as both topical and transdermal skin delivery systems. Neat OA showed the lowest TMZA-HE flux through hairless mice skin (Table 4), but it can deliver drug extensively to accumulate in the skin; therefore, OA is likely to be a promising vehicle for topical delivery of TMZA-HE. In contrast, IPM ME systems might be more suitable for transdermal delivery.

Conclusion

A topical formulation of the prodrug TMZA-HE for treatment of skin cancer is feasible since it demonstrates enhanced stability compared with the parent compound. Microemulsion systems with OA or IPM as the oil phase and VE-TPGS as the surfactant demonstrated beneficial solubilising ability for TMZA-HE and provided a stable environment for the prodrug TMZA-HE. Using IPM as the oil phase, and inclusion of the co-surfactant IPA, significantly increased TMZA-HE permeation through silicon membranes and rat skins, resulting in less drug retention within the skin. Therefore IPM ME systems are promising for transdermal delivery of TMZA-HE. For a topical formulation of TMZA-HE, OA ME systems may be more effective due to their higher solubilising ability and increased retention of drug within the skin.

References

Agatonovic-Kustrin, S., Glass, B. D., Wiseh, M. H., Alany, R. G. (2003) Prediction of a stable microemulsion formulation for the oral delivery of a combination of antitubercular drugs using ANN methodology. *Pharm. Res*. **20**: 1760–1765

Alany, R. G., Tucker, I. G., Davies, N. M., Rade, T. (2001) Characterizing colloidal structure of pseudoternary phase diagrams of formed by oil/water amphiphile systems. *Drug Develop. Ind. Pharm*. **27**: 31–38

Baroli, B., Arturo López-Quintela, M., Begoña Delgado-Charro, M., Fadda, A. M., Blanco-Méndez, J., (2000) Microemulsion for topical delivery of 8-methoxsalen. *J. Control. Release* **69**: 209–218

Bodet, J.-F., Bellare, J. R., Davis, H. T., Scriven L. E., Miller W. G. (1988) Fluid microstructure transition from globular to bicontinuous in midrange microemulsion. *J. Phys. Chem*. **92**: 1898–1902

Bolzinger, M. A., Carduner, T. C., Poelman, M. C. (1998) Bicontinuous sucrose ester microemulsion: a new vehicle for topical delivery of niflumic acid. *Int. J. Pharm*. **176**: 39–45

Burauer, S., Belkoura, L., Stubenrauch, C., Strey, R. (2003) Bicontinuous microemulsions revisited: a new approach to freeze fracture electron microscopy (FFEM). *Colloids Surf. A; Physicochem. Eng. Aspects* **228**: 159–170

Chen, H., Chang, X., Weng, T., Zhao, X., Gao, Z., Yang, Y., Xu, X., Yang, X, (2004) A study of microemulsion for transdermal delivery of triptolide. *J. Control. Release* **98**: 427–436

De Rosa, F. S., Tedesco, A. C., Lopez, R. F. V., Pierre, M. B. R., Lange, N., Marchetti, J. M., Rotta, J. C. G., Bentley, M. V. L. B. (2003) *In vitro* skin permeation and retention of 5-aminolevulinic acid ester derivatives for photodynamic therapy. *J. Control. Release* **89**: 261–269

Delgado-Charro, B. M., Iglesias-Vilas, G., Blanco-Méndez, J., Arturo López-Quintela, M., Marty, J.-P., Guy, R. H. (1997) Delivery of a hydrophilic solute through the skin from novel microemulsion systems. *Eur. J. Pharm. Biopharm*. **43**: 37–42

Du, L. (2005) *Preparation of microemulsion on TDS and pharmacology study for anticancer prodrug TCE*. Master dissertation of Shenyang Pharmaceutical University, May 2005

Gupta, R. R., Jain, S. K., Varshney, M. (2005) AOT water-in-oil microemulsions as a penetration enhancer in transdermal drug delivery of 5-fluorouracil. *Colloids and Surfaces B: Biointerfaces*. **41**:25–32

Heimberger, A. M., Archer, G. E., Mclendon, R. E., Hulette, C., Friedman, A. H., Friedman, H. S., Bigner, D. D., Sampson, J. H, (2000) Temozolomide delivery by intracerebral microinfusion is safe and efficacious against malignant gliomas in rats. *Clin. Cancer Res*. **6**: 4148–4153

Jahn, W., Strey, R. (1988) Microstructure of microemulsions by freeze electron microscopy. *J. Phys. Chem*. **92**: 2294–2301

Kawakami, K., Yoshikawa, T., Moroto, Y., Kanaoka, E., Takahashi, K., Nishihara, Y., Masuda, K, (2002) Microemulsion formulation for enhanced absorption of poorly soluble drugs. I. Prescription design. *J. Control. Release* **81**: 65–74

Ke, W.-T., Lin, S.-Y., Ho, H.-O., Sheu, M.-T. (2005) Physical characterization of microemulsion systems using tocopheryl polyethylene glycol 1000 succinate (TPGS) as a surfactant for the oral delivery of protein drugs. *J. Control. Release* **102**: 489–507

Kreilgaard, M. (2002) Influence of microemulsions on cutaneous drug delivery. *Adv. Drug Del. Rev*. **54**: S77–S98

Larrucea, E., Arellano, A., Santoyo, S., Ygartua, P. (2001) Combined effect of oleic acid and propylene glycol on percutaneous penetration of tenoxicam and its retention in the skin. *Eur. J. Pharm. Biopharm*. **52**: 113–119

Lawrence, M. J., Rees, G. D. (2000) Microemulsion-based media as drug delivery systems. *Adv. Drug Del. Rev*. **45**: 89–121

Middleton, M. R., Grob, J. J., Aaronson, N., Fierlbeck, G., Tilgen, W., Seiter, S., Gore, M., Aamdal, S. Cebon, J., Coates, A., Dreno, B., Henz, M., Schadendorf, D., Kapp, A., Weiss, J., Fraass, U., Statkevich, P., Muller, M., Thatcher, N. (2000) Randomised phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. *J. Clin. Oncol*. **18**: 158–166

Narishetty, S. T. K., Panchagnula, R. (2004) Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. *J. Control. Release* **95**: 367–379

Peltola, S., Saarinen-Savolainen, P., Kiesvaara, J., Suhonen, T. M., Urtti, A. (2003) Microemulsion for topical delivery of estradiol. *Int. J. Pharm*. **254**: 99–107

Sampson, J. H., Archer, G. E., Villavicencio, A. T., McLendon, R. E., Friedman, A. H., Bishop, W. R., Bigner, D. D., Friedman, H. S. (1999) Treatment of neoplastic meningitis with intrathecal temozolomide. *Clin. Cancer Res*. **5**: 1183–1188

Schmalfusz, U., Neubert, R., Wohlrab, W. (1997) Modification of drug penetration into human skin using microemulsion. *J. Control. Release* **46**: 279–285

Sintov, A. C., Shapiro, L. (2004) New microemulsion vehicle facilitates percutaneous penetration in vitro and cutaneous drug bioavailability in vivo. *J. Control. Release* **95**: 173–183

Suppasansatorn, P., Wang, G., Conway, B. R., Wang, W., Wang, Y. (2006) Skin delivery potency and antitumor activities of temozolomide ester prodrugs. *Cancer Lett*. **244**: 42–52

Wu, S. H., Hopkins, W. K. (1999) Characteristics of D-α-tocopheryl PEG-1000 succinate for application as an absorption enhancer. *Pharm. Technol*. **23**: 2–10

Wheelhouse, R. T., Stevens, M. F. G. (1993) Decomposition of antitumour drug temozolomide in deuteriated phosphate buffer: methyl group transfer in accompanied by deuterium exchange. *J. Chem. Soc., Chem. Commun*. **15**: 1177–1178