International Journal of Pharmaceutics 366 (2009) 58–64

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Permeation of quinine across sublingual mucosa, *in vitro*

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ARTICLE INFO

Article history:
Received 23 June 2008
Received in revised form 28 August 2008
Accepted 28 August 2008
Available online 12 September 2008

Keywords:
Quinine
Malaria
Leg cramps
Sublingual
Tongue

ABSTRACT

Quinine is the first line treatment in severe *P. falciparum* malaria and nocturnal leg cramps and a fast, convenient delivery method of this drug quinine is needed. The purpose of this study was to investigate *in vitro* the sublingual route for the delivery of quinine. Permeation studies were carried out with Franz diffusion cells containing sublingual mucosa membranes with PBS receptor phase and dosed with solutions of quinine hydrochloride or quinine/2-hydroxypropyl-β-cyclodextrin complexes. Receptor phase samples were taken 2 hourly over a 12 h period and quinine was determined by reverse-phase HPLC analysis. The ventral surface of the tongue was significantly more permeable than porcine floor of the mouth ($p < 0.05$) and there was no significant effect of freezing on the ventral surface of the tongue (p 0.2444). The presence of saliva caused a decrease in the permeation of quinine across the ventral surface of the tongue by up to 68%. Inclusion complexation between quinine and 2-HP-β-CD was supported by ^{1}H NMR spectral data, and an ethanol vehicle provided the highest quinine flux from the inclusion complex solutions compared to deionised water and PEG. Overall, the data support further investigations into the clinical use of sublingual quinine, particularly for children with falciparum malaria or patients with nocturnal leg cramps. Use of quinine/cyclodextrin inclusion complexes may circumvent compliance issues due to bitter taste.
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1. Introduction

Quinine is a relatively inexpensive drug derived from cinchona tree bark and was used to treat fever in the 17th century (Huston and Levinson, 2006). Growing resistance to chloroquine means that the use of quinine has increased for the treatment of *P. falciparum* malaria, and intravenous infusion of quinine is the first line treatment in severe falciparum malaria in both adults and children (Lalloo et al., 2007). The patient switches to oral therapy when able to swallow. Parenteral administration of quinine requires a trained health technician, proper equipment and hygienic conditions, which are not always available in rural Africa. WHO (2007) recommends that anti-malarial treatment commences within 24 h of the onset of symptoms but the lack of local clinics means that many children in rural Africa die before hospital admission (Fawaz et al., 2004). Furthermore, it may not always be feasible to administer oral quinine prior to hospital admission for intravenous administration. Patients suffering from malaria may experience nausea, vomiting and convulsions; in severe malaria, the patient may even be unconscious. Therefore, there is a need for an alternative dosage form of quinine which has a rapid onset of action, does not require specific skills and facilities and avoids the enteral route of administration.

Quinine is also used to treat nocturnal leg cramps, although questions have been asked concerning its safety and efficacy in such cases (Man-Son-Hing and Wells, 1995; Man-Son-Hing et al., 1998). Quinine sulphate tablets are currently prescribed in the UK, although the FDA recently banned the drug for this indication due to a potentially unfavourable risk/benefit profile (Huston and Levinson, 2006). Nocturnal leg cramps are more common among the elderly (Hall, 1947), many of whom will be receiving complicated oral regimens. An alternative method for delivering quinine, administrable upon need and with rapid action onset would clearly be beneficial. There has been some research into the development of rectal quinine (Fawaz et al., 2004; Koffi et al., 2008), although this route of administration is neither readily accessible nor highly accepted by patients.

Oral mucosal membranes are currently of interest as targets for a range of drug delivery systems. The oral mucosa can be divided into the masticatory mucosa, specialised mucosa and lining mucosa. Some 60% of the oral mucosa consists of the lining mucosa (Collins and Dawes, 1987), a thin, non-keratinised, stratified squamous epithelium, which includes the lips, soft palate, buccal mucosa, the floor of the mouth and the ventral surface (underside) of the tongue. Sublingual is the area under the tongue, but in *in vitro* sublingual permeation studies, the sublingual mucosa frequently refers to the floor of the mouth (Chen et al., 1999; Zhang et al., 2002). There have been very few studies on the permeation of drugs across the ventral surface of the tongue, even though drugs administered sublingually, whether as tablets or

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doi:10.1016/j.ijpharm.2008.08.048

sprays are equally exposed to this region as well as the floor of the mouth.

Drugs delivered by the sublingual route have been shown to have a faster clinical onset of action than their oral equivalents (Zhang et al., 2002). Sublingual drug delivery is more commonly used to treat acute disorders, whereas the buccal route is chosen when a prolonged release of drug is needed in chronic disorders (Rossi et al., 2005). The sublingual mucosa is thinner (approximately 190 μm compared to 580 μm of the buccal mucosa) (Squier and Wertz, 1996) and more permeable than the buccal mucosa (Squier and Hall, 1985a; Lesch et al., 1989), which makes it more favourable if a rapid onset of pharmacological effect is required. Furthermore, it is more difficult to retain a buccal tablet in the pouch of the cheek than it is to administer a spray under the tongue.

The sublingual mucosa is highly vascularised and so drugs delivered sublingually are absorbed directly into the bloodstream thereby avoiding gastrointestinal tract degradation and first pass metabolism (Rathbone et al., 1996). This means that sublingual delivery could increase the bioavailability of the drug as compared to the orally administrated drug. Gastric emptying and the presence of food in the gastrointestinal tract will affect oral drug absorption, but not drugs delivered sublingually. Moreover, the sublingual route is non-invasive and not as intimidating as other delivery routes, thus encouraging patient compliance.

Sprays and fast dissolving tablets are the two most widely used formulations for sublingual delivery. Sublingual sprays have a faster onset of action compared to tablets because time is needed for the dissolution of tablets (Parker et al., 1986; Reisin et al., 1988; Marmor, 1990; Ducharme et al., 1999). Furthermore, the time needed for tablet dissolution is variable from one individual to another (Noonan and Benet, 1985; Reisin et al., 1988; Ducharme et al., 1999). Young children may also find it difficult to retain the sublingual tablet under the tongue for sufficient time.

Quinine is well known for its bitter taste which could result in compliance issues. Bitter taste buds are located at the rear of the dorsal surface of the tongue (Szejtli and Szente, 2005), and quinine sprayed under the tongue should in theory avoid their stimulation. However, this assumes all the quinine is instantly absorbed and none migrates to the dorsal surface. The sublingual area is clearly a moist environment with constant efflux of saliva from the salivary glands in the mouth floor and, in practice, it will be very difficult to prevent all quinine molecules migrating to the bitter taste bud region. Taste maskers can be added to formulations to modulate the bitter sensation, although the similar effects can also be achieved by drug encapsulation. Cyclodextrins can modulate the bitter taste of drugs either by enwrapping the drug in the cavity to limit the interaction of the bitter drug with the taste buds, or by directly interacting with taste buds (Szejtli and Szente, 2005).

This work tested *in vitro* the hypothesis that quinine can be effectively delivered via the sublingual route. The *in vitro* permeation of quinine across porcine ventral surface of the tongue and the floor of the mouth were compared to determine the most effective region for delivery. Further comparisons were made between frozen versus non-frozen ventral tongue membranes, and the absence versus presence of saliva. Finally, the permeation of quinine from quinine/2-hydroxypropyl-β-cyclodextrin inclusion complexes was determined from three vehicles.

2. Materials and methods

2.1. Materials

Quinine hydrochloride dihydrate, 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD), phosphate buffered saline (PBS) and polyethylene glycol 400 (PEG) were purchased from Sigma–Aldrich Company (Poole, UK). Methanol, ethanol, acetonitrile, ammonium formate and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific (Loughborough, UK). Saliva was obtained from a volunteer and sterilised by filtration through a 0.22 μm membrane. Porcine ears, prior to steam cleaning, were obtained from a local abattoir and stored at −20 °C. Porcine heads and tongues were bought either from the local abattoir or a local market, and used either directly or stored at −20 °C.

2.2. Preparation of porcine membranes

Porcine and human oral membranes are similar in composition (Heaney and Jones, 1978), structure (Squier et al., 1991) and permeability measurements (Squier and Wertz, 1996), making porcine oral mucosa suitable as a model for human oral mucosa. Permeability across the porcine oral mucosa is not metabolically linked therefore it is not important for the tissue to be viable (Squier and Hall, 1985b). Porcine floor of mouth and ventral (underside) tongue mucosa membranes were excised by blunt dissection using a scalpel. The excised mucosa were cut into approximately 1 cm squares and frozen on aluminium foil at −20 °C until used (<2 weeks). For non-frozen ventral surface of porcine tongue, the mucosa was used in the permeation studies within 3 h of excision.

2.3. Preparation of quinine hydrochloride solutions

Quinine is available in two main chemical forms, the lipophilic free base or hydrophilic salt, e.g., hydrochloride or phosphate. Due to the hydrated nature of the oral mucosa and low solubility of quinine in PBS, quinine hydrochloride was used in this work. Solutions of quinine HCl were prepared at two concentrations for use in the permeation experiments. Quinine hydrochloride dihydrate (100 or 300 mg) was weighed out and made up to 10 mL with ethanol, producing 10 and 30 mg mL^{-1} solutions respectively.

2.4. Preparation of quinine/2-hydroxypropyl-β-cyclodextrin (Q/2-HP-β-CD) complex

Quinine hydrochloride dihydrate and 2-HP-β-CD were co-precipitated in equal molar proportions. 1.81 g of quinine hydrochloride dihydrate (MW = 396.92, 5 mmol) and 7 g of 2-HP-β-CD (MW = 1400, 5 mmol) were weighed. The quinine hydrochloride and 2-HP-β-CD were then dissolved in 200 mL deionised water, stirred using a magnetic stirrer and then transferred to a rotary evaporator and reduced until a dry, white solid was formed. Saturated solutions of Q/2-HP-β-CD in deionised water, ethanol and polyethylene glycol (PEG) were prepared in an incubator at 32 °C. The Q/2-HP-β-CD complexes were dosed to the sublingual membranes as saturated solutions to provide comparability in terms of thermodynamic activity. Aliquots of 1 mL of deionised water, ethanol or PEG were added to Eppendorf vials and the Q/2-HP-β-CD complex added until further complex was unable to dissolve in the solvent and precipitation was formed at the bottom of the vial. The saturated solutions were centrifuged at 13.2 × 1000 rpm for 10 min. The supernatant was sampled and applied to the donor compartments of the Franz diffusion cells.

2.5. In vitro permeation studies

The permeability of the membranes to quinine was determined using all-glass Franz diffusion cells with a nominal receptor volume of 3.6 mL and diffusional area of 0.2 cm^2. The cell flanges were greased with high performance vacuum grease and the membranes

mounted between the receptor and donor compartments, with the mucosal surface uppermost. Clamps were used to hold the membranes into position before the receptor compartments were filled with degassed phosphate buffered saline (PBS), pH 7.4. Micromagnetic stirrer bars were added to the receptor compartments and the complete cells were placed in a water bath at 37 °C. The membranes were equilibrated with PBS applied to the donor compartments for 20 min before being aspirated with a pipette. Aliquots of 5 μL of the quinine solution or 100 μL of the saturated solutions of Q/2-HP-β-CD complex in different vehicles were applied to each of the donor compartments. In the study to determine the effect of saliva on the permeation of quinine across the ventral surface of the tongue, 100 μL of sterile saliva was added to the donor compartments before adding 5 μL of the quinine solution.

At 2, 4, 6, 8, 10 and 12 h, the receptor phases were withdrawn from the sampling ports and aliquots of 1 mL samples were transferred to HPLC autosampler vials, before being replaced with fresh PBS stored at 37 °C. Apart from the studies involving Q/2-HP-β-CD saturated solutions (where an infinite dose was applied at the start of the experiments), 5 μL of the respective quinine solution was reapplied to the donor phase up to 10 h. The purpose of this was to represent a hypothetical in-use finite dosing regimen based upon an interval of 2 h between doses. At least 3 replicates were carried out for each study.

2.6. HPLC analysis

The amount of quinine in the receptor phases was determined using an Agilent 1100 Series chromatograph, fitted with a Gemini 5 μm C18 150 mm × 4.6 mm column (Phenomenex, Macclesfield, UK). The HPLC method was adapted from a previous report (Cole and Heard, 2007), with a mobile phase consisting of methanol: acetonitrile: 0.7% (w/v) ammonium formate solution, 35:20:45% (v/v). The flow rate was 1 mL min^{-1}, injection volume 20 μL and UV detection was at 281 nm. Quinine retention time was 6.5 min.

2.7. NMR analysis

Nuclear magnetic resonance (NMR) can be used to determine the successful formation of a guest–host inclusion complex (Wang and Chen, 1995; Consonni et al., 2004). In this case, the guest molecule is quinine and the host molecule is 2-HP-β-CD. ^{1}H NMR spectra of quinine hydrochloride, 2-HP-β-CD, and Q/2-HP-β-CD complexes in DMSO were measured using a Bruker Avance 500 spectrometer at 298.1 K.

2.8. Data analysis

Cumulative amounts of quinine permeated per unit area (i.e., μg cm^{-2}) were plotted against time (h) over the 12 h experimental period. The gradient of the linear section of the curve (generally between 4 and 12 h) was calculated to provide the value for the steady-state flux (J_{ss}). All J_{ss} values were reported as the mean ± S.D. (μg cm^{-2} h^{-1}).

2.9. Statistical analysis

Statistical analyses were carried out using Instat 3 for Macintosh GraphPad Software, Inc. (Hercules, CA, USA). Unpaired *t*-tests with Welch correction were used to investigate differences between two data sets. A *p*-value of <0.05 was considered significant, and a *p*-value of <0.0001 was considered extremely significant.

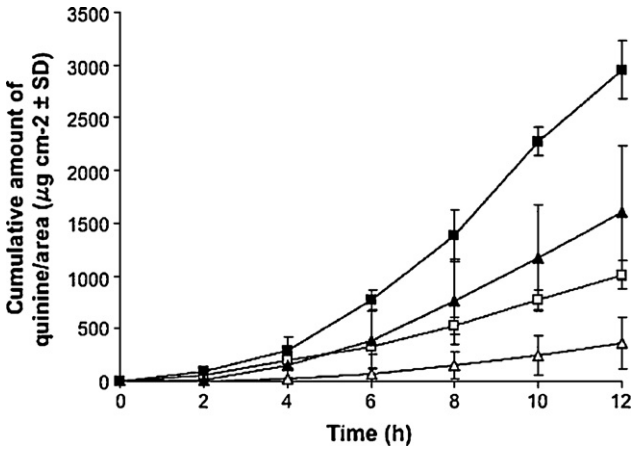

Fig. 1. Plot comparing the permeation of quinine across the ventral surface of the tongue and floor of the mouth at two different concentrations: (□) tongue, 10 mg mL^{-1}, (■) tongue 30 mg mL^{-1}, (△) floor, 10 mg mL^{-1}, (▲) floor, 30 mg mL^{-1}, ($n \geq 3$, ±S.D.).

3. Results

3.1. Permeation of quinine across different porcine membranes

Membranes from two sublingual zones were examined: the ventral surface of the tongue, the floor of the mouth. The permeation profiles of quinine across the ventral surface of the tongue and floor of the mouth at two dosage levels are illustrated in Fig. 1. It is clear from the permeation profiles and Table 1 that the ventral surface of the tongue was more permeable than the floor of the mouth at both concentrations of quinine used in this study.

An unpaired *t*-test with Welch correction showed that there was a very significant difference between the flux of quinine across the ventral surface of the tongue and the floor of the mouth with both 10 and 30 mg mL^{-1} quinine solutions (*p* 0.0037 and 0.0055 respectively). For the 10 mg mL^{-1} quinine solution, the ventral surface of the tongue was approximately twice as permeable compared to the floor of the mouth. For the 30 mg mL^{-1} quinine solutions, there was a 1.8-fold difference between the ventral surface of the tongue and the floor of the mouth in terms of the steady state flux of quinine.

As expected, there was a significant statistical difference between the flux of quinine from the 10 and 30 mg mL^{-1} quinine solutions across the ventral surface of the tongue (*p* 0.0108). With the floor of the mouth, there was a very significant difference between the permeation of quinine from the 10 and 30 mg mL^{-1} quinine solutions (*p* 0.0027). Increasing the quinine concentration thus resulted in a 3.2-fold increase in flux across the ventral surface of the tongue, whereas increasing the quinine concentration from 10 to 30 mg mL^{-1} resulted in a 3.6-fold increase in flux across the floor of the mouth.

Table 1
Steady-state flux of quinine from an ethanol vehicle across the ventral surface of the tongue, floor of the mouth, with and without 100 μL saliva.

Region	[Quinine] (mg mL^{-1})	J_{ss} (μg cm^{-2} h^{-1})
Floor of the mouth	10	58.05 ± 32.89
Ventral surface of tongue	10	115.11 ± 21.12
Ventral surface of tongue + 100 μL saliva	10	36.70 ± 4.98
Floor of the mouth	30	210.49 ± 69.89
Ventral surface of tongue	30	372.96 ± 44.40
Ventral surface of tongue + 100 μL saliva	30	128.80 ± 17.05
Ventral surface of tongue, non-frozen	30	315.19 ± 53.18

Previously frozen tissue unless otherwise stated ($n \geq 3$, ±S.D.).

3.2. Effect of freezing on the permeation of quinine across the ventral surface of the tongue

Although Table 1 suggests that the frozen ventral surface of the tongue may have been slightly more permeable to quinine compared to the non-frozen tissue, no statistically significant difference was found at the 5% level (p 0.2444).

3.3. Effect of saliva on the permeation of quinine across the ventral surface of the tongue

The purpose of this experiment was to mimic an *in vivo* scenario, where the dose would be administered in the presence of saliva. Table 1 shows that the presence of 100 μL saliva in the donor phase decreased the permeation of quinine by 68%. According to the unpaired t-test with Welch correction, there was an extremely significant difference between the permeation of quinine from the 10 mg mL^{-1} quinine solution across the ventral surface of the tongue in the presence and absence of saliva in the donor phase (p 0.0003). A significant statistical difference was observed in the permeation of quinine from the 30 mg mL^{-1} quinine solution across the ventral surface of the tongue in the presence and absence of saliva (p 0.012).

3.4. Complexation of quinine and 2-HP-β-CD

Co-precipitation of quinine and 2-HP-β-CD produced a white solid compound. ^{1}H NMR spectroscopy can be used to confirm if a guest molecule has been successfully taken up by the cyclodextrin as the formation of an inclusion complex will produce chemical shifts of the protons, and representative spectra are shown in Fig. 2. Table 2 shows the effect of quinine on the ^{1}H chemical shifts of 2-HP-β-CD in DMSO. H_1, H_3, H_5 and H_6 of the 2-HP-β-CD experienced relatively larger downfield shifts; H_4 experienced a relatively greater upfield shift compared to H_2.

3.5. Permeation of quinine from Q/2-HP-β-CD complex in different vehicles

It is clear from Fig. 3 and Table 3 that the highest steady-state flux for quinine from the Q/2-HP-β-CD complex across the ventral surface of the tongue was observed from the ethanol vehicle. Unpaired t-test with Welch correction between ethanol with deionised water; and ethanol with PEG provided p-values of 0.0156 and 0.0154 respectively, showing that there was a significant difference between ethanol with water and PEG. Statistically, a significant difference was not seen between water and PEG as vehicles for the complex (p 0.4495). The permeation of quinine from Q/2-HP-β-CD in ethanol was some 40 times higher than with deionised water as

Table 2
^{1}H NMR chemical shift ($\Delta\delta$) values for 2-HP-β-CD in the free and complex states measured in DMSO.

Proton	$\delta_{2\text{-HP-β-CD}}$ (ppm)	$\delta_{Q/2\text{-HP-β-CD}}$ (ppm)	δ (ppm)
H_1	4.825	4.843	0.018
H_2	3.319	3.317	−0.002
H_3	3.741	3.760	0.019
H_4	3.230	3.213	−0.017
H_5	3.550	3.566	0.016
H_6	3.620	3.640	0.020

Table 3
Steady-state flux of quinine from Q/2-HP-β-CD complex across the ventral surface of tongue in deionised water, ethanol and PEG (n = 3, ±S.D.).

Vehicle	J_{ss} (μg cm^{-2} h^{-1})
Deionised water	31.22 ± 9.89
Ethanol	1259.2 ± 268.27
PEG	22.36 ± 14.67

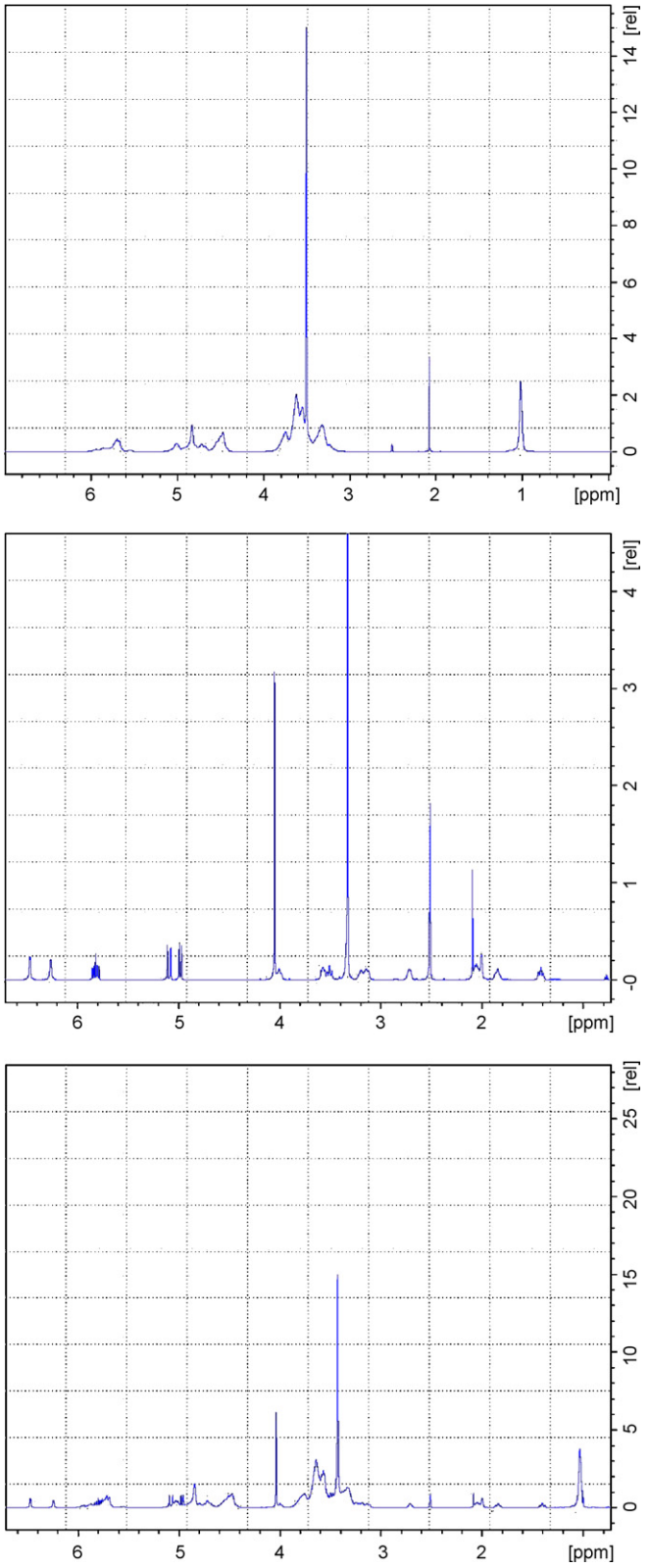

Fig. 2. ^{1}H NMR spectra of 2-HP-β-CD (upper), quinine HCl and Q/2-HP-β-CD (lower) in DMSO.

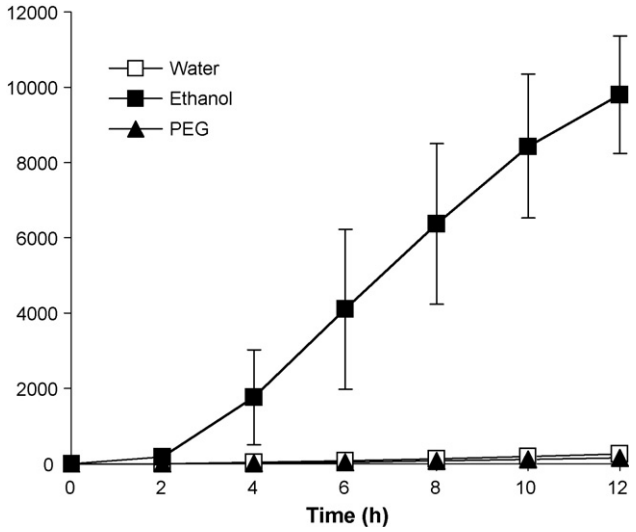

Fig. 3. Plot comparing the permeation of quinine from Q/2-HP-β-CD complex in different vehicles (deionised water, ethanol and PEG) across the ventral surface of the tongue ($n = 3$, ±S.D.).

a vehicle for the complex. The flux of quinine from the Q/2-HP-β-CD complex in ethanol was approximately 56-fold higher than with PEG as the vehicle for the inclusion complex.

4. Discussion

Most drugs are absorbed through the oral mucosa by passive diffusion (Kurosaki et al., 1998; Zhang et al., 2002). The most important factor in determining the rate and extent of drug permeation across the oral mucosa membrane is the physicochemical properties of the drug, which includes the molecular weight or size, lipophilicity and charge of the drug. Smaller drugs with a molecular weight of less than 500 Da generally permeate more efficiently than large molecular weight drugs, although this generalisation does not apply to all drugs (Squier and Johnson, 1975; Rathbone et al., 1996). A lipophilic drug should permeate across the lipid-filled sublingual mucosa much faster than a hydrophilic drug (Squier and Johnson, 1975; Wang et al., 2008). The charge of a drug greatly influences its lipophilicity, aqueous solubility and permeability and an uncharged drug is more lipophilic and permeates more effectively across the sublingual mucosa compared to the charged species. The degree of ionisation of a weakly acidic or basic drug would depend on both the pK_a of the drug and the pH of the aqueous solution. Quinine has a molecular weight of 361.9, which makes it a good drug candidate for permeation across the sublingual membranes (ventral surface of the tongue and floor of the mouth). However, despite the hydrophilicity of the drug, it was shown to permeate significantly across the sublingual membranes. If the absorption of the drug across the mucosa is too low, a proportion will be swallowed and this may affect the bioavailability of the drug.

The surface area of the ventral surface of the tongue and floor of the mouth is considerably smaller (26.5 cm^2) than the surface area of the intestinal membrane (350,000 cm^2) (Collins and Dawes, 1987), which means that only a small amount (a few milligrams) of a highly potent drug can be delivered using the sublingual route at any one time (Zhang et al., 2002). It would be difficult to calculate the exact amount of quinine that can be absorbed by the sublingual membranes as quinine administered as a sublingual spray will not be uniformly distributed across the ventral surface of the tongue and floor of the mouth, which is complicated further by the significantly different permeabilities of the two membranes.

4.1. Permeability across different regions of oral tissues

Different regions of the oral mucosa have been reported to have different permeabilities (Squier and Hall, 1985b; Lesch et al., 1989). In two previous studies, the permeabilities of porcine gingiva, buccal mucosa and floor of the mouth were compared, where the floor of the mouth was found to be the most permeable region, followed by the buccal mucosa and lastly, the gingiva. However, the current study yielded conflicting evidence suggesting that the ventral surface of the tongue was significantly more permeable to quinine than the floor of the mouth by a factor of about 2, as illustrated in Table 1.

The difference in the permeability of the floor of the mouth and the ventral surface of the tongue is likely to be attributed to the difference in lipid composition of the epithelium. Glycosylceramide which is present only in the non-keratinised epithelium and not the keratinised epithelium may contribute to the permeability of the floor of the mouth and ventral surface of the tongue (Squier et al., 1991). The buccal mucosa, which is less permeable than the floor of the mouth, contains proportionately more glycosylceramide than the floor of the mouth. The ventral surface of the tongue was not studied by Squier et al. (1991), but it may be that this region contains less glycosylceramide than the floor of the mouth membrane.

Many sublingual *in vitro* permeation studies only investigate the permeation of drugs across the floor of the mouth even though drugs administered sublingually will be absorbed across both the floor of the mouth and the ventral surface of the tongue. This study has shown that the permeability of the ventral surface of the tongue was significantly different from the floor of the mouth. Collins and Dawes (1987) calculated the surface area of the ventral surface of the tongue and floor of the mouth to be 26.5 ± 4.2 cm^2. The surface area of the ventral surface of the tongue is somewhat larger than the surface area of the mouth floor. Due to the higher permeability and surface area of the ventral surface of the tongue, it is likely that a higher percentage of drug molecules will be absorbed through the ventral surface of the tongue compared to the mouth floor following sublingual administration of the drug.

The ventral surface of the tongue is highly vascularised (Rathbone et al., 1996), where the blood flow of the ventral surface of the tongue expressed in terms of weight or volume are higher than the blood flow in the floor of the mouth (Squier and Nanny, 1985). However, blood supply is generally not considered a limiting factor in the absorption of drugs across the oral mucosa membranes (Squier and Johnson, 1975; Rathbone et al., 1996).

4.2. Effect of freezing on the permeation of quinine across the ventral surface of the tongue

It is common practice to freeze membranes for *in vitro* permeation studies. There are contradictory reports on the effect of freezing on the *in vitro* permeation of drugs across biological membranes, which may be attributed to the region from which the membrane was excised, the storage length, temperature and conditions, and the physicochemical properties of the drug in the study. Different regions of the oral mucosa (human and porcine) could be frozen at −85 °C for up to 6 months without affecting their permeabilities (Lesch et al., 1989). It is possible that freezing the ventral surface of the tongue at −20 °C for up to 2 weeks did not affect the permeation of quinine across the membrane because of the relatively short length of storage time. It has been reported that frozen tissue may deteriorate and affect its permeability the longer it is stored (Babu et al., 2003).

4.3. Effect of saliva on the permeation of quinine across the ventral surface of the tongue

Saliva functions to keep the mouth moist, protect teeth from decay, control bacterial flora in the oral cavity and aid digestion (Sudhakar et al., 2006). Intuitively, it is possible for saliva to have two opposing effects on the absorption of drugs depending on drug lipophilicity. On the one hand, saliva may act as an additional barrier to the permeability of lipophilic drugs across the mucosal membrane (Squier, 1991). On the other hand, polar drugs are more likely to experience modulated driving force as a consequence of dilution, although as saliva hydrates the oral mucosa this may act to increase permeability (Squier and Johnson, 1975). Dry mouth (xerostomia) has been reported to decrease the permeation of a drug across the sublingual mucosa (Collins and Dawes, 1987; Reisin et al., 1988; Ducharme et al., 1999).

From Table 1, it is evident that the presence of 100 μL saliva significantly decreased the permeation of quinine across the ventral surface of the tongue, due to simple dilution of this highly polar solute. The thickness of the salivary film in the oral mucosa in both adults and children was calculated to be approximately 0.07–0.10 mm, but will vary depending on the region of the oral mucosa (Collins and Dawes, 1987; Rathbone et al., 1996; Weatherell et al., 1996); speaking and breathing through the mouth may also decrease the salivary film thickness. The presence of 100 μL of saliva in the donor phase could represent an accumulation of saliva between the floor of the mouth and the ventral surface of the tongue. Sublingual and submandibular saliva and other oral fluids drain into the floor of the mouth, resulting in a pool of fluid in the mouth floor (Weatherell et al., 1996). Tongue movement also increases salivary turnover, thus further increasing the accumulation of fluid in the floor of the mouth. Although this study showed that saliva decreased the permeation of quinine by about two-thirds, the thickness of the salivary film and the volume of the saliva in the mouth are not constant, and the relatively high amounts used in this work could indicate the upper limit of this effect. Lastly, by consideration of the enzyme systems within human saliva (http://en.wikipedia.org/wiki/Saliva) it is highly unlikely that quinine would be degraded within a salivary film.

4.4. Permeation of quinine from the Q/2-HP-β-CD complex in different vehicles

Changes in chemical shifts can provide information on the interaction between the guest molecule and the 2-HP-β-CD to confirm the formation of the inclusion complex (Consonni et al., 2004; Polyakov et al., 2004; Jullian et al., 2007). H_3 and H_5 protons are located inside the toroid cavity of the cyclodextrin, and would therefore experience higher ^1H shifts compared to H_1, H_2, H_4 and H_6 which are found on the exterior of the cyclodextrin (Consonni et al., 2004; Polyakov et al., 2004). The downfield shifts experienced by H_3, H_5 and H_6 located inside or on the rim of the cavity of the cyclodextrin indicates that an inclusion complex could have formed between quinine and 2-HP-β-CD. It is also likely that the relatively larger chemical shifts with the exterior protons, H_1 and H_4, suggests interaction between the drug and these protons of the cyclodextrin.

It has been suggested there is no need for initial complexation if the drug is able to form complexes with cyclodextrins because the saliva gets rapidly saturated with the large excess of cyclodextrin, and the inclusion complex is formed immediately (Szejtli and Szente, 2005). Although generally weak, the chemical shifts observed in the current work (Table 2) were consistent, indicative of a complexation event. However, in a saturated solution of cyclodextrin inclusion complexes, the guest molecule quickly exchanges between the bound and unbound states to give a mixture of inclusion and non-inclusion complexes (Consonni et al., 2004; Loftsson and Duchêne, 2007). The unavoidable use of solvent (DMSO) in the NMR tubes can shift the equilibrium, potentially explaining the weakened signal.

2-HP-β-CD has a high molecular weight of 1400 and large numbers of hydrogen bond donors and acceptors, features that are typical of compounds that do not permeate easily across biological membranes (Loftsson and Duchêne, 2007). This means that the drug in the inclusion complex has to be released from the cavity of the cyclodextrin before permeation across the biological membrane is feasible. Although potentially a rate-limiting step, it has been reported that the formation and break down of the drug/cyclodextrin inclusion complex is so rapid and continuous that it is not the case (Loftsson and Duchêne, 2007). Studies suggest that cyclodextrins increase the permeation of a drug by solubilising the lipophilic drug in the donor solution (Salehian et al., 1995), although others report that cyclodextrins act as permeation retarders (Williams, 2003), but this may depend on the aqueous solubility and permeability of the drugs (Loftsson and Duchêne, 2007).

The highest permeation of quinine across the ventral surface of the tongue was observed in ethanol, which has been previously reported to be an effective vehicle for increasing the penetration of compounds across the oral epithelium (Squier and Johnson, 1975; Ungphaiboon and Maitani, 2001). In skin studies, ethanol was a better vehicle compared to water and PEG (Oh et al., 2001; Kikwai et al., 2002). Although it is possible to extrapolate the skin permeation effects of the vehicles to the oral mucosa, the differences in the lipid composition and presence or absence of the keratinised layer should be taken into account (Ganem-Quintanar et al., 1997).

The traditional view for the mechanism for the permeation enhancement of ethanol suggests that it transiently increases the lipid fluidity to enhance the permeation of the drug (Ganem-Quintanar et al., 1998). However, recent findings demonstrating enhanced flux of mefenamic acid across isolated (non-epithelial) dermis by ethanol supports the pull effect (or convective mass transport) on drug/ethanol solvated complex as the most probable mechanism in skin membranes (Heard and Screen, 2008), which is equally likely to occur with sublingual membranes.

4.5. Clinical considerations of sublingual quinine

The minimum therapeutic concentration for total (bound and unbound) blood quinine for the treatment of severe falciparum malaria remains uncertain. The value given by The Hospital for Tropical Diseases (London, UK) is 5 mg L^{-1}, although others quote 20 mg L^{-1} as being therapeutic (Flanagan et al., 2006). Whatever the concentration that is recommended, the blood volume of the patient will also determine the amount of quinine needed to achieve therapeutic effect. The body weight and thus the blood volume in children are much smaller than in adults.

The mean blood volume per body weight of a prepubertal stage male child was calculated to be 78.5 mL kg^{-1} (Raes et al., 2006) and the mean weight of a 7-year-old child is 23 kg (British National Formulary, 2007). Therefore, the blood volume of 7-year-old child would be about 1.81 L. For a male adult with a weight of 68 kg (British National Formulary, 2007) and blood volume per body weight of 71 mL kg^{-1} (Lentner, 1984), the blood volume would be 4.828 L. The blood volume of a 7-year-old child would therefore be more than half of a male adult, resulting in about more than half the amount of quinine needed to reach clinical therapeutic effect in severe falciparum malaria.

For an average 7-year-old child with a blood volume of 1.81 L, approximately 9.05 mg of quinine is needed to reach the minimum therapeutic concentration for severe *P. falciparum* malaria of 5 mg L^{-1}. For an adult with a blood volume of 4.8 L, 24.14 mg of

quinine is needed to reach the minimum effective concentration for severe falciparum malaria of 5 mg L^{-1}. Rudimentary determinations in our laboratory found the surface area of the ventral surface of the tongue to be approximately 13 cm^2. Assuming the total area can be targeted by a spray device then, based upon the parameters used in this work, administration of 30 mg mL^{-1} quinine HCl to the ventral surface of the tongue, where the steady-state flux from an ethanol vehicle was 372.96 μg cm^{-2} h^{-1}, a dose of (373 μg cm^{-2} h^{-1} × 13 cm^{-2} × 12 h) approximately 58 mg could be achieved after 12 h. Moreover, a therapeutic dose for an adult could be deliverable within 6 h; for a child a time of ∼2 h is determined, although this appears to be with the lag phase (Fig. 1). The amount deliverable from the cyclodextrin complex in ethanol would be even greater, on the basis of the flux data. The oral quinine dose for nocturnal leg cramps is about a sixth to a ninth less than the oral quinine dose for adults with severe *P. falciparum* malaria (British National Formulary, 2007).

In conclusion, one aim of this work was to determine the maximum dose of quinine deliverable by the sublingual route. With this known, it would be a less arduous task for future development work to reformulate and to optimise other vehicles and dosages. The data support further investigations into the clinical use of sublingual quinine, particularly in emergency cases, where oral delivery is not possible and facilities for intravenous delivery not at hand. Use of quinine/cyclodextrin inclusion complexes may circumvent issues of bitter taste.

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