IN VITRO TRANSDERMAL DIFFUSIONAL PROPERTIES OF TETRACAINE FROM A TOPICAL FORMULATION

K.J. Miller Jr., Y.K. Rao, D.O. Shah, and S.R. Goodwin

Center for Surface Science and Engineering
Departments of Chemical Engineering and Anesthesiology
University of Florida, Gainesville, Fl., USA 32611

In general, local anesthetics do not penetrate the skin. There is considerable need for a formulation that can allow the transdermal delivery of local anesthetics. Using a combination of the salt and base forms of tetracaine as well as mixed solvents of saline and propylene glycol, several compositions have been developed which are effective in the transdermal delivery of local anesthetics through full-thickness nude mouse skin in vitro. A 40% (v/v) propylene glycol solution is found to produce a flux of drug of approximately $200 \mu g/cm^2/hr$ while the corresponding flux from a pure saline or pure propylene glycol solution is approximately $100 \ \mu g/cm^2/hr$. The mixed solvent and mixed drug system is used to control the partitioning behavior and solubility of the drug mixture. The presence of the HCl form of the drug enhances the solubility of the free base and the presence of the free base seems to enhance the diffusion of the HCl form. Specifically, the solubility of tetracaine base in a 40% propylene glycol solution is $4.5 g/l$. The presence of tetracaine HCl in the aqueous solution, however increases the solubility of the free base beyond $100 g/l$. In a 70% propylene glycol solution the flux of a 3:2 mixture of tetracaine free base and tetracaine HCl is the same as that of a 100% tetracaine free base solution of the same overall drug concentration (flux from a 100% tetracaine HCl solution is negligible) indicating that the diffusion rate of the mixture is greater than those of the components. The solubility of tetracaine free base increases with propylene glycol content, but higher concentrations of propylene glycol also decrease the diffusion rate of the drug into the skin. Consequently, there must exist an optimum propylene glycol concentration as well as HCl:free base ratio for maximum transdermal flux.

1. K. J. Miller Jr., Y. K. Rao, S. R. Goodwin, and D. O. Shah, *In Vitro* Transdermal Diffusional Properties of Tetracaine From Topical Formulation, In Prediction of Percutaneous Penetration Proceedings, April 1991, R. C. Scott, R. H. Guy, and J. Hadgraft (eds), IBC Technical Services, London, (in press), (1992).

2. K. J. Miller Jr., G. B. Westermann-Clark, and D. O. Shah, Quasi-Steady State Model for Percutaneous Penetration of Local Anesthetics, In Prediction of Percutaneous Penetration Proceedings, April 1991, R. C. Scott, R. H. Guy, and J. Hadgraft (eds), IBC Technical Services, London, (in press), (1992).

IN VITRO TRANSDERMAL DIFFUSIONAL PROPERTIES OF TETRACAINE FROM A TOPICAL FORMULATION

K J Miller Jr[1], Y K Rao[1], S R Goodwin[2,3], and D O Shah[1,2]

Center for Surface Science and Engineering
Departments of [1]Chemical Engineering, [2]Anesthesiology, and [3]Pediatrics , University of Florida, Gainesville, FL 32611

1.   Introduction

A renewed interest in the transdermal delivery of medications has recently emerged. The transdermal route of administration offers many advantages over conventional routes. These include ease of administration, longer duration of action, slow continuous dosing, and the lack of pain which is often associated with parenteral routes. While the physicochemical properties of many drugs permit rapid and dependable percutaneous absorption, others such as local anesthetics are quite resistant to penetration through the stratum corneum barrier of the skin.[1-3] The benefits of transdermally effective local anesthetics are easily recognized. Both children and adults could greatly benefit from the ability to perform venepuncture, lumbar puncture, and other painful procedures without the normally associated trauma.

The in vitro approach (using a flow-through Franz diffusion cell) was used to obtain quantitative results of drug flux versus time and reduce the variability encountered clinically. The reduction of variability facilitated comparisons between different preparations. The test membrane was full thickness nude mouse skin.

A water miscible system was used to avoid the complications that would occur as water diffused through the skin <u>into</u> the drug preparation. If separation occurred as water entered the drug solvent, the drug would have had an additional interface to cross (i.e. a water barrier on the skin surface) and drug flux would be decreased.

2. Materials

Two forms of tetracaine are used in the experiments, tetracaine free base (a hydrophobic ester) and tetracaine HCl (a hydrophilic salt of the free base). Tetracaine base penetrates the neuron more effectively,[4] but has very low aqueous solubility preventing the use of clinically relevant concentrations. Tetracaine salt, however, has almost unlimited solubility in aqueous solutions (>200g/l). Tetracaine salt is also much more stable than the free base which must be kept refrigerated and dry.[4]

To achieve a compromise between the favorable diffusion characteristics of the base form and the high aqueous solubility of the salt form, a mixture of the base and salt forms was used. Such a mixture takes advantage of the tetracaine salt:tetracaine free base equilibrium. It should be noted that mixing a drug and its HCl salt in solution is equivalent to adding HCl acid to a preparation containing only free base (conversely, adding NaOH to a preparation containing only the salt form).[4]

The solvent in which the drugs are dissolved is a mixture of propylene glycol and saline. Propylene glycol is used to increase the solubility of tetracaine base in aqueous solutions and is also considered to be a penetration enhancer.

3. Methods

3.1 Solubility

Solubility was determined by allowing a solution in contact with excess drug to equilibrate at least 18 hours at room temperature. A sample was then withdrawn, filtered, and analyzed by HPLC to get the total drug concentration in solution.

For the 50% base/50% salt mixture, solubility was found by slowly dissolving solute until turbidity was observed. The estimated saturation concentration was that of the solution before turbidity.

3.2 Drug Partitioning

The term vehicle is used here and elsewhere to indicate the mixture of propylene glycol and water (saline) in which the drug is dissolved. To simulate the environment encountered by the drug when applied to the skin, the anesthetic preparation is placed in contact with n-octane. The partition coefficients for vehicle/n-octane systems were found by means similar to those described for solubility. Vehicle formulations were allowed to contact an equal volume of n-octane for at least 18 hours. Total drug concentration in the non-polar phase relative to that in the vehicle was found by HPLC. N-octane was used since the vehicle and 1-octanol (a preferable solvent for partition function evaluation)[5] are miscible above 60% (v/v) propylene glycol. Since the alcohol and the vehicle are miscible in some cases, a much less polar solvent was deemed more appropriate for estimating partition coefficients.

3.3 In vitro diffusion through mounted mouse skin

The diffusion of tetracaine was measured using flow-through type Franz diffusion cells (Figure 1). The diffusion cells had four parts (body, cap, o-ring, and clamp). The cell body was modified to include a magnetic stirring "tee" which greatly increased mixing efficiency and reduced the tendency to form a stagnant boundary layer adjacent to the skin surface. The cell body, (surrounded by a water jacket to maintain constant temperature), contained the lower (receptor) compartment into which the drug diffused. The receptor compartment initially contained 0.9% w/w saline. The cell cap contained the upper (donor) phase (source of diffusing drug) and held the skin in place. A rubber o-ring sat between the cell body and the inside surface of the skin. A clamp held the entire assembly together.

Donor solution (2 ml) was applied to the external surface of the skin, and the donor compartment sealed to prevent evaporation. To assure constant sampling intervals for multiple diffusion cells (generally three), experiments were staggered 5 minutes.

At regular intervals (1 or 2 hrs.), a 0.2 ml to 0.3 ml sample was withdrawn from the center of the receptor volume through the upper sample port using a long, thin needle and a

3 ml syringe. This sample was sealed in an autosampler vial for later analysis by HPLC. The sample volume extracted was replaced by fresh saline injected by a syringe through the upper sample port. (To insure that no air was drawn into the receptor compartment, the sample volume extracted was less than the volume in the upper sample port arm.)

The concentrations obtained from HPLC were used to calculate the total mass transferred through the skin. The following mass balance accounts for the sampling process.

$$M(t_n) = C(t_n)V + V_s \left(\sum_{x=0}^{n-1} C(t_x) \right)$$

Where:

$M(t_n)$ Total mass transferred at time t_n

$C(t_n)$ Measured concentration at time t_n

V Volume of receptor compartment

V_s Sampled Volume

x Summation index

The total mass obtained from this equation can then be converted to a corrected concentration by dividing by the receptor compartment volume (V) or flux by dividing by the mass transfer area (diameter = 25 mm) and sampling interval.

4. Results and Discussion

Prior to these studies, we conducted experiments to determine whether untreated skin could be used for short term transdermal diffusion. In these experiments, the diffusion of lidocaine salt from saline through nude mouse skin was carried out for 72 hours. The flux was monitored during this period to determine if any inflection occurred. Analysis of the data indicated that the barrier properties of fresh, untreated, nude mouse-skin did not change appreciably over 72 hours (with regard to lidocaine salt). Results for this experiment are illustrated in Figure 2. The error bars for this and all subsequent figures represent the maximum observed deviation from the average value which is plotted.

4.1 Solubility

Propylene glycol, although widely considered to be a penetration enhancer, did not enhance the transdermal diffusion of tetracaine salt relative to a saline solution (Figure 3). For solutions containing combinations of tetracaine base and tetracaine salt, however, the behavior was dramatically different. Figure 4 shows measured solubilities of tetracaine salt, tetracaine base, and a 50% base/50% salt mixture in propylene glycol-water solvents. The solubility of tetracaine base in aqueous solution is

negligible. By adding propylene glycol, however, the solubility of the base peaks at about 700 g/l then falls to 575 g/l in pure propylene glycol. The solubility of tetracaine salt decreases slightly as the propylene glycol fraction increases, but does not change much overall. The solubility of the 50% base/50% salt mixture peaks near 50% propylene glycol with a solubility greater than the sum of the salt and base solubilities in 50% propylene glycol. This non-additivity near 50% propylene glycol shows that HCl acid can enhance solubility above what would be expected from the pure component solubility curves (pure salt in solution is an equimolar mixture of base and HCl acid).

4.2 Drug Partitioning

Drug partitioning between the stratum corneum and the vehicle influences the transdermal diffusion of tetracaine.[5] The ratio of drug concentrations in the vehicle and n-octane at equilibrium was taken as the partition coefficient for that vehicle, as in Figure 5. The partition coefficient of tetracaine in propylene glycol-water solutions seems to decline steadily with increasing propylene glycol content up to 70% propylene glycol. Above 70%, however, partitioning into the oil phase decreases. It is inferred from these data that a minimum partition coefficient exists at about 70% propylene glycol.

4.3 In vitro diffusion through mounted mouse skin

Figure 6 compares the cumulative transdermal flux over eight hours for vehicles consisting of propylene glycol and 0.9% saline with an acidified tetracaine solute. In all cases, the concentration of tetracaine is 0.36 M (0.227 M tetracaine base, 0.133 M tetracaine salt). The cumulative fluxes for six propylene glycol-water solutions are shown (10%, 20%, 40%, 50%, 60%, and 70% propylene glycol).

The key features of the cumulative flux plot are the extremes (two minima at 20 and 65% propylene glycol and the maximum at 50% propylene glycol). The minimum in cumulative flux at 70% propylene glycol (Figure 6), corresponds to the minimum in the partition coefficient (Figure 5). At 70% propylene glycol, the decrease in the oil phase partitioning of the drug may be responsible for the decrease in the measured flux through the skin. Such straightforward explanations are not readily available for the other extremes in the measured flux. The partition coefficient does not change appreciably in the range 0 to 30% propylene glycol, yet the cumulative flux of tetracaine has a pronounced minimum at 20% propylene glycol. The solubility of the drug mixture (Figure 4) decreases steadily below 60% propylene glycol which suggests a decrease in the flux with decreasing propylene glycol fraction. The high flux at 10% propylene

glycol indicates that there is another factor that favors the transdermal diffusion of this drug at low propylene glycol content. The maximum in the flux at 50% is equally curious since it has no correlation to either the solubility (rising to a maximum at 60% propylene glycol) or the partition coefficient (falling to a minimum at 70% propylene glycol). The increasing solubility of base in the vehicle may enhance transdermal flux up to 50% propylene glycol, at which point the decreasing partition function begins to dominate and the flux decreases (i.e. the effects may compete to create a maximum).

5.   Conclusions

5.1 Solubility

1.   Amount of HCl added (the proportion of tetracaine salt relative to tetracaine base) has a complex effect on the solubility of tetracaine in propylene glycol-saline mixtures.

2.   Mixtures of propylene glycol and saline can increase the solubility of tetracaine base by as much as 700 g/l over saline alone.

5.2 Drug Partitioning

The n-octane/vehicle partition coefficient has a minimum at 70% propylene glycol which corresponds to a minimum in cumulative transdermal flux.

5.3 In vitro drug diffusion through mounted mouse skin

1.   Propylene glycol does not enhance the diffusion of aqueous tetracaine salt through untreated nude mouse skin.

2.   The highest fluxes measured (40% and 50% propylene glycol) may result from competing effects of increasing solubility and decreasing oil-phase partitioning.

3.   The minimum in flux at 20% propylene glycol does not correspond to either the solubility or partitioning behavior, indicating that it is caused by some unknown factor.

Acknowlegements

REFERENCES

1. Woolfson A D, McCafferty D F, McGowan K E, Percutaneous local anesthesia: comparison of in vitro predictions with clinical response in, Prediction of Percutaneous Penetration Proceedings April 1989, IBG Technical Services, London, pp 192-198 (1990).

2. Adriani J, Dalili H, Pentration of localanesthetics through epithelial barriers, Anesth Analg, 50(5) pp 834-841 (1971).

3. Monash S, Topical anesthesia of the unbroken skin, Arch Dermatol, 76, pp 752-756, (1957).

4. de Jong R H, Physiology and Pharmacology of Local Anesthesia, Charles C. Thomas, Springfield, pp. 73-79, (1970).

5. Hadgraft J, Mathematical models of skin absorption in, Prediction of Percutaneous Penetration Proceedings April 1989, IBG Technical Services, London, pp 252-262 (1990).

CAPTIONS

FIGURE 1: Schematic diagram of flow-through Franz diffusion cell.

FIGURE 2: Receptor phase concentration versus time for transdermal diffusion of aqueous lidocaine salt through untreated nude mouse skin.

FIGURE 3: Receptor phase concentration of tetracaine salt versus time for transdermal diffusion through untreated nude mouse skin from saline and 50% (w/w) propylene glycol.

FIGURE 4: Solubility of tetracaine salt, base, and 50% (w/w) mixture in solutions of propylene glycol and water.

FIGURE 5: N-octane/vehicle partition coefficients of tetracaine in acidified propylene glycol-saline solutions.

FIGURE 6: Cumulative flux of tetracaine through nude mouse skin in vitro from acidified propylene glycol-saline solutions at eight hours.

Sample Ports

Stirrer

Water Jacket

Wax Film

Cell Cap

Skin

Cell Body

Concentration (mg/l)

Time (hrs)

Concentration (mg/l) vs **Time (hrs)**

Aqueous ●

50% PG ○

Salt ○

Base ●

50% Mixture ▲

% Propylene Glycol (v/v)

Solubility (g/l)