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A Study on the In-vitro Percutaneous Absorption of Propranolol from Disperse Systems

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Abstract

Transdermal administration of propranolol can be used to avoid hepatic first-pass metabolism of the drug. The effect of polysorbate 80 concentration on the permeation of propranolol incorporated into micelles of polysorbate 80 in water, oil-in-water microemulsions of isopropyl myristate-polysorbate 80-sorbitol-water and oil-in-water emulsions of isopropyl myristate-polysorbate 80-sorbitan monooleate-water has been investigated by use of an artificial double-layer membrane, composed of a barrier foil and a lipid barrier, in Franz-type diffusion cells. Reversed-phase high-performance liquid chromatography, with celiprolol as internal standard, was used to determine the concentration of propranolol in the receptor compartment and a logarithmic equation was used to estimate the apparent permeability coefficient of propranolol from disperse systems.

For each disperse system the apparent permeability coefficient of propranolol decreased with increasing polysorbate 80 concentration. Moreover, for a given polysorbate 80 concentration the apparent permeability coefficient of propranolol increased when the disperse system was changed from emulsion to microemulsion and then to solubilized system, because of the increasing interfacial area of total disperse phase.

The results show that transdermal permeation of propranolol is greater when it is diffused from solubilized systems rather than from microemulsions or emulsions.

Propranolol, a non-selective β-adrenoceptor blocker, is used to treat hypertension, angina pectoris, cardiac arrhythmias, myocardial infarction and migraine (Physicians' Desk Reference 1993). Its oral bioavailability is low and variable because of extensive and variable first-pass metabolism in the liver. The transdermal route of drug administration by-passes first-pass hepatic metabolism leading to higher systemic bioavailability of the drug. Several formulations have been proposed for transdermal delivery of propranolol (Kai et al 1993; Kobayashi et al 1996; Krishna & Pandit 1996).

It is easily demonstrated that the vehicle in which the drug is applied influences the rate and extent of the percutaneous absorption of drugs (Loth 1991). Shamim et al (1995, 1996) evaluated the in-vitro increase of percutaneous penetration of propranolol by prodrug formation. Non-ionic surfactants are widely used for solubilizing water-insoluble drugs and the technique might have the advantage of protecting the drug against hydrolysis (Attwood & Florence 1983).

The internal phase of oil-in-water microemulsions could be a reservoir for lipophilic drugs and their infinite stability could increase the physical maintenance of the system for a prolonged time (Kreuter 1994). The incorporation of a lipophilic drug into the internal phase of an oil-in-water microemulsion has recently become an attractive technique for solubilizing these drugs and using microemulsions as topical drug-delivery vehicles (Gasco et al 1991; Osborne et al 1991). The incorporation of a lipophilic drug into the internal phase of oil-in-water emulsions is a well-known technique for percutaneous administration of oil-soluble drugs (Lalor et al 1994; Schwarz et al 1995).

In this study propranolol was incorporated into micellar systems, microemulsions and emulsions. The apparent permeability coefficient of propranolol through an artificial hydrophilic-lipophilic barrier was estimated and the influence of the

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polysorbate 80 concentration on the permeation of propranolol from different types of disperse system was determined. Reversed-phase high-performance liquid chromatography (HPLC), using celiprolol as internal standard, was used to determine the concentration of propranolol in the receptor compartment.

Materials and Methods

Materials

Polysorbate 80 (polyoxyethylene 20 sorbitan mono-oleate, tween 80), sorbitan mono-oleate (span 80), isopropyl myristate (98% pure), D-sorbitol (research grade) and propranolol hydrochloride were used as supplied by Sigma. The propranolol base was prepared from propranolol hydrochloride. 1-Dodecanol (for synthesis), disodium hydrogen phosphate (analytical reagent), potassium dihydrogen phosphate (analytical reagent), HPLC-grade acetonitrile and methanol, reagent-grade monobasic potassium phosphate, *n*-octylamine, 85% phosphoric acid and dialysis tubing cellulose membrane were used as supplied by Merck. Cellulose nitrate membrane filter (0·1 μm pore size) was supplied by Micron Separation.

Preparation of solubilized systems

Solutions were prepared by mixing the required amount of propranolol and polysorbate 80 at 40°C before diluting with warm water to the required total concentration (Attwood et al 1989). Solutions were left to equilibrate at 37°C for 24 h before being used for permeation measurements.

Preparation of microemulsions

A 2.5% w/w solution of propranolol in isopropyl myristate was prepared with gentle stirring at 40°C. Oil-in-water microemulsions were prepared by adding the appropriate amount of a sorbitol solution to mixtures containing the isopropyl myristate solution of propranolol and polysorbate 80 in a stirred beaker at 40°C (Ktistis 1990). Microemulsions were left to equilibrate at 37°C for 24 h before being used for permeation measurements. In all microemulsions the mass ratio of polysorbate 80 to sorbitol was maintained at 1 : 2·5.

Preparation of emulsions

Propranolol was dissolved in isopropyl myristate with gentle stirring at 40°C. Oil-in-water emulsions were prepared by adding the required amount of a polysorbate 80 solution to propranolol mixtures, isopropyl myristate and sorbitan mono-oleate in a stirred beaker at 40°C. Emulsions were left to equilibrate at 37°C for 24 h before being used for permeation measurements. In all emulsions the mass ratio of polysorbate 80 to sorbitan mono-oleate was maintained at 1 : 0·4 to give a HLB value of 12.

Permeation studies

The in-vitro permeation studies were performed using Franz diffusion cells (FDC-400 25OR; Crown Glass Company). Donor chambers were filled with the examined dispersion system (5 mL) and closed with paraffin laboratory film to restrict evaporation of water. The receptor phase was 15 mL de-aerated phosphate-buffered saline (0.13 M, pH 7·4), continually stirred with a magnetic stirrer and equilibrated with circulating water at 37 ± 1°C.

An artificial double-layer membrane comprising of two layers, a barrier foil and a lipid barrier, was used. The barrier foil was cellulose membrane-type dialysis tubing, soaked in water for 24 h. The lipid barrier was a cellulose nitrate membrane filter of 0·1 μm pore size impregnated with 1-dodecanol. These layers were placed together to form a hydrophilic-lipophilic membrane. The area of the membrane available for permeation, S, was 4·91 cm^2. The hydrophilic side was placed toward the donor chamber.

Receptor-compartment samples (0·2 mL) were taken at predetermined times up to 48 h and analysed by HPLC. Extracted volumes were replaced with phosphate-buffered saline.

Propranolol analysis

Propranolol concentrations in receptor samples were determined by reversed-phase high-performance liquid chromatography (HPLC) using celipropol as internal standard. Stock standard solutions of propranolol (600 $\mu g\,mL^{-1}$) and internal standard (300 $\mu g\,mL^{-1}$) were prepared in methanol and stored at −20°C. A solution of internal standard in methanol (300 $\mu g\,mL^{-1}$; 100 μL) was added to a 1·5-mL Eppendorf polypropylene tube containing receptor sample (100 μL). After mixing on a vortex mixer, 20-μL was injected on to the column.

The HPLC system (Varian, Palo Alto, CA) consisted of a pump (model 2510), a variable-wavelength UV-Vis detector (model 2550), a manual injection valve (model 7125) with a 20-μL fixed loop (Reodyne, Cotati, CA), an integrator (model 4290) and a LiChrospher RP-18 reversed-phase analytical column (250 × 4·0 mm i.d.), 5 μm particle size, protected by a dry-packed guard column (RP-18, 37–53 μm, 30 × 4·6 mm i.d.).

Chromatography was performed at room temperature. The mobile phase was acetonitrile-0·1 M monobasic potassium phosphate-*n*-octylamine

(30:69·9:0·1, v/v) adjusted to pH 3·3 with phosphoric acid; this was pumped isocratically at a flow rate of 0·9 mL min^{-1}. Detection of propranolol was accomplished at 290 nm, close to the absorption maximum of the compound. The retention times of celiprolol (internal standard) and propranolol were 4·5 and 7·1 min, respectively.

Quantitation of propranolol samples was determined by comparison of peak-height ratios with standard curves, which were prepared daily. The calibration graphs were linear with a correlation coefficient (r) of 0.999 or better for concentrations between 25 and 300 $\mu g\,mL^{-1}$. The within-day precision of the method, expressed as the coefficient of variation (CV%), was found to be better than 4%.

Results and Discussion

Measurements of concentration, C_R, in the receiver compartment of the Franz diffusion cells were performed for solubilized systems, microemulsions and emulsions containing 10% w/v isopropyl myristate and 25 mg/100 mL of propranolol with the compositions given in Table 1. The concentration of propranolol is near to its solubility in isopropyl myristate at 25°C. Stable microemulsions with polysorbate and sorbitol concentration outside the range 32 to 56% w/v could not be prepared under the experimental conditions used in this study.

In Figures 1–3 the cumulative amount of propranolol permeating across the hydrophilic-lipophilic barrier is shown as a function of time, t. The figures show that the rate of diffusion of the drug from dispersed systems decreases with increasing surfactant concentration. Attwood & Florence (1983), explaining the effect of surfactants on membrane permeability, noted that there are three main types of surfactant behavior when surfactant concentration is increased: an increase in drug permeation up to the critical micelle concentration followed by a decrease when the drug is solubilized in the surfactant micelles; an overall decrease in drug permeation when solubilization occurs; and an overall increase in drug permeation when the drug is not associated with the micelles. All preparations used in this work contained an amount of the surfactant sufficient for complete solubilization of propranolol and so the results observed are those expected for micellar systems.

An explanation of the observed reduction in propranolol permeation with increasing surfactant concentration, in all formulations, might be because of the reduction of the concentration of the monomolecular form of the drug in the continuous phase. Because the volume of dispersed phase is increased, the amount of propranolol in all the preparations used in this work is the same and the partition coefficient of propranolol between dispersed and continuous phase is much greater than unity. The distribution of drug between continuous and disperse phases is instantaneous at any moment and only the monomolecular form of the drug in the aqueous continuous phase permeates through the double-layer membrane. Under these conditions an increase in surfactant concentration reduces the concentration of the monomolecular form of the drug in the continuous phase, with a resulting decrease in the rate of diffusion of the drug.

Table 1. Composition of solubilized systems (A), microemulsions (B) and emulsions (C) containing 10% w/v of isopropyl myristate and 25 mg/100 mL propranolol

Designation	Amount of polysorbate 80 (% w/v)	Amount of span 80 (% w/v)	Amount of sorbitol (% w/v)
A1	2·0	0·0	0·0
A2	4·0	0·0	0·0
A3	8·0	0·0	0·0
A4	12·0	0·0	0·0
A5	16·0	0·0	0·0
B1	12·0	0·0	30·0
B2	13·0	0·0	32·5
B3	14·0	0·0	35·0
B4	15·0	0·0	37·5
B5	16·0	0·0	40·0
C1	2·0	0·8	0·0
C2	4·0	1·6	0·0
C3	6·0	2·4	0·0
C4	8·0	3·2	0·0
C5	10·0	4·0	0·0

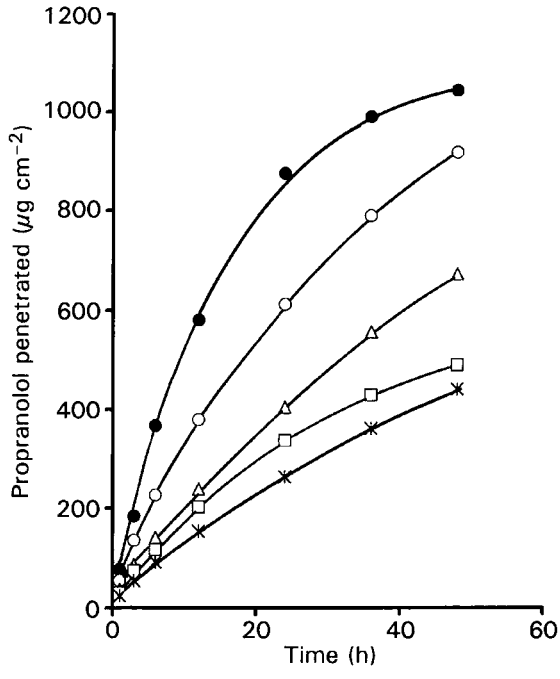

Figure 1. Amount of propranolol permeating as a function of time for solubilized systems containing ● 2%, ○ 4%, △ 8%, □ 12% and * 16% w/v polysorbate 80.

The permeation rate of the drug is given by Fick's law, which can be written:

$$dM/dt = PS(C_D - C_R) \quad (1)$$

where M is the amount of diffused drug, t the time of diffusion, P the permeability coefficient, S the cross-sectional area of the membrane, and C_D and C_R are, respectively, the concentrations of drug in the donor and receiver sides of the membrane.

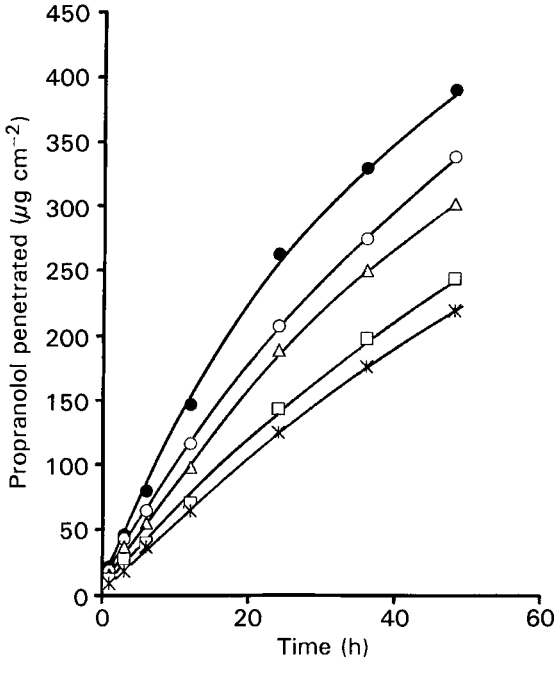

Figure 2. Amount of propranolol permeating as a function of time, for microemulsions containing ● 12%, ○ 13%, △ 14%, □ 15% and * 16% w/v polysorbate 80.

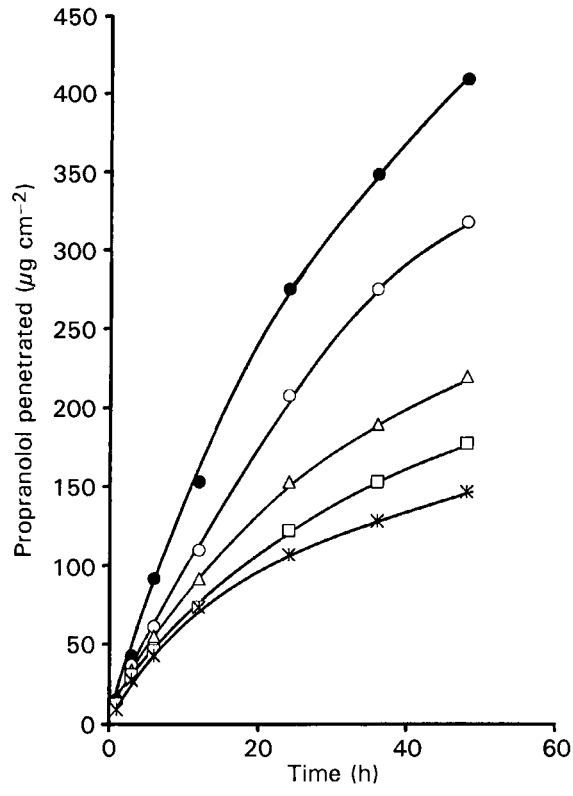

Figure 3. Amount of propranolol permeating as a function of time, for emulsions containing ● 0.2%, ○ 0.4%, △ 0.6%, □ 0.8% and * 1.0% w/v polysorbate 80.

The concentration of drug in the donor side of the membrane is equal to its concentration in the continuous phase. This is difficult to measure in disperse systems, so usually the drug concentration in the whole donor compartment is used. In this case an apparent permeability coefficient, P_a, is calculated instead of the permeability coefficient, P.

Because the amount of diffused drug is the difference between the mass of the drug in the receiver compartment and the initial amount of the drug in the donor compartment:

$$M = M_{D(o)} - M_R \quad (2)$$

and the volume of the receiver compartment, V_R, was three times that of the donor compartment,

$$C_D = C_{D(0)} - 3C_R \quad (3)$$

where $C_{D(0)}$ is the initial concentration of drug in the donor compartment.

Integration of equation 1, after the above substitutions, gives

$$-\ln [1 - (4C_R/C_{D(0)})] = (4P_aS/V_R)t \quad (4)$$

P_a can then be obtained from the slope of a linear plot of $-\ln(1 - 4C_R/C_{D(0)})$ against t.

Plots of equation 4 for the solubilized system A3, the microemulsion B2 and the emulsion C4 are shown in Figure 4. The plots are linear; the slopes of each line were derived by linear regression analysis and the apparent permeability coefficients, P_a, were calculated from these slopes.

The results are shown in Figure 5 as plots of apparent permeability coefficients of propranolol from solubilized systems, microemulsions and emulsions as functions of polysorbate 80 concentration. An apparent permeability coefficient of $26{\cdot}68 \times 10^{-5}$ cm min^{-1} was determined for a suspension of 25 mg propranolol in 100 mL water. This value was the maximum of the logarithmic curve of P_a against polysorbate concentration, where the apparent permeability coefficient of propranolol decreased logarithmically with increasing polysorbate concentration in solubilized systems. For microemulsions and emulsions also the value of P_a decreased logarithmically with increasing polysorbate concentration. Moreover, at a given value of polysorbate concentration the apparent permeability coefficient of propranolol increased as the dispersed system was changed from an emulsion to a microemulsion and then to a solubilized system. An explanation of this increase is the increasing total interfacial area of the disperse phase from emulsions to microemulsions to solubilized system, because the particle sizes are larger for the emulsion droplets than for the microemulsion cores or micelles. The total interfacial area of the disperse phase plays an important role in the quantity of propranolol transferred from the internal to the continuous phase. Indeed a larger interfacial area enables quicker replacement of permeated drug through the barrier.

Consequently, the transfer of the drug from dispersed to continuous phase increased from an emulsion to a microemulsion to a solubilized system with the result that the permeability of the drug through the barrier increases from an emulsion to a microemulsion or solubilized system.

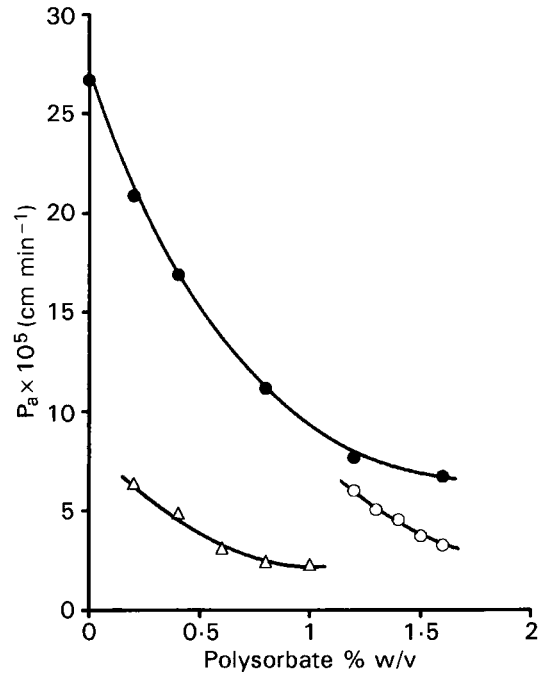

Figure 5. Apparent permeability coefficient, P_a, of propranolol as a function of polysorbate 80 concentration in dispersed system for ● solubilized systems, ○ microemulsions and △ emulsions.

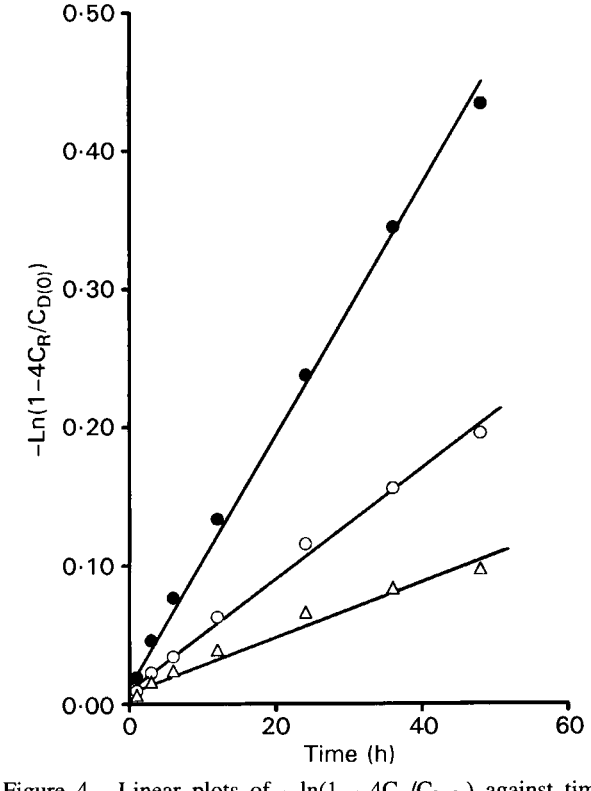

Figure 4. Linear plots of $-\ln(1 - 4C_R/C_{D(0)})$ against time (equation 4) for: ● a solubilized system containing 8% w/v polysorbate 80, ○ a microemulsion containing 13% w/v polysorbate 80 and △ an emulsion containing 8% w/v polysorbate 80.

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