Influence of Supersaturation on the Pharmacodynamic Effect of Bupranolol After Dermal Administration Using Microemulsions as Vehicle

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Transdermal absorption of drugs is limited by the stratum corneum, which serves as a diffusion barrier. This barrier might be overcome by enhancing the thermodynamic activity of the drug vehicle. Thermodynamic activity is particularly high in supersaturated systems because it is directly correlated with the degree of saturation. Since supersaturated systems are not stable, they were formed *in situ* by application of water-free microemulsion bases. These water-free microemulsion bases saturated with the drug were applied to New Zealand albino rabbits with an occlusive patch. Occlusion leads to water uptake from the skin due to hydratation and changes the microemulsion base into a microemulsion. The microemulsion will become supersaturated as a result of decreasing solubility of the drug with increasing water content. The pharmacodynamic effect of the model drug bupranolol *in vivo* was investigated over a 10-hr time period. The *in vitro* solubility of bupranolol was examined with respect to the water content. The solubility vs water content curves were compared to the effect vs time curves. The microemulsions and their individual components were studied, and the effect vs time curves were inversely correlated with the solubility vs water content curves.

KEY WORDS: transdermal; bupranolol; *in vitro; in vivo;* rabbit; microemulsion; pharmacodynamic effect; supersaturation.

INTRODUCTION

The absorption of drugs via the transdermal route is limited mostly by the generally poor penetration rate of drugs through the stratum corneum (1). According to Higuchi (2,3) the absorption of drugs can be accelerated by increasing the thermodynamic activity of the drug in the vehicle. The thermodynamic activity can be expressed approximately in terms of relative solubility (= current concentration in vehicle:concentration of saturated vehicle) (4). For this reason the absorption rate should be high from supersaturated vehicles (relative solubility, >1). Supersaturated systems cannot be stored because their physical instability leads to recrystallization. Hence, supersaturated preparations must be formed *in situ* by application of systems that become supersaturated during the application period. Suitable systems are the microemulsions used in this work.

Microemulsions consist of an aqueous component, a lipophilic component, and a surfactant or a surfactant/cosurfactant mixture. They are thermodynamically stable, self-emulsifying, clear or slightly opalescent, isotropic, and of a low viscosity. These microemulsions possess two favorable characteristics as vehicles for transdermal absorption. (i) They show a decreasing solubility for apolar drugs with increasing water content (5–7). (ii) *In vitro* crystallization of drugs in supersaturated microemulsions occurs only very slowly and with a delay of some days after preparation. It takes 10 to 14 days to reach equilibrium, and no crystals were observed in a 2- or 3-day period (= a possible application period *in vivo*) after forming the supersaturated systems (7,8).

Thus, application of a saturated, water-free microemulsion base with an occlusive patch should lead to water uptake from the skin and change the water-free microemulsion base into a microemulsion. Thereby, the solubility of the incorporated drug decreases and a particularly high absorption rate because of an enhanced thermodynamic activity is expected.

Bupranolol was used as a model compound because previous *in vivo* studies (9) with different β-adrenoceptor antagonists in microemulsion systems showed bupranolol to be an effective drug in dermal administration. The aim of the present study was to examine the correlation between the pharmacodynamic effect and the solubility of bupranolol in the microemulsions. Investigations were carried out with microemulsions and the single components of the microemulsion bases with respect to the water content. The measured pharmacodynamic parameter was the extent of tachycardia produced by a standard dose of isoproterenol.

MATERIALS AND METHODS

Drugs and Excipients

Bupranolol as free base was kindly donated by Schwarz Pharma GmbH, Monheim, FRG. The surfactants [polysorbate 85 = Tween 85, Atlas Chemicals AG, Essen, FRG, and polyoxyethylene(20) glycerol monooleate = Tagat O2, T. Goldschmidt AG, Essen, FRG] and the cosurfactants (poloxamer 101 = Pluronic L31, C. H. Erbslöh, Düsseldorf, FRG, and Tegin 4600, a mixture of mono- and diglycerides of C8 and C10 fatty acids, T. Goldschmidt, AG) were kindly donated by the manufacturers. Isopropylpalmitate (Rilanit) was a gift from Henkel KGaA (Düsseldorf, FRG). All other reagents and solvents were of analytical grade, and double-distilled water was used throughout the study. All substances were used as received without further purification. For application of the *in vivo* preparations, occlusive patches [50 × 60 mm, 2 mm in thickness (10)] were kindly produced for this investigation by 3M Medica (Borken/Westf., FRG).

Preparations for *in Vitro* Investigations

Composition of microemulsion I was as follows: polysorbate 85, 35%; poloxamer 101, 25%; and isopropyl-

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palmitate, 40%. Composition of microemulsion II was as follows: polyoxyethylene(20) glycerol monooleate, 35%; Tegin 4600, 45%; and isopropylpalmitate, 20%.

The water-free microemulsions I and II were used to produce various microemulsions with increasing water contents by dilution with water in increments of 5 and 10%, respectively. Bupranolol, 15%, was added to all systems. To increase the velocity of solubility the mixtures were heated to 40°C for 5 hr and were frequently well shaken. Crystallization was accelerated by adding some bupranolol crystals when no sediment was observed. The systems were then allowed to equilibrate in a water bath at 25°C over a period of 14 days.

In the same way, the binary mixtures of polysorbate 85, poloxamer 101, and isopropylpalmitate with different amounts of water were saturated with bupranolol.

Determination of Drug Solubility

Determination of bupranolol was carried out using a HPLC method. The chromatograph consisted of a Gynkotek 300B constant-flow pump, a Gynkotek SP-4 UV detector with variable wavelength (Gynkotek, Munich, FRG), and a Shimadzu C-R18 integrator (Shimadzu, Kyoto, Japan). Injections were made with a Kontron MSI 600 autosampler (Kontron, Zurich, Switzerland) equipped with a 100-μl Rheodyne injection valve (Rheodyne, Cotati, USA).

For separation 5-μm Shandon SAS Hypersil RP 2,4,6 (Shandon, Runcorn, UK) was used as stationary phase in a 250 × 5-mm column. The mobile phase consisted of acetonitrile/methanol/phosphoric acid/water (350/50/0.1/590.9). The chromatograph operated at 25°C with a flow rate of 2 ml/min. The eluent was monitored spectrophotometrically at a wavelength of 225 nm. The assay of bupranolol was carried out by integration of the peak areas and comparison with areas of known amounts of external standards.

After equilibration 0.2 g of the supernatant fraction (2 min, 13,000 rpm, in a Biofuge A, Heraeus Sepatech, Osterode, FRG) of the test preparations was diluted in 50.00 ml of methanol. One milliliter of this solution was diluted with 19 ml of the eluent and used for injection. Three dilutions of each sample were made and analyzed in triplicate.

Preparations for *in Vivo* Investigations

Isoproterenol solution (2.5 μg/ml free base) was prepared from Aludrin solution (Boehringer GmbH, Ingelheim, FRG) by dilution with 0.9% sterile sodium chloride solution. Heparin solution (500 IU/ml) was prepared from Lipo-Hepin 25000 (3M Medica, Borken/Westf., FRG) by dilution with 0.9% sterile sodium chloride solution.

The tested preparations were nearly saturated solutions of bupranolol in water-free microemulsions I and II and in pure polysorbate 85, poloxamer 101, and isopropylpalmitate. To avoid crystallization the drugs were dissolved only up to 95% in their *in vitro* solubility. These nearly saturated solutions are subsequently referred to as "saturated."

Evaluation of the Pharmacodynamic Effect

Analysis of the pharmacodynamic effect after dermal application of β-receptor antagonists was reported previously (7,9). An area of the dorsal skin of well-conditioned male New Zealand albino rabbits was clipped. Two stainless-steel electrodes were set subcutaneously for registration of the ECG. The animals were immobilized in a pyrogen test box and a catheter was set to the ear vein. Four to six i.v. bolus injections of a standard dose of isoproterenol (0.25 μg/kg) were given at intervals of 15–20 min. The catheter was rinsed with the heparin solution to overcome the dead volume and to keep the catheter open. The ECG was plotted from 2 min before to approximately 5 min after the bolus injection. Heart rate was determined by numbering the "R peaks" of the ECG. The differences in heart rate before and at the maximum heart rate of each injection were calculated. The mean of these differences was taken as an individual response to the standard dose of isoproterenol.

The test preparations were applied with the occlusive patch, which contains an application chamber of about 7 cm^2. A piece of viscose fleece (0.5 cm^2/100 mg) soaked with an amount of preparation which corresponded to a bupranolol dose of 2 mg/kg body weight was placed in the chamber. Also, isoproterenol effects were measured in varying intervals over a 10-hr time period after application of the patches. Total inhibition of the isoproterenol-induced tachycardia corresponded to 100% effect. Three runs of every preparation were made with different rabbits. The β-blocker effect versus time curves were fitted to the following function [Eq. (1)] using the mean values of the three runs:

$$E = E_{max} \times \left[1 + \frac{1}{k_1 - k_2}\left(k_2 e^{-k_1 t} - k_1 e^{-k_2 t}\right)\right] \quad (1)$$

where E is the effect at time t; E_{max} is the maximum effect; k_1, k_2 are first-order time constants, describing changes of effects with time (fictive constants, used here as an aid for determination of the parameters named below); and t is the time after application of the preparations.

Fits were computed with a nonlinear-regression program according to the algorithm of Ebert and Ederer (11). The algorithm was programmed in GFA-Basic (GFA Systemtechnik, Düsseldorf, FRG) on an Atari mega ST4. The following parameters were calculated from the fitted function: E_{max} = maximum effect; t_{max} = time to reach 98% of the maximum effect; $t_{50\%}$ = time to reach 50% inhibition of

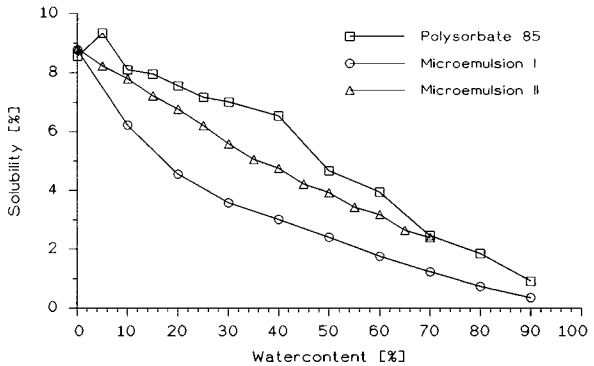

Fig. 1. Solubility of bupranolol with respect to the water content of several test preparations. Data shown are the means of three determinations; the coefficient of variation was ±0.53 to ±1.17%. Microemulsion I data from Ref. 6; microemulsion II data from Ref. 5.

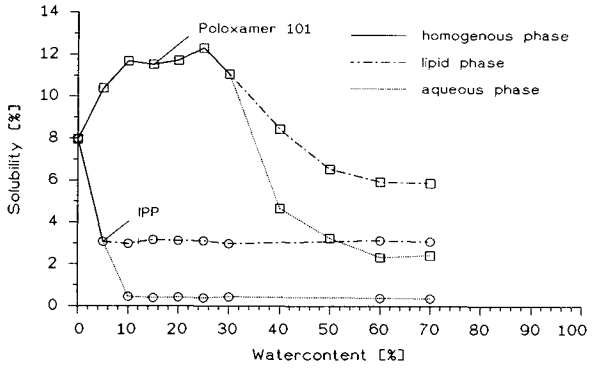

Fig. 2. Solubility of bupranolol with respect to the water content of several test preparations. Means of three determinations; the coefficient of variation was ±0.53 to ±1.17%.

the isoproterenol effect; and S = slope of the curve when 50% of the maximum effect was reached.

RESULTS

Solubility of Bupranolol in the Different Systems

Curves of the solubility of bupranolol in the tested systems plotted against the water content are shown in Figs. 1 and 2. The coefficients of variation of the determinations were in the range of ±0.53 to ±1.17%. Solubility of bupranolol in the microemulsion I systems declines nearly exponentially (coefficient of exponential regression = 0.9586) with increasing water content, whereas it decreases in the microemulsion II systems nearly linearly (coefficient of linear regression = 0.9936) (Fig. 1). In the polysorbate 85 systems a maximum is observed at 5% water content but decline of the curve is also linear (coefficient of linear regression = 0.9767).

Poloxamer 101 and isopropylpalmitate (IPP) can be mixed with water up to 40% (poloxamer 101) and 5% IPP, respectively, to form homogeneous solutions. Higher water content leads to double-phased systems, with both phases having their distinct solubility pattern. Solubility of bupranolol base is lower in the aqueous phase than in the lipophilic phase (Fig. 2). Solubility in the single phase range of the IPP system strongly decreases with increasing water content, whereas it increases in the single phase range of the poloxamer 101 system, with a maximum observed at 25% water.

Pharmacodynamic Effect After Dermal Application

Parameters calculated according to Eq. (1) are summarized in Table I. A placebo preparation does not show any statistically significant effect (7,9). Comparison of microemulsions I and II shows a steeper rise of bupranolol effect after application in water-free microemulsion I ($t_{50\%}$, about 30 min) than in microemulsion II ($t_{50\%}$, about 90 min). But the maximum effect of both preparations differs only a little (Table I and Fig. 3).

Effects after dermal application of bupranolol in the water-free pure components of microemulsion system I are depicted in Fig. 4. The curve after application of the IPP preparation shows a course similar to that of microemulsion I. The maximum effect of the polysorbate preparation is smaller than the IPP or microemulsion effect but the difference is not statistically significant. However, the rise of the effect is slower. The poloxamer preparation shows the smallest effect of all systems investigated in this study.

DISCUSSION

A decrease in the solubility of apolar drugs in microemulsion I with increasing water content has been reported previously by Kleinebudde and Müller (12,13). According to Yalkowsky (14), drugs can be divided into polar, semipolar, and apolar drugs with respect to the lipophilicity of the solvent/cosolvent mixture. The solubility of apolar drugs declines linearly with increasing water content of the solvent (= increasing polarity) as observed for pindolol, indomethacin, propranolol, and metipranolol in microemulsion system I and bupranolol in microemulsion system II (6,7). The higher the lipophilicity of the drug, the steeper the decrease in the plot of solubility versus water content. It is assumed that for drugs which were very apolar with respect to the solvent, the linear decrease in the solubility curve changes into an exponential decrease as observed for bupranolol (Fig. 1), carazolol (6), and penbutolol (7) in microemulsion I systems. The difference in lipophilicity of bupranolol and microemulsion II seemed to be smaller so that the decrease in the solubility plot is only linear (Fig. 1).

Solubility in polysorbate (Fig. 1) also shows a linear decrease but deviation from the straight line is a little higher. This is possibly due to liquid crystal structures observed in the range of 15 to 35% water content. Further, there is a primary increase in solubility. Maxima at small amounts of water and a linear decrease in solubility at higher water content were also observed with other β-adrenoceptor blocking

Table I. Bupranolol Concentration of the *in Vivo* Preparations and Calculated Parameters of the *in Vivo* Experiments[a]

Preparation	C	E_{max}	t_{max}	$t_{50\%}$	S
Microemulsion I	7.65	86.4	97	28	1.696
Microemulsion II	8.37	80.1	315	92	0.482
Polysorbate 85	7.65	74.1	328	103	0.407
Poloxamer 101	7.65	52.9	182	163	0.470
Isopropylpalmitate	7.65	81.5	38	14	3.623

[a] C = β-blocker concentration of the applied microemulsion base (%, w/w), E_{max} = maximum effect (% of isoproterenol inhibition), t_{max} = time to reach 98% of the maximum effect (min), $t_{50\%}$ = time to reach 50% isoproterenol inhibition (min), S = slope of the curve when 50% of the maximum effect was reached. All values are means of n = 3.

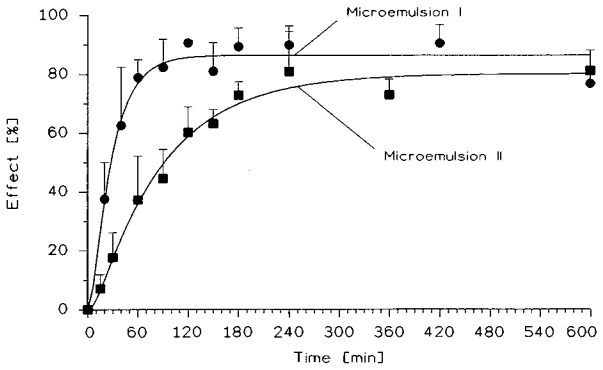

Fig. 3. Effect versus time curves after dermal application of water-free microemulsion bases saturated with bupranolol (dose, 2 mg/kg). Data shown are means and standard deviations of three separate experiments; curves were fitted according to Eq. (1).

drugs [metipranolol, propranolol (7)] in microemulsion I systems.

Binary mixtures of water and poloxamer 101 or IPP, respectively, are homogeneous at a low water content and double phased at a higher water content (Fig. 2) because poloxamer 101 and IPP are poorly miscible with water. In the double-phase systems the solubility of bupranolol is higher in the lipophilic phase than in the aqueous phase. This is due to the lipophilicity of bupranolol. In the homogeneous phase of IPP–water mixtures there is a strong decrease in solubility with increasing water content, whereas in the homogeneous binary systems of poloxamer 101 and water, the solubility of bupranolol first increases with increasing water content. A maximum is observed at 25% water. Due to this increasing solubility, a decrease in the thermodynamic activity could be expected.

Although the solubility of bupranolol base will be affected by the pH, the aqueous phase in the *in vitro* experiments was not buffered because only nonionic compounds were used, and therefore no considerable shift of the pH of purified water would occur, and only water-free systems were applied *in vivo*.

The *in vivo* data were fitted to Eq. (1), which is also used in reaction kinetics (15). Other mathematical models for plotting the possible course of the effect versus time curves were also carried out [e.g., the two-compartment model as described by Guy and Hadgraft (16)], but for different reasons they were less successful (7,9). Therefore Eq. (1) was used for fitting the effect vs time curves. The validity of this *in vivo* model, dose dependency of the effects, and influence of lipophilicity and concentration of several β-adrenoceptor blocking drugs were documented in previous papers (7,9). The observation period was set to 10 hr (7,9), and elimination was not documented.

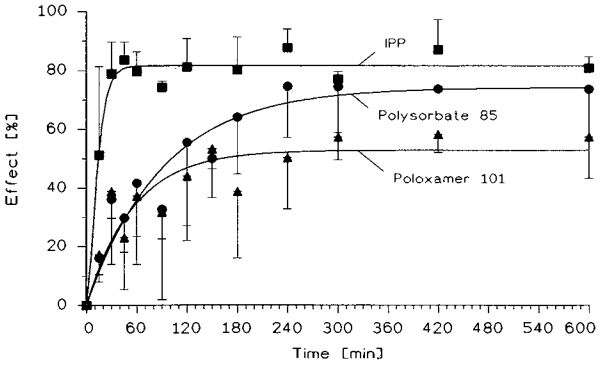

Fig. 4. Effect versus time curves after dermal application of several water-free test preparations saturated with bupranolol (dose, 2 mg/kg). Data shown are means and standard deviations of three separate experiments; curves were fitted according to Eq. (1).

The faster increase in the effect after application of bupranolol in microemulsion I compared to microemulsion II (Fig. 3) could be explained by the faster decrease in solubility occurring in water uptake from the skin due to occlusion (7). Assuming the same rates of water uptake from the skin with time in microemulsion I and microemulsion II, the relative solubility in microemulsion I is higher each time than in microemulsion II. Therefore supersaturation as the driving force for drug diffusion across the stratum corneum is higher and leads to a faster increase in the effect.

The effect–time curves obtained with the solution of bupranolol in polysorbate 85 (Fig. 4) is similar to the curve achieved with microemulsion II (Fig. 3), which reflects the similar course of the *in vitro* solubility curves (Fig. 1). The steep rise of the effect *in vivo* after application of bupranolol in IPP (Fig. 4) is correlated with the sharp decrease in the *in vitro* solubility curve (Fig. 2). Supersaturation arises fast because even small amounts of water taken from the skin lead to a supersaturated system. Additionally, there might be an absorption enhancing effect of the IPP as reported by Barry (17).

The smallest effects were observed after application of the poloxamer 101 preparation. This result might be due to the increasing solubility with increasing water content and, therefore, the decreasing thermodynamic activity of bupranolol in the applied system. A decreasing thermodynamic activity *in vitro* for octanol in mixtures of water with different poloxamers was reported by Cappel and Kreuter (18), and no penetration enhancing effect of the poloxamers occurred. Therefore, poloxamer itself is not a good vehicle for dermal administration of drugs.

In conclusion, the *in situ* formation of supersaturated microemulsions from microemulsion bases applied water-free enhance drug flux through the skin. Further, the rise of pharmacodynamic effects *in vivo* correlates with the decline of the solubility versus water content curves *in vitro*.

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