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Effect of Skin Binding on Percutaneous Transport of Benzocaine from Aqueous Suspensions and Solutions

U. G. DALVI * and J. L. ZATZ ˣ

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* Present Address: E. R. Squibb & Sons, Inc., New Brunswick, NJ 08903.

Abstract □ Various aqueous suspensions of benzocaine containing nonionic surfactants exhibited the same rate of *in vitro* penetration through hairless mouse skin. Saturated solutions yielded a lower rate of benzocaine penetration due to depletion of drug from the donor portion of the diffusion cell. Extensive skin binding was responsible.

Keyphrases □ Benzocaine—effect of skin binding on percutaneous transport from aqueous suspensions and solutions □ Skin binding—effect on percutaneous transport of benzocaine from aqueous suspensions and solutions □ Percutaneous transport—effect of skin binding, of benzocaine from aqueous suspensions and solutions

The effect of surfactants on drug penetration through skin is an important aspect of formulation of topical drug products. The influence of these additives depends on the rate-limiting step in penetration, so that the type of preparation and conditions of application to the skin are important considerations. Penetration from aqueous solutions containing a fixed benzocaine concentration depended on the extent of benzocaine solubilization by surfactant included in the formula (1). Penetration flux was directly proportional to the unbound benzocaine concentration. A series of surfactant solutions saturated with benzocaine had the same rate of benzocaine penetration. No alteration of membrane permeability was found. This work was extended to aqueous gel suspensions to determine the rate limiting step in penetration and the influence of nonionic surfactants in these systems. The suspensions were similar to those utilized in previous studies of benzocaine release (2, 3).

EXPERIMENTAL

Materials and apparatus used for the *in vitro* penetration experiments were reported previously (1). Surfactants used in the formulations were commercially available polyoxyethylene nonylphenols[1]. The membrane was whole hairless mouse skin. The receptor solution (normal saline containing 0.25% chlorobutanol) was maintained at 37° and stirred at 500 rpm. The donor compartment was not stirred. Further details concerning experimental procedure appeared previously (1). All donor systems were studied in triplicate.

All the suspensions were prepared on a w/v basis. Accurately measured surfactant stock solution was transferred to a wide mouth glass jar containing a magnetic stirring bar. To that solution, the required amount of benzocaine was added and stirring was continued for ~15 min until all lumps were broken. A preweighed quantity of gelling agent was sprinkled on the top and stirring was continued for ~3 hr to yield a smooth homogeneous suspension. A control suspension without the drug was prepared for every formulation. All the suspensions were analyzed for benzocaine content and were stored at 30° until needed.

[1] Igepal CO series, GAF Corp., N.Y.

RESULTS AND DISCUSSION

Details of the suspension formulations are listed inTable I. In the case of all the formulations studied, when the cumulative amount of benzocaine in the receptor compartment was plotted against time, a linear relationship with a small lag time was observed. A typical plot is shown in Fig. 1. The gradual rise in penetration rate (Fig. 1) until steady state was achieved is typical of membrane controlled diffusion. When transport through the vehicle is rate limiting, the flux is initially high and then gradually decreases with time. A second indication that diffusion across the stratum corneum was the slow step in benzocaine transport is high flux values obtained from the suspension formulations, which will be discussed.

The flux values were calculated for all formulations (Table I). ANOVA showed that drug concentration, gelling agent, and surfactant concentration had no statistically significant effect on flux. No influence of the surfactants on skin membrane integrity was evident.

These results are similar to those with saturated benzocaine solutions (1). However, there is a surprising difference in the magnitude of the penetration values when saturated solutions are compared to suspensions. Mean benzocaine flux from saturated solutions was ~60 $\mu g\ hr^{-1}\ cm^{-2}$ (1), whereas the average flux for suspensions was 101 $\mu g\ hr^{-1}\ cm^{-2}$. This difference was unexpected since the free benzocaine concentration, which provides the driving force for diffusion, should have been the same in both types of preparation.

In penetration experiments, the amount of drug transported during the course of an experiment is usually small enough to justify the assumption that the donor concentration is constant. In conducting experiments on benzocaine solutions, there had been no reason to question the validity of this basic assumption. However, in view of the difference in benzocaine flux from saturated solutions as compared to suspensions, it was hypothesized that depletion of drug from the donor must have been taking place in the solution systems during the course of the penetration experiments.

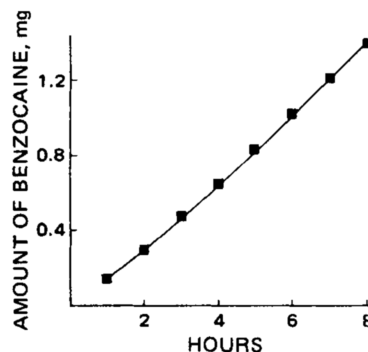

Figure 1—*Penetration of benzocaine through hairless mouse skin from a 5% suspension containing 0.0227* M *polyoxyethylene (15) nonylphenol.*

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Table I—Skin Penetration of Benzocaine from Aqueous Suspensions

Formulation	Benzocaine Concentration, % w/v	Gelling Agent, % w/v Hydroxypropyl Cellulose	Gelling Agent, % w/v Xanthan Gum	Surfactant Concentration $M \times 10^3$ $n = 50$	Surfactant Concentration $M \times 10^3$ $n = 15$	Flux ± *SD*, mg hr^{-1} cm^{-2} $\times 10^3$
A	1	2	—	—	22.7	97.3 ± 6.5
B	5	2	—	—	22.7	109.8 ± 4.3
C	10	2	—	—	22.7	98.6 ± 8.1
D	1	—	0.4	—	22.7	88.9 ± 7.3
E	5	—	0.4	—	22.7	97.3 ± 8.7
F	5	—	0.4	22.7	—	108.6 ± 6.3
G	5	—	0.4	—	2.27	107.8 ± 6.2

To test this hypothesis, a set of skin penetration experiments was conducted using a saturated aqueous benzocaine solution as donor, and at regular intervals the donor and receptor solutions were sampled and analyzed for benzocaine content. Figure 2 shows the results of these experiments. From the start, drug concentration in the donor dropped rapidly. The rate of drug transport to the receptor under sink conditions dropped during the 24-hr period for which data were collected. However, for the first 8–10 hr, the usual time allotted to previous experiments, the penetration rate was pseudo zero-order.

Assuming that the drug is distributed between donor, receptor, and skin at any given time, the donor and receptor were analyzed and the amount of benzocaine present in skin as a function of time was calculated by difference. Results are plotted in Fig. 2. There was significant uptake of benzocaine by the membrane. At the end of 24 hr, the skin contained 1.2 mg of benzocaine, while only 0.65 mg of benzocaine was left in the donor compartment.

To confirm the fact that a significant amount of benzocaine was actually retained in the skin, a new set of experiments was performed. A saturated aqueous solution was placed on hairless mouse skin and left undisturbed for 24 hr. At the end of 24 hr, the donor and receptor solutions were completely removed from the diffusion cell and analyzed for benzocaine content. The amount of benzocaine retained in the skin was estimated as the difference between the original quantity in the donor and that found in the analyses. The donor solution was then replaced by distilled water and the experiment was continued with regular withdrawal and replacement of receptor solution. The results are plotted in Fig. 3. Benzocaine release continued for the duration of the experiment (144 hr). Approximately 70% of the benzocaine estimated to be in the skin was removed during this period. These data confirm that a significant quantity of benzocaine was bound by the skin.

The mineral oil–water partition coefficient of benzocaine is ~0.9. Based on the relative volumes of the skin and donor compartments, the apparent skin–donor partition coefficient is many times higher than this value. Apparently, proteinaceous material in the skin is responsible for benzocaine uptake. Although no data on protein binding of benzocaine was found, other anilide local anesthetics such as lidocaine, mepivacaine, and bupivacaine are bound by proteins (4). Investigation of skin binding of drugs has not been extensive. *In vitro* binding of testosterone and benzyl alcohol to human dermis was reported previously (5, 6).

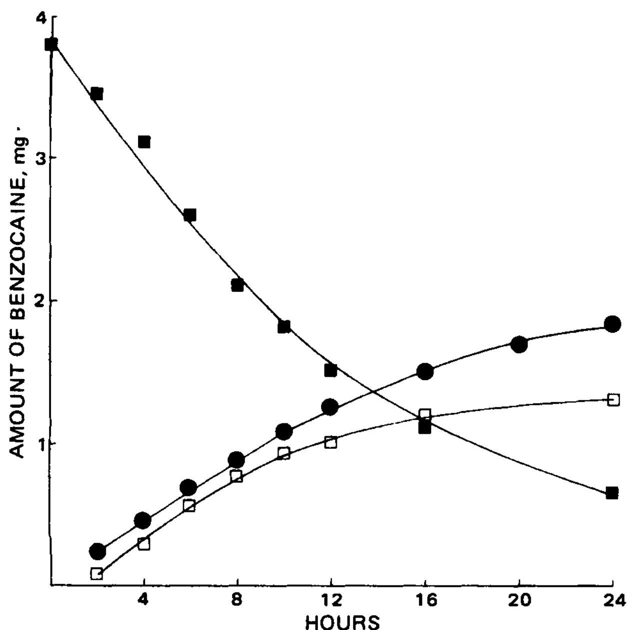

Figure 2—*Skin penetration of benzocaine from saturated aqueous solution. Key: (■) donor; (●) receptor; (□) skin.*

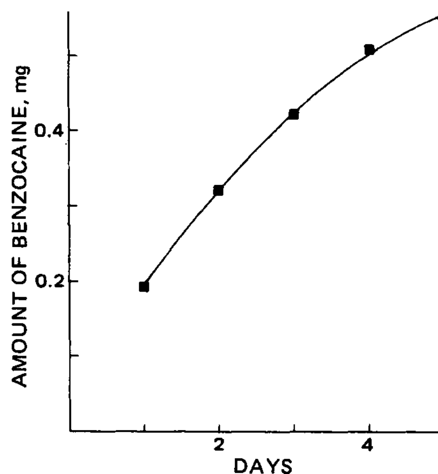

Figure 3—*Benzocaine release from the skin after complete withdrawal of donor solution.*

A dual sorption model was used (7) to explain the sorption and skin penetration behavior of aqueous scopolamine solutions. The same model is in qualitative agreement with the findings on benzocaine transport through hairless mouse skin. A significant portion of the benzocaine that enters the stratum corneum is immobilized there without contributing to the flux. This material, together with the benzocaine that partitions in the membrane and diffuses into the receptor, causes loss of benzocaine from the donor. If the donor is a suspension, the solid particles act as a reservoir to replenish lost benzocaine and maintain a constant concentration. Sorbed benzocaine equilibrates with nonimmobilized drug, the system eventually reaches diffusional steady state and the sorption of benzocaine by the membrane has no effect on the penetration flux. However, with benzocaine solutions, drug that is lost from the donor by penetration and sorption is not replaced and the driving force for penetration is reduced. The result is a decrease in the apparent steady-state

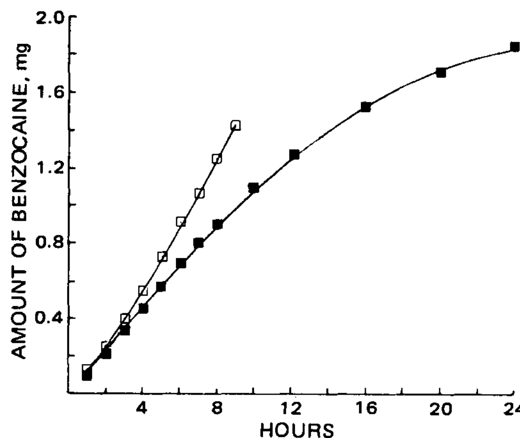

Figure 4—*Skin penetration of benzocaine from saturated aqueous solution. Key: (■) saturated solution; (□) saturated solution with 1 mg/ml of excess drug.*

flux value, in comparison to suspensions, as well as a continuing reduction in flux with time.

As a final test of this explanation, and to ensure that the surfactant used in the suspension formulations did not influence the results, experiments were performed using a saturated solution of benzocaine in water to which excess benzocaine (1 mg/ml) was added.

The results are plotted in Fig. 4 for the penetration profiles with and without excess drug in donor. It is evident that the slopes for the two curves are different. The flux for the donor with excess drug (100×10^{-3} mg hr^{-1} cm^{-2}) was very close to the values obtained for the suspension systems (Table I).

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Degradation of Melphalan in Aqueous Solutions—Influence of Human Albumin Binding

HANS EHRSSON ˣ **and ULLA LÖNROTH**

Received April 28, 1981, from the *Karolinska Apoteket, S-104 01 Stockholm, Sweden.* Accepted for publication September 28, 1981.

Abstract □ The protein binding and degradation rate of melphalan in human albumin solutions and plasma have been investigated. In plasma, melphalan is bound 69.0 ± 3.4% (25°, melphalan concentration 25 μg/ml). The stability of melphalan when bound to albumin is about three times higher ($k = 3.07 \times 10^{-2} \pm 0.48 \times 10^{-2}$ hr^{-1}) than unbound in solution ($k = 1.14 \times 10^{-1} \pm 0.01$ hr^{-1}).

Keyphrases □ Melphalan—degradation studies in aqueous solutions, influence of human albumin binding □ Binding—human albumin to melphalan, degradation of melphalan in aqueous solutions □ Albumin, human—influence in binding with melphalan, degradation in aqueous solutions

Melphalan, an alkylating agent of the nitrogen mustard type, can be bound to plasma proteins as a result of a chemical reaction with the protein molecules as well as by a process of reversible adsorption (1). At least 60% of melphalan was bound to serum proteins (26°) as studied by an equilibrium dialysis technique (2). However, the quantitative determinations were performed using an unselective technique, and it is unclear to what extent the results are affected by a codetermination of degradation products of melphalan formed during the dialysis procedure. Melphalan (30%) was observed previously to be undialyzable in human plasma (4°), the quantitative determinations being performed by high-performance liquid chromatography (1). In the present study the reversible protein binding of melphalan in albumin solutions and human plasma has been determined by a modified ultrafiltration technique (3). Since the binding of melphalan to albumin might have a profound effect on its stability *in vivo* (4), the rate constants for the degradation of protein-bound and free melphalan have been evaluated.

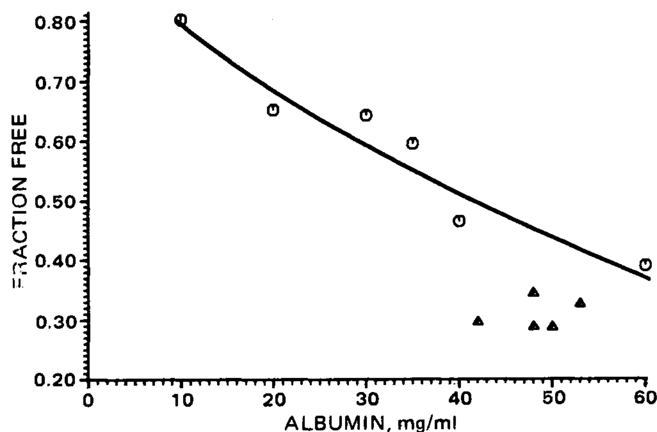

Figure 1—*Influence of albumin concentration on fraction free melphalan (25°; melphalan concentration: 25 μg/ml). The fraction free melphalan is a mean of four determinations. Key:* (○) *albumin solutions;* (▲) *plasma samples.*

EXPERIMENTAL

Degradation Studies—Melphalan[1], dissolved in 0.1 *M* HCl, was diluted 100 times with a solution of human albumin[2] in isotonic phosphate buffer pH 7.35 (NaCl concentration 0.095 *M*) or plasma[3] to a final melphalan concentration of 25 μg/ml. The mixture was incubated at 25.0 ± 0.1° and at appropriate times aliquots were analyzed by liquid chromatography.

Protein-Binding Studies—The protein binding was determined using a modified ultrafiltration technique (3). The studies were carried out at 25 ± 2° using albumin or plasma solutions containing 25 μg/ml of melphalan, unless otherwise stated. The degradation of melphalan in the ultrafiltrate was minimized by collecting it in albumin solutions containing the same concentration of albumin as the solution inside the dialysis tubing. No binding of melphalan to the dialysis membrane could be observed as studied by ultrafiltration of melphalan dissolved in isotonic phosphate buffer pH 7.35.

Quantitative Analysis—The quantitative determinations of melphalan were performed by liquid chromatography according to previous

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Human albumin, essentially fatty acid free, Sigma Chemical Co. (A 1887), St. Louis, Mo.
[3] Blood was obtained from drug-free volunteers and collected in tubes containing heparin. The plasma was separated by centrifugation and stored at −20° until used.

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