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Effect of Surfactants on Percutaneous Absorption of Naproxen II: *In Vivo* and *In Vitro* Correlations in Rats

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Abstract □ The effect of surfactants on percutaneous absorption of naproxen was studied using rat *in vivo* models. The *in vivo* normalized relative absorption rates were in good agreement with the *in vitro* relative flux. An antipyretic model in the rat could not show relatively small increases in percutaneous absorption caused by the surfactants. Based on these results, it is apparent that reliance on the rat, using either *in vitro* or *in vivo* models, may lead to erroneous conclusions when considering the effect of surfactants on human percutaneous absorption of drugs such as naproxen.

Keyphrases □ Surfactants, various—effect on percutaneous absorption of naproxen, *in vivo* and *in vitro* correlations in rats □ Naproxen—percutaneous absorption, effect of various surfactants, *in vivo* and *in vitro* correlations in rats □ Absorption, percutaneous—naproxen, effect of various surfactants, *in vivo* and *in vitro* correlations in rats □ Anti-inflammatory agents—naproxen, percutaneous absorption, effect of various surfactants, *in vivo* and *in vitro* correlations in rats

Percutaneous absorption generally is studied by two types of model systems: those employing excised skin in some type of a diffusion cell and those using living animals or humans *in situ*. Many studies suggested that these two model systems may differ in their mode of percutaneous absorption, namely, the *in vitro* measurement of steady-state flux *versus* the pharmacological activity mainly induced by the small amount of drug penetrating through the appendageal route in the early stages of diffusion.

BACKGROUND

Penetration through sweat ducts under a potential gradient occurred within 1–5 min, with no comparable transport through the stratum corneum within this period (1). Histological studies (2) also demonstrated follicular diffusion occurring within 5 min. Similarly, perifollicular wheals were observed 5 min after the application of 10% histamine free base (3). Some studies also related the *in vitro* steady-state flux to the *in vivo* steady-state flux. For example, the *in vitro* and *in vivo* transport of alkylmethyl sulfoxide across rabbit skin was studied, and the steady-state urinary elimination rate was about one-half of the *in vitro* steady-state flux (4).

Because of the question of the absorption mechanism, *i.e.*, intra-appendageal or transepidermal, in *in vivo versus* model systems and further complications due to variations in skin permeability from site to site, human to human, and species to species, comparable quantitative data between *in vivo* and *in vitro* model systems are sparse. A notable exception may be data (5) showing a similarity between the *in vitro* skin penetration and *in vivo* vasoconstriction response of fluocinonide and fluocinolone acetonide.

Previously (6), an *in vitro* model using excised skin from laboratory animals and humans was used to study the effect of surfactants on percutaneous naproxen absorption. The results suggested that excised skin of laboratory animals may lead to erroneous conclusions about the effect of surfactants on excised human skin.

This paper reports the effect of surfactants on percutaneous naproxen absorption in the rat *in vivo*. Comparisons between the *in vivo* normalized relative absorption rates and the *in vitro* relative flux in the rat indicate good correlation between the two model systems. Since surfactants showed only a small increase in percutaneous absorption in the rat, an antipyretic bioassay model in the rat could not show a similar correlation.

EXPERIMENTAL

Materials—The surfactants sodium lauryl sulfate[1], sodium laurate[2], and methyldecyl sulfoxide[3] were used as received.

Nonlabeled naproxen[4] was at least 99% pure. The tritiated naproxen was purified by radiochromatography and was at least 98% pure. The two solvent systems used were hexane–ethyl acetate (85:15) and benzene–tetrahydrofuran–acetic acid (90:9:3). All other chemicals were analytical reagent grade unless otherwise indicated.

Preparation of Creams—The method for the preparation of creams was as described previously (6).

***In Vivo* Percutaneous Absorption in Rats**—Male Sprague–Dawley rats[5], 250–300 g, were employed. Hair was removed[6] from the skin of the dorsal area between the forelegs and hindlegs on both sides of the spine, 18 hr prior to the application of cream. Throughout the shaving and dosing operations, the animals were kept under light anesthesia with anesthetic grade ether[7].

A cardboard template with an outside measurement of 4 × 5 cm and an inside measurement of 2 × 3 cm was devised to restrict and control the contact area between the cream and skin of the rat. The cardboard was covered on both sides with waterproof plastic tape[8] and was held in position by medical adhesive tape[9]. An accurately measured dose (0.5 ml) was delivered with a 1-ml tuberculin syringe to a 2 × 3-cm area. A

[1] E. I. du Pont de Nemours & Co., Menlo Park, Calif.
[2] Eastman Kodak Co., Rochester, N.Y.
[3] Institute of Organic Chemistry, Syntex Research, Palo Alto, CA 94304.
[4] *d*-2-(6′-Methoxy-2′-naphthyl)propionic acid, Syntex Research, Palo Alto, CA 94304.
[5] Simonsen Laboratories, Gilroy, Calif.
[6] Model-A2 animal electric clipper with size 40 blade, John Oster Manufacturing Co., Milwaukee, WI 53200.
[7] Mallinckrodt Chemical Works, St. Louis, MO 63100.
[8] Scotch No. 471, 3M Co., St. Paul, Minn.
[9] Zonar Porous Tape, Johnson & Johnson.

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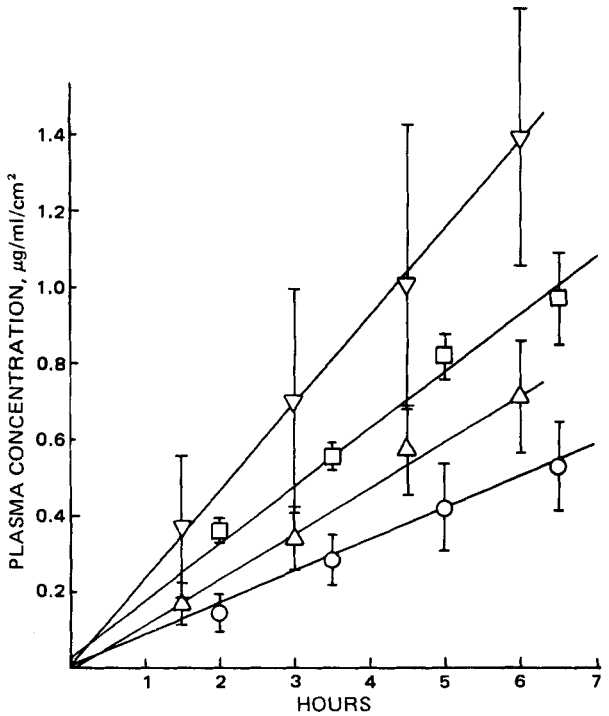

Figure 1—*Effect of surfactants on the* in vivo *percutaneous absorption of naproxen in the rat. Experimental points are averages of three rats, and vertical bars represents standard errors. Lines are the regression lines. Key:* ○, *control,* $y = -0.008 + 0.083x$, $r = 0.999$; △, *2% sodium lauryl sulfate,* $y = -0.007 + 0.121x$, $r = 0.998$; ▽, *2% sodium laurate,* $y = 0.029 + 0.150x$, $r = 0.996$; *and* □, *1% methyldecyl sulfoxide,* $y = 0.01 + 0.231x$, $r = 1.00$.

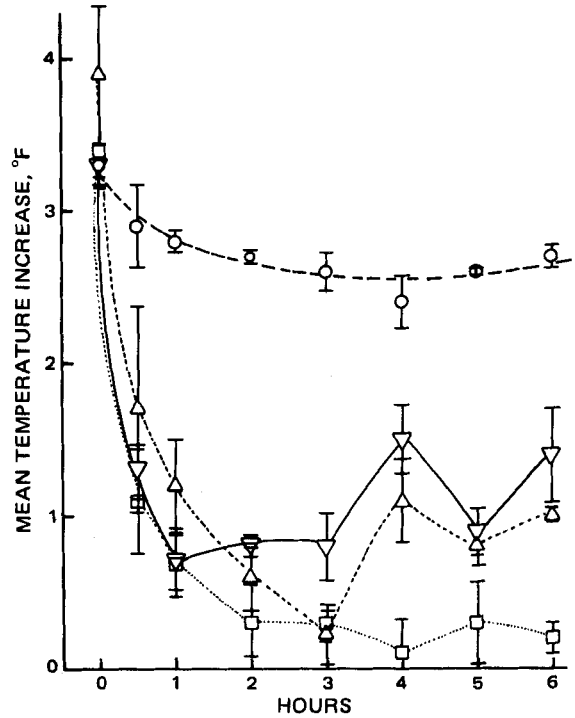

Figure 2—*Antipyretic activity of naproxen following topical application of cream formulations with and without surfactants. The curves indicate onset, intensity, and duration of antipyretic activity. Key:* ○, *placebo control;* △, *naproxen cream without surfactant;* ▽, *naproxen cream containing 1% methyldecyl sulfoxide; and* □, *naproxen cream containing 2% sodium lauryl sulfate. Points are averages of five rats, and vertical lines represent standard error.*

plastic film[10], 4 × 5 cm, was placed over the dose and the template and taped in place.

The cream was uniformly spread in the restricted area with a stainless steel spatula, and the rats were then transferred to individual cages. At appropriate intervals, blood was withdrawn by making an incision in the tail vein. Heparinized microhematocrit capillary tubes[11] were used to collect blood samples. These tubes were centrifuged immediately, and 50 μl of plasma was added to 15 ml of scintillation cocktail contained in a scintillation vial. Then the radioactive counts were determined[12].

The counts per minute of plasma samples were corrected for the quench effect, and the total radioactivity was converted to micrograms of naproxen per milliliter of plasma using an internal standard. Three animals were used for each evaluation.

***In Vivo* Studies Using a Rat Antipyretic Model**—The procedure described by Roszkowski *et al.* (7) was used. The dorsal fur of female rats[5], 90–100 g, was clipped 1 day prior to taking the "normal" rectal temperature at 0 hr, after which pyresis was induced by subcutaneous administration, dorsally and ventrally, of 2 ml of a 44% suspension of yeast (one 0.6-oz cake of bakers yeast[13] in 22 ml of 0.9% NaCl). The injection sites were then massaged to spread the suspension beneath the skin.

At 17 hr, the rats were massaged again to stimulate a further increase in body temperature. At 18 hr, the rectal temperature was recorded for the second time, which served as the baseline from which antipyresis was determined. An accurately measured dose (0.1 ml) of naproxen cream was delivered to a specific skin area and spread uniformly over an area 2 cm². Subsequent rectal temperatures were recorded at appropriate intervals.

RESULTS

The initial plasma naproxen concentration *versus* time profiles obtained following topical application of the four cream formulations are given in Fig. 1. The relative rates were obtained from the linear regression lines. These rates were normalized for making comparisons to the *in vitro* relative flux data. Table I shows that excellent correlation exists between the *in vitro* flux and the *in vivo* normalized rates, with the possible exception of the formulation containing 1% methyldecyl sulfoxide. These results suggest that the *in vitro* skin penetration model provides a good index of the *in vivo* percutaneous naproxen absorption in the rat.

A bioassay model using rats was developed (7) for determining antipyretic effects of naproxen. This model has been utilized to investigate the differences, if any, in the antipyretic activity of naproxen due to the presence of surfactants in the formulation applied topically. The mean temperature increase *versus* time plots following injection of a yeast suspension and the application of the placebo control and creams under study are given in Fig. 2. A definite antipyretic effect occurred from topical application of creams containing naproxen. However, the effect of the surfactants in enhancing percutaneous absorption of naproxen could not be demonstrated. The creams with and without surfactants showed similar onset, intensity, and duration of antipyretic activity. This result is probably because of the lack of sensitivity of the bioassay model to show small enhancement in percutaneous absorption in the rat from creams containing surfactants.

DISCUSSION

Percutaneous absorption studies using an *in vitro* skin penetration model assume that the living processes and the skin surface conditions do not affect medicament penetration. The main barrier to skin penetration is the stratum corneum; *i.e.*, the model also assumes that the steady-state flux obtained after a long lag time may serve as a good index of the *in vivo* situations, where percutaneous absorption is frequently measured during the lag time. In view of these assumptions of the *in vitro* model, it is important to establish a correlation between *in vitro* and *in vivo* models. The results of this study show some correlation between the results of *in vitro* and *in vivo* models in the rat. Additional studies directed specifically at the correlation of *in vitro* and *in vivo* topical absorption are clearly required to clarify this point.

[10] Saran Wrap, Dow Chemical Co., Midland, Mich.
[11] Sherwood Medical Industries, St. Louis, Mo.
[12] Unilux II, Nuclear-Chicago, Chicago, Ill.
[13] Fleischmann's

Table I—Comparison of the *In Vitro* Relative Flux of Naproxen Using Excised Rat Skin and the Normalized Relative Rates of Percutaneous Absorption in the *In Vivo* Rat

Formulation	*In Vitro* Relative Flux[a]	*In Vivo* Normalized Relative Rates[b]
No added surfactant (control)	1	1
Sodium lauryl sulfate (2%)	1.3	1.5
Sodium laurate (2%)	1.7	1.8
Methyldecyl sulfoxide (1%)	1.5	2.8

[a] Data from Table II, Ref. 6. [b] Slopes of the linear regression lines in Fig. 2 were normalized.

It is also apparent that reliance on the rat using either *in vitro* or *in vivo* models may lead to erroneous conclusions regarding the effect of surfactants on human percutaneous absorption of drugs similar to naproxen. A great degree of caution should be exercised in using animal bioassay models in evaluating the effect of formulation changes on topical absorption.

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Rapid GLC Determination of Chlordiazepoxide and Metabolite in Serum Using On-Column Methylation

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Received December 12, 1977, from the *Pharmaceutical Products Division, Abbott Laboratories, North Chicago, IL 60064.* Accepted for publication March 13, 1978.

Abstract □ A rapid GLC method was developed for the assay of chlordiazepoxide in serum. After chlordiazepoxide was extracted with ether, it was methylated with trimethylanilinium hydroxide in the injection port and detected by electron capture. The assay is simple and sensitive and can be automated for large-scale clinical analysis.

Keyphrases □ Chlordiazepoxide—electron-capture GLC analysis in serum □ GLC, electron capture—analysis, chlordiazepoxide in serum □ Tranquilizers—chlordiazepoxide, electron-capture GLC analysis in serum

Electron-capture GLC determination of chlordiazepoxide in biological fluids was accomplished by assaying for either its hydrolysis product (1, 2) or the intact moiety (3, 4). These procedures were somewhat tedious and required large amounts of sample. The following method is rapid and has a sensitivity of about 45 ng/ml using 0.4 ml of serum.

EXPERIMENTAL

Reagents—Chlordiazepoxide hydrochloride[1] (I), trimethylanilinium hydroxide[2] in methanol (0.1 *M*), and 1,3-dimethyl-6-methoxy-4-(*p*-chlorophenyl)-1*H*-pyrazolo[3,4-*b*]pyridine[3] (II) as the internal standard were used as supplied. Ether[4] was anesthetic grade, and other chemicals were analytical reagent grade.

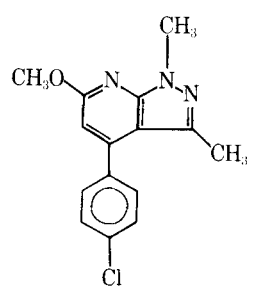
Instrumentation—A gas chromatograph[5], equipped with a ^{63}Ni-electron-capture detector containing a 2-mCu ^{63}Ni-β-ionization source, and an electronic integrator were used. The glass column was 1.21 m (4 ft) × 4 mm (i.d.), packed with 5% OV-225[6] on 80–100-mesh Gas Chrom Q[6]. The column was conditioned at 265° for at least 18 hr with a carrier gas [argon–methane[7] (95:5)] flow rate of 50 ml/min. The cylinder of the carrier gas was fitted with an oxygen trap filter[8].

The operating conditions were: column oven temperature, 265°; electron-capture detector temperature, 300°; injection port temperature, 270°; detector pulse interval, 150 μsec; electrometer range, 10^3; recorder presentation, 2 mv; and slope sensitivity, 0.3 mv/min. Under these conditions, the internal standard and chlordiazepoxide had retention times of 1.8 and 3.0 min, respectively (Fig. 1).

Table I—Assay Reproducibility

Serum Chlordiazepoxide Concentration, μg/ml	Calculated Serum Concentration[a], μg/ml	*RSD*, %
0	0	—
0.10	0.099 ± 0.0098	9.9
0.25	0.229 ± 0.062	27.2
0.75	0.758 ± 0.013	1.7
1.00	1.04 ± 0.14	13.7
1.50	1.48 ± 0.068	4.6

[a] Mean ± *SD* of triplicate determinations at each concentration.



II

[1] Lot 351074, Hoffmann–La Roche, Nutley, N.J.
[2] Eastman Kodak Chemicals, Rochester, N.Y.
[3] Lot 3827-LRS-25, synthesized from methylation of Compound 22 in *J. Heterocycl. Chem.*, **12**, 1137 (1975).
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[5] Hewlett-Packard model 7620A.
[6] Applied Science Laboratories, State College, Pa.
[7] Matheson Gas Products, Elk Grove Village, Ill.
[8] Altech Associates, Arlington Heights, Ill.

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