Improved Delivery through Biological Membranes X: Percutaneous Absorption and Metabolism of Methylsulfinylmethyl 2-Acetoxybenzoate and Related Aspirin Prodrugs

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Abstract □ Oxidative–reductive interconversion of the methylthiomethyl ester of aspirin and the corresponding sulfoxide and sulfone derivatives can be detected in rat liver homogenate, in addition to the extremely facile hydrolysis of these esters. The methylthiomethyl and methylsulfinylmethyl 2-acetoxybenzoates penetrate freshly excised hairless mice skin rather easily with the simultaneous hydrolysis of the two ester functions. Contrary to *in vivo* observations in dogs, where significant amounts of aspirin formed, the prodrugs cleave to salicylic acid and/or salicylate esters rather than aspirin.

Keyphrases □ Methylsulfinylmethyl 2-acetoxybenzoate—percutaneous absorption and metabolism, aspirin prodrugs □ Aspirin prodrugs—percutaneous absorption and metabolism □ Prodrugs—methylsulfinylmethyl 2-acetoxybenzoate and related aspirin agents, percutaneous absorption and metabolism

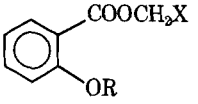
In the preceding papers of this series, novel prodrugs of aspirin (I) were described, together with the corresponding salicylic acid (II) derivatives as summarized by III–VIII. Although significant differences in the chemical and plasma hydrolyses of the double esters III–V were observed, *in vivo* studies clearly indicated that at least IV cleaves to aspirin; III and V might do so, but aspirin could not be detected in the dogs in these cases.

BACKGROUND

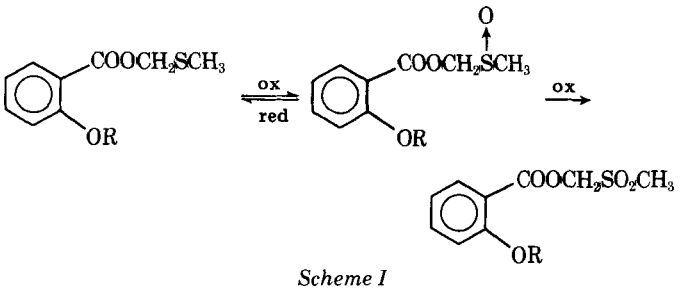
In addition to the direct hydrolysis of the ester functions, oxidative interconversion of the sulfur-containing alkyl groups also is possible, which might alter the expected hydrolytic behavior. Thus, similarly to the case of sulindac (1), these esters can be expected to participate in the *in vivo* redox system shown in Scheme I. Other sulfides and sulfoxides undergo similar transformations (2), which most likely occur in the liver. Thus, one objective of the present studies was to determine whether the oxidized forms, IV and V, can be detected if III is incubated with liver homogenate.

The possible topical use of the aspirin prodrugs, III–V, or the salicylic acid derivatives, VI–VIII, also is of interest. The primary use of salicylic acid in topical medication, aside from its antiseptic and germicidal uses, has been as a keratolytic and anti-inflammatory agent. Keratolytic drugs act to damage the cornified layer of the skin, which is then sloughed off to whatever depth the agent has acted. The traditional keratolytic agent has been salicylic acid in concentrations up to 6% in various creams and ointments (3).

Steroids commonly are applied topically to alleviate inflammatory



III: R = CH_3CO, X = CH_3S–
IV: R = CH_3CO, X = CH_3SO–
V: R = CH_3CO, X = CH_3SO_2–
VI: R = OH, X = CH_3S–
VII: R = OH, X = CH_3SO–
VIII: R = OH, X = CH_3SO_2–



Scheme I

states such as some allergic skin conditions, but only one nonsteroidal anti-inflammatory agent, bufexamac, is marketed as a topical cream (4). In a recent study (5), the anti-inflammatory effects of several steroidal and nonsteroidal drugs were tested topically on guinea pig skins in which UV dermatitis was induced. Salicylic acid was just as good an anti-inflammatory agent as bufexamac, and aspirin was even more active and, in some cases, was superior to hydrocortisone (6).

In the present study, the apparent permeability of the potential prodrugs of aspirin, III and IV, through excised hairless mice skin was studied and compared to aspirin. Since the skin, as all other portals of entry into the body, possesses metabolic activity (7), simultaneous metabolism–absorption processes were considered.

EXPERIMENTAL

Metabolism Studies in Rat Liver Homogenate—The liver homogenate was prepared following the method of Schneider (8). Two male Sprague–Dawley rats were killed by decapitation, and the livers were removed and chilled on an ice-cold glass plate. After cooling, the livers were weighed (total weight 28.4 g) and homogenized with a polytef pestle homogenizer in a glass mortar in 113.6 ml of 0.25 *M* sucrose (total volume 142 ml). The homogenate was centrifuged for 10 min at 600×*g*, and the supernate was decanted and centrifuged for 20 min at 24,000×*g*. The supernate, which was then 120 ml, was decanted and used undiluted for the oxidation–cleavage studies.

The supernate was stored in an ice bath and used within 5 hr after the killing of the animals. A 10-μl sample of a standard solution of the compound in acetonitrile was added to 3.0 ml of the supernate ($<10^{-3}$ *M*). Samples of 0.50 ml were removed, mixed with 0.50 ml of acetonitrile, and centrifuged for 20 min. The supernate was analyzed by high-pressure liquid chromatography (HPLC) as described previously (9).

Permeability–Metabolism Studies Using Hairless Mice Skin—Full-thickness dorsal skin of 12–14-week-old female hairless mice[1] was used. The mice were sacrificed by snapping of the spinal cord at the neck, and the whole dorsal skin then was removed. Adhering fat and other visceral debris were removed carefully from the undersurface. The excised skin was stretched gently over the lower opening of the plexiglass lid of a diffusion cell and secured with a rubber gasket, and excess skin was trimmed away.

The diffusion cells were manufactured[2] from plexiglass and consisted of a lower chamber (45 ml) with a side arm to allow sampling of the receptor phase, a lid, and a rubber gasket. A plastic-coated magnetic bar provided efficient mixing. The opening in the lid left exposed an 8.04-cm^2 area on the epidermis side through which penetration was measured. The

[1] Obtained from Jackson Laboratories, Bar Harbor, ME 04609.
[2] Kercso Engineering Consultants, Palo Alto, Calif.

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Table I—Permeability Coefficient and Lag Times for Apparent Permeability of Aspirin (I) and Methylthiomethyl (III) and Methylsulfinylmethyl (IV) 2-Acetoxybenzoates through Excised Hairless Mice Skin at 31°

Compound Applied	Compound Detected in Receptor Phase[a]	Permeability Coefficient, cm/hr	Lag Time, hr
Aspirin	I	$4.9 \pm 1.0 \times 10^{-3}$	0.82
	II	$1.4 \pm 0.1 \times 10^{-2}$	0.72
	Total	$1.9 \pm 0.2 \times 10^{-2}$	0.77
III	II	$4.0 \pm 0.5 \times 10^{-2}$	0.70
IV	VII	$9.6 \pm 4.0 \times 10^{-3}$	1.18
	II	$1.2 \pm 0.6 \times 10^{-2}$	0.33
	Total	$2.2 \pm 1.0 \times 10^{-2}$	0.87

[a] The compounds detected in the receptor phase were aspirin (I), salicylic acid (II), and methylsulfinylmethyl 2-hydroxybenzoate (VII).

receptor was filled with pH 5.4 isotonic sodium acetate–boric acid buffer (60 mg and 1.84 g, respectively, in 100 ml of deionized water). As a preserving agent, 0.1% formaldehyde was added. All air bubbles were removed carefully from the dermal surface of the skin by tipping the cell.

A solution of the test compound was made in acetonitrile containing 10% polyethylene glycol 400. Then 200 μl of this solution was applied to the skin; and the acetonitrile was evaporated by blowing air on the surface, leaving a 2 *M* solution of the compound in polyethylene glycol 400 spread evenly on the skin. Each cell then was placed on a magnetic stirrer in a 31° incubator in which a high constant humidity was maintained. Samples of 1 ml were removed from the receptor phase *via* the side arm and replaced with fresh buffer. The samples were analyzed by HPLC.

RESULTS AND DISCUSSION

The rat liver homogenate metabolism studies revealed that the various ester-type prodrugs of aspirin (III–V) and the corresponding salicylates (VI–VIII) were metabolized extremely fast. The disappearance rates of III and IV were 2.5 and 0.8 min^{-1}, corresponding to half-lives of 0.3 and 0.9 min, respectively. These values reflect significantly faster metabolism in the liver than in plasma. [The corresponding half-lives in human plasma at 37° were 1.2 (III) and 14 (IV) min, respectively.] The analysis of the liver homogenate mixtures indicated that III was hydrolyzed mainly to VI and then to salicylic acid. The main product from IV also was salicylic acid. However, despite the fast hydrolysis rates, small fractions of both IV and V could be detected after incubation of the homogenate with III. In the same way, small fractions of VII could be detected after incubation with VI, and small fractions of V could be detected after incubation of the homogenate with IV. This finding clearly indicates that some oxidative interconversion of the species occurs.

The skin permeability studies also indicated extensive metabolism of III and IV. This result is not surprising; although the stratum corneum is the main diffusion barrier (10), the conventional drug permeation mathematical models (11–13) had to be modified to account for simultaneous metabolism–permeation when prodrugs were used (14, 15). The whole skin, particularly the epidermis, contains many highly active enzyme systems (7), and some esterase activity has been found in the stratum corneum of pig skin (16).

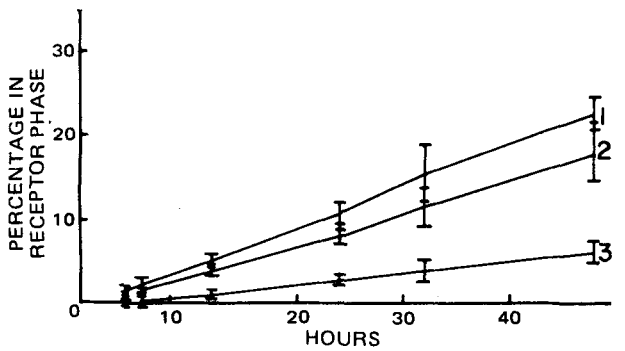

Figure 1—*Permeability of aspirin (I) through hairless mice skin as percentage of aspirin applied. Key: 1, total appearance in the receptor phase; 2, salicylic acid (II); and 3, aspirin.*

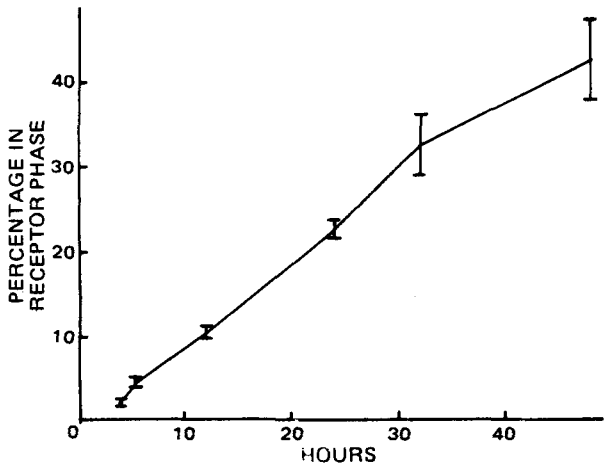

Figure 2—*Permeability of methylthiomethyl 2-acetoxybenzoate (III) through hairless mice skin as percentage of dose applied and appearance as salicylic acid (II) in the receptor phase.*

The main enzyme systems of interest in this study were the esterases, which can attack both the aromatic carboxyl esters and the acetyl ester in aspirin and its derivatives, and oxygenases, which could possibly oxidize the sulfur in the thiomethyl and sulfoxide derivatives.

The methylsulfonylmethyl 2-acetoxybenzoate (V) had low solubility in polyethylene glycol 400 and tended to precipitate after application to the skin. Therefore, it showed little permeability in the initial experiment and was not studied further. The obtained permeation profiles for I, III, and IV in terms of percentage in the receptor phase of the total amount applied to the skin as a function of time are displayed in Figs. 1–3. Permeability coefficients were calculated from the slopes of the amounts diffused *versus* time profile according to Eq. 1 in the time interval 5.5–24 hr:

$$P = \frac{V(dc/dt)}{AC_d} \qquad \text{(Eq. 1)}$$

where P is the permeability coefficient, V is the volume of the receptor phase, A is the surface area of the exposed skin, and C_d is the concentration of the solute in the donor phase.

The lag times were estimated graphically; they were quite sensitive to small differences in slopes, and slight experimental variability in slopes produced marked variability.

Each experiment was repeated five times, and the results given represent average values. The error bars in Figs. 1–3 represent the standard error of the mean. The permeability coefficients and lag times are listed in Table I.

The observed hydrolysis rate constant for aspirin in the receptor phase (pH 5.44) was measured separately at 31.0° and found to be $3.74 \pm 0.06 \times 10^{-4}$ min^{-1} or a half-life of 31 hr. These results indicate that when aspirin is applied to the skin, ~30% penetrates the skin unmetabolized,

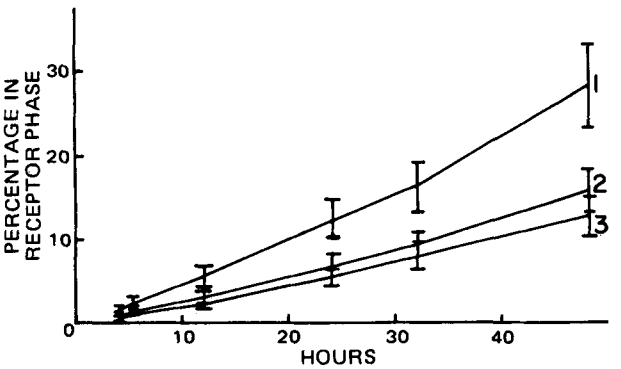

Figure 3—*Permeability of methylsulfinylmethyl 2-acetoxybenzoate (IV) through hairless mice skin as percentage methylsulfinylmethyl 2-hydroxybenzoate (VII) (curve 2), salicylic acid (curve 3), and total appearance (curve 1) in the receptor phase relative to the total amount applied to the skin.*

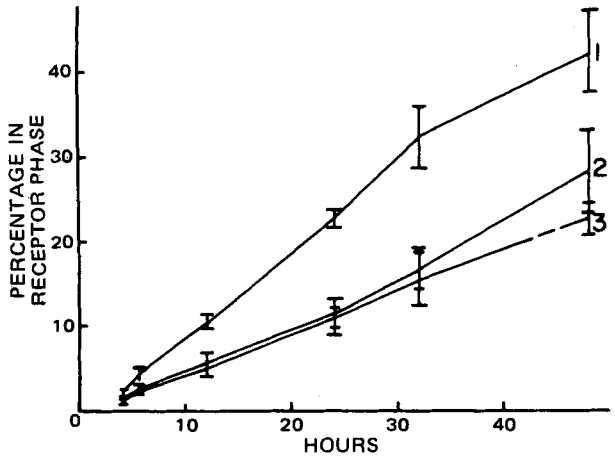

Figure 4—*Comparative permeability of aspirin (curve 3), methylthiomethyl 2-acetoxybenzoate (III) (curve 1), and methylsulfinylmethyl 2-acetoxybenzoate (IV) (curve 2) through hairless mice skin.*

while the major part, ~70% of the dose penetrating the skin, is hydrolyzed in the skin to salicylic acid during absorption (Fig. 1).

The hydrolysis half-life of the methylthiomethyl derivative (III) to aspirin was ~10 min and that of VI to salicylic acid, at 30° and pH 5.4, was ~9 min. If any significant amounts of aspirin are formed during passage through the skin or if III penetrates the skin without being metabolized, aspirin would be detected in the receptor phase. On the other hand, only salicylic acid was found when III was applied to the skin, which means that III is metabolized in the skin to VI or salicylic acid or both (Fig. 2).

The hydrolysis half-life of the methylsulfinylmethyl derivative (IV) to aspirin was ~10 days and that of VII to salicylic acid, at 30° and pH 5.4, was ~6 days. Thus, very little hydrolysis should occur in the receptor phase and almost all metabolites of IV detected in the receptor phase must be formed in the enzymatic cleavage in the skin. No aspirin was detected in the receptor phase after application of IV to the skin; only large amounts of VII and salicylic acid were found (Fig. 3). These results indicate that ~57% of IV penetrating the skin is metabolized to VII and that ~43% of IV is metabolized to salicylic acid.

The comparative cumulative penetration profiles for aspirin, III, and IV are shown in Fig. 4. The methylthiomethyl derivative (III) of aspirin is absorbed at a rate about two times faster than that of aspirin, while the methylsulfinylmethyl derivative (IV) is absorbed at the same rate as aspirin within experimental error.

It is clear that significant metabolism of all salicylic acid derivatives, including aspirin and its prodrugs, occur in the fresh mouse skin. The various ester functions hydrolyze with different rates and in a different order than what was shown to be their chemical hydrolysis or enzymatic cleavage in plasma or after the intravenous *in vivo* administration. The amounts penetrating the skin, however, are significant. It is possible to achieve therapeutic levels either as keratolytic or anti-inflammatory or analgesic agents. Further *in vivo* studies should be performed to answer these questions.

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Aluminum Chlorohydrate I: Structure Studies

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Abstract □ X-ray diffraction and IR and ^{27}Al-NMR spectroscopy indicate that aluminum chlorohydrate is composed of a central aluminum in a tetrahedral configuration surrounded by 12 aluminum atoms in octahedral configuration. The complex, $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$, is essentially spherical, with the +7 charge equally distributed on the surface. Seven chloride ions are associated with the complex as counterions. This structure is consistent with both the method of synthesis and the proposed mechanisms of antiperspirant activity.

Keyphrases □ Aluminum chlorohydrate—structure proposed by X-ray diffraction and IR and ^{27}Al-NMR spectroscopy □ X-ray diffraction—aluminum chlorohydrate, structure proposed □ Spectroscopy, IR—aluminum chlorohydrate, structure proposed □ ^{27}Al-NMR spectroscopy—aluminum chlorohydrate, structure proposed □ Antiperspirant activity—aluminum chlorohydrate, structure proposed based on X-ray diffraction and IR and ^{27}Al-NMR spectroscopic studies

Aluminum chlorohydrate, a basic aluminum complex, is widely used for its antiperspirant activity, for sealing porous strata in oil drilling operations, and to control the viscosity of kaolinite clays. It is known as aluminum chlorohydrate, aluminum hydroxychloride, basic aluminum chloride, or chlorhydrol. The empirical formula, $Al_2(OH)_5Cl{\cdot}2H_2O$, is known (1), but the structure has not been characterized. The chemistry of partially hydrolyzed

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