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Effect of sodium salicylate on in vitro percutaneous penetration of isopropamide iodide through mouse skin

Chul-Soon Young, Chang-Koo Shim, Min-Hwa Lee and Shin-Keun Kim

College of Pharmacy, Seoul National University, Seoul (Korea)

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Summary

The effects of temperature, concentration, hydration, and sodium salicylate (SA) on the permeability of isopropamide iodide (ISP) through the excised abdominal mouse skin were investigated using the in vitro diffusion cell technique. The permeability of ISP was increased as the temperature of the diffusion medium increased, but was independent of ISP concentration, which suggests a simple diffusion process of ISP through the skin. It was increased about 2-fold by hydration of the skin for 3 days in normal saline. The permeability of ISP through the hydrated skin increased in the presence of SA. SA itself did not seem to affect the permeability of the skin for ISP in an irreversible manner, since the increase of the permeability was not seen after SA pretreatment of the skin followed by repeated washing. The apparent *n*-octanol/water partition coefficient (APC) of ISP increased as the concentration of SA in the aqueous phase increased. The permeability of ISP through the skin increased as the APC of it increased. The increase of the permeability in the presence of SA might be due to the formation of lipophilic ion-pair complex between ISP and SA.

Introduction

Both experimental findings and various theoretical relationships have been published to elucidate the characteristics of the skin. The principal barrier function of the skin has been reported to reside almost entirely in the stratum corneum, the thin coherent membrane of flattened, compact, keratonized, metabolically inactive cells that comprised the dead surface of the epidermis (Nemanic and Elias, 1980; Scheuplein, 1976). The discovery that stratum corneum is the rate-limiting barrier to the skin penetration has led to numerous in vitro experiments using the isolated skin. The applicability of the in vitro technique is largely due to the non-cellular nature of the stratum corneum (Durrheim et al., 1980; Flynn et al., 1981; Pitman and Rostas, 1982). The phenomenon of the percutaneous absorption is essentially one of adsorption on to the stratum corneum, diffusion through it and through the viable epidermis, and finally through the papillary dermis and into the microcirculation. Among the aforementioned steps, diffusion through the stratum corneum itself is the rate-limiting step for the majority of substances (Idson, 1975; Scheuplein and Blank, 1971; Zatz, 1983). A simplistic two-phase model at the macromolecular level has been proposed which describes

Correspondence: C.K. Shim, College of Pharmacy, Seoul National University, Seoul 151, Korea.

the stratum corneum as a hydrophilic protein gel in a continuous lipid matrix through which penetrants migrate by dissolution and Fickian diffusion (Michales et al., 1975; Pitman and Rostas, 1982; Scheuplein and Blank, 1971).

There are many factors that can alter the rate of absorption into the skin, namely, temperature (Arita et al., 1970; Idson, 1971), hydration (Durrheim et al., 1980; Idson, 1971), surfactant (Grasso and Lansdown, 1972), solvent and vehicle (Dicolo et al., 1980; Scheuplein and Ross, 1970; Idson, 1983), APC and solubility (Durrheim et al., 1980), pH and ionization state (Arita et al., 1970; Idson, 1971), regional variation (Holbrook and Odland, 1974; Feldmann and Maibach, 1967) and species variation (Bartek et al., 1972). If all but APC of the above-mentioned factors can be kept constant, then it will be possible to determine the effect of APC on the permeability of the skin. The purpose of this study is to show how in vitro percutaneous penetration of ISP can be correlated with APC of it in the presence of various concentrations of SA. The idea is based in part on the well known fact that many ion-pair complexes are much more lipophilic than either of the interacting constituents and that the skin has the characteristics of a lipoidal barrier.

Materials and Methods

Materials

Isopropamide iodide (ISP; Yuhan Co.), sodium salicylate (SA; Kokusan Chemicals), *n*-octanol (Junsei Chemicals), chloroform (Tedia Co.), Methyl orange (Shinyo Pure Chemicals), gentamicin sulfate (Dong-A Pharm. Co.), hydrochloric acid (Junsei Chemicals), and ethanol (Merck) were used. All materials were of reagent grade. Male ICR mice (20–30 g) were obtained from the Experimental Animal Farm of Seoul National University.

Apparatus

A UV-spectrophotometer (Hitachi), pH-meter (Corning M-7), diffusion cell (Fig. 1), flash mixer (model D7-SK1801) and centrifuge (Kokusan Type H-36A) were used.

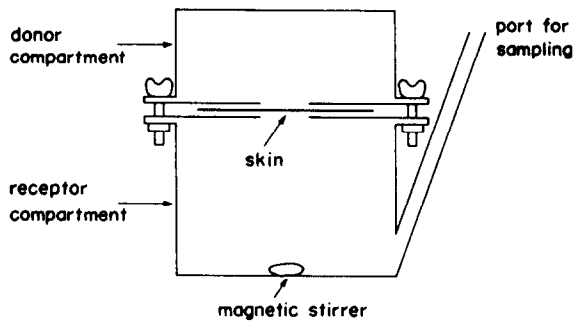

Fig. 1. Schematic illustration of the diffusion cell for in vitro study of the skin penetration of ISP.

Preparation of mouse skin

Clipped abdominal skin specimens excised from male mice were cleaned of adhering fat and visceral debris by blunt dissection and scraping, care being taken to avoid injuring the skin.

Quantitation of isopropamide iodide (ISP)

For the analysis of ISP, a modification of Santoro (1960) was used. A 2 ml aliquot of the sample was added to a screw-capped test tube containing 5 ml of Methyl orange buffer (pH 10.2) (Gaginella et al., 1973) and 10 ml of chloroform. After vigorous agitation for about 5 min, two phases were separated by centrifugation at 3000 rpm for about 5 min and a 5 ml aliquot of the chloroform layer was added to 2 ml of 0.5 N HCl in ethanol. Then the absorbance was measured spectrophotometrically at 525 nm.

Partition studies

n-Octanol saturated with 0.1 M phosphate buffer (pH 7.4) and 0.1 M phosphate buffer (pH 7.4) saturated with *n*-octanol were used. ISP and SA were dissolved in 0.1 M phosphate buffer (pH 7.4) saturated with *n*-octanol. The concentration of ISP was kept constant at 2.9×10^{-2} M, with only the concentration of SA being varied between 0 and 2.9×10^{-1} M. A 10 ml aliquot of the aqueous buffer solution containing ISP and SA was mixed vigorously with 10 ml of *n*-octanol in a screw-capped test tube for about 5 min and was shaken for 2 h at 25 °C in a water bath. After standing for 30 min at 25 °C in the water bath, two phases were separated by centrifugation at

3000 rpm for 10 min and the organic phase was discarded by aspiration. The aqueous phase was assayed for ISP. The concentration of ISP in the aqueous phase before and after partitioning was measured and APC and ISP was calculated by the difference.

Permeability studies

The permeability of ISP through the mouse skin was measured in the diffusion cell (Fig. 1); a piece of the skin was mounted to separate two compartments: the donor compartment and the receptor compartment. The stratum corneum surface of the skin was directed to the donor compartment. The volume of the receptor compartment and donor compartment were 94 ml and 30 ml, respectively. The receptor compartment has a port for sampling. The exposed area of the skin was 2.0 cm^2. The skin was hydrated for 3 days in the saline solution (37° C) containing 200 ppm of gentamicin sulfate prior to being mounted on the cell. Gentamicin sulfate was reported not to affect the membrane permeability and to prevent bacterial growth during skin hydration and subsequent experiments (Galey et al., 1976). To ensure a good sealing of the interphase between the two compartments, a small amount of high-vacuum silicone grease was coated onto the interphase prior to the skin mounting. After the skin was mounted, normal saline was added completely to the receptor compartment and then allowed to equilibrate for half-an-hour in the 37° C water bath. Then, ISP dissolved in the saline (30 ml) was added to the donor compartment and 2 ml aliquots were periodically withdrawn from the receptor compartment and analyzed for ISP concentration. The volume of the fluid removed was replaced by the fresh saline (37° C). The receptor solution was stirred by a teflon-coated magnetic bar at 300 rpm.

Calculation of the permeability constant

The permeability constant of each run was calculated from the ISP concentration of the receptor compartment versus time profile using (Durrheim et al., 1980):

$$K_p = V_r(dc/dt)/A\Delta C \quad (1)$$

where V_r is the volume of the solution in the receptor compartment, A is the exposed cross-sectional area of the skin between compartments, dc/dt is the rate of concentration change in the receptor compartment, and ΔC is the concentration difference between the two compartments.

The value of ΔC effectively is the donor concentration, since in these experiments the concentration of the receptor compartment is so low compared to the concentration of the donor compartment that it may be considered zero.

Results and Discussion

Effect of SA on the APC of ISP

As shown in Fig. 2, APC of ISP was increased by SA, which indicates the possible formation of the lipophilic ion-pair complex between ISP and SA. ISP, a quaternary ammonium salt, was reported to be fully ionized and form an ion-pair complex with SA at this pH.

The formation constant and true partition coefficient of the complex were reported to be 11.7 M^{-1} and 11.7, respectively (Shim et al., 1981; Shim, 1983).

Effect of hydration, temperature and ISP concentration on the permeability of the skin for ISP

The permeability of ISP was increased by hydration in normal saline at 37° C (Fig. 3). Any

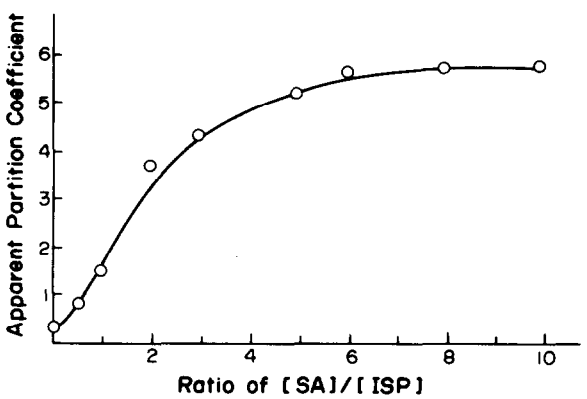

Fig. 2. Effect of SA on APC (*n*-octanol/pH 7.4 phosphate buffer) of ISP at 25° C. The concentration of ISP was kept constant at 2.9×10^{-2} M.

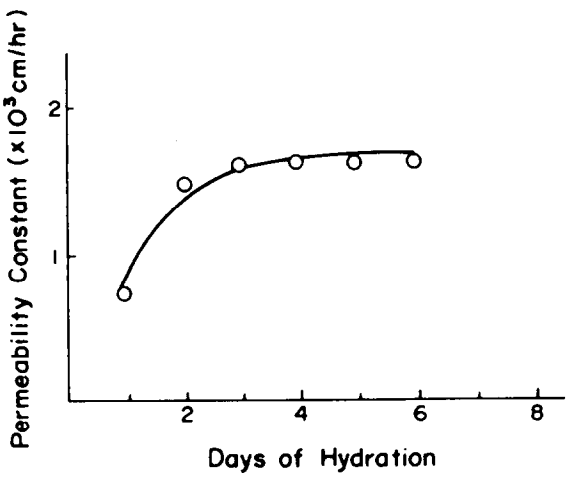

Fig. 3. Effect of hydration of the skin in the saline at 37 °C on the permeability of ISP.

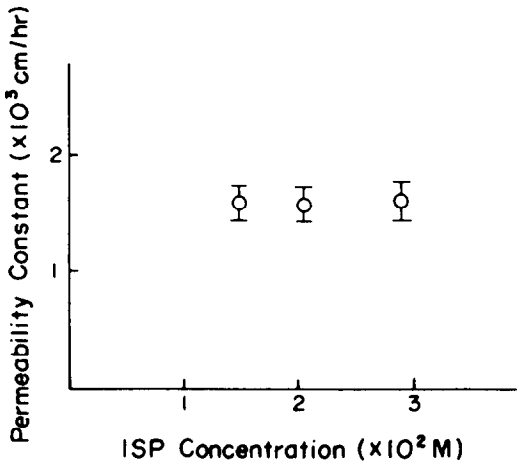

Fig. 5. Effect of ISP concentration on the permeability of itself at 37 °C. The data were expressed as the mean ± S.D. of experiments.

bacterial degradation of the skin was not observed during the hydration. A constant flux was achieved after 3 days of hydration. The permeability increased as the temperature of the diffusion medium increased (Fig. 4). But it was not affected by the concentration (1.5×10^{-2}–3.0×10^{-2} M) of ISP itself (Fig. 5), which suggests the simple diffusion for the mechanism of ISP permeation through the skin (Arita et al., 1970; Scheuplein and Ross, 1970; Flynn et al., 1974).

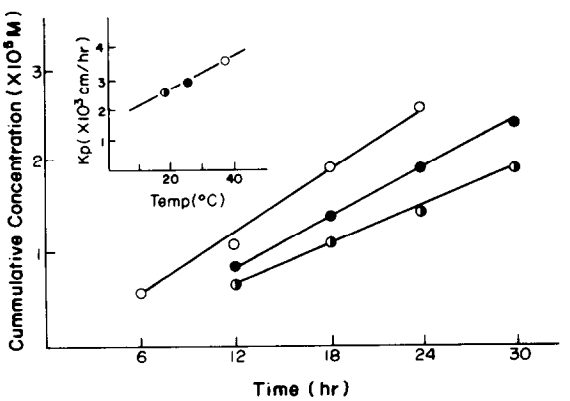

Fig. 4. Effect of temperature on the permeability of ISP. The inset indicates the correlation between temperature and permeability constant (Kp). [$K_p = (0.052° C + 1.59) \times 10^3$, $r = 0.999$, $P < 0.01$.] Key: ◑, 18 °C; ●, 25 °C; ○, 37 °C.

Effect of SA on the permeability of ISP through the skin

Immersion of the skin in SA solution (2.3×10^{-1} M) for 24 h followed by repeated washing did not affect the permeability of the skin (Fig. 6). This suggests that SA itself does not change the permeability of the skin by direct breakdown of the skin barrier. The permeability of ISP increased as the concentration of SA added increased (Fig.

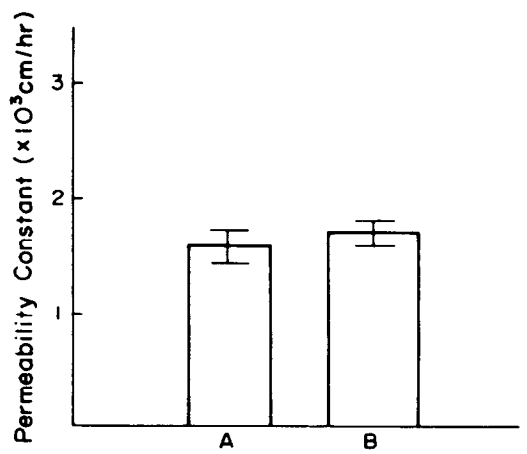

Fig. 6. Effect of SA pretreatment on the permeability of ISP through the skin at 37 °C. The data were expressed as the mean ± S.D. of 4 experiments. Key: A = permeability of the skin before pretreatment; B = permeability of the skin after pretreatment with SA (2.3×10^{-1}M) followed by repeated washing with the saline solution.

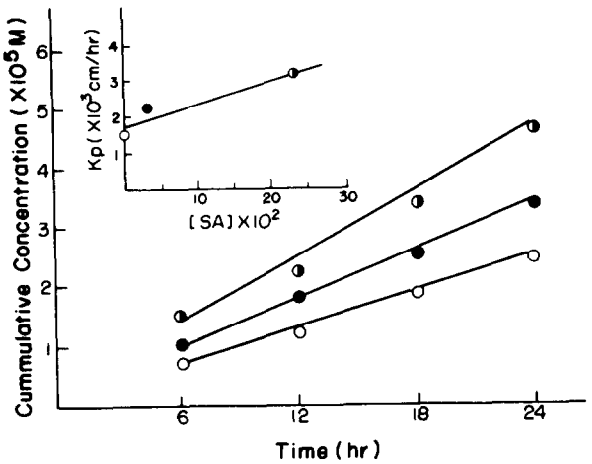

Fig. 7. Effect of SA on the permeability of ISP (2.9×10^{-2} M) at 37 °C. The inset indicates the correlation between molar concentration of SA, [SA], and permeability constant (Kp). [$K_p = (0.12[SA] + 1.78)\times10^3$, $r = 0.942$, $P < 0.4$.] Key: ○, ISP alone; ●, ISP with 2.9×10^{-2} M of SA; ◑, ISP with 2.3×10^{-1} M of SA.

7). The pH of the ISP solution (2.9×10^{-2} M) in the donor compartment was in the range of pH 7.0–7.6 in the presence of SA (0–2.3×10^{-1} M), which does not seem to affect the formation of ion-pair complex between ISP and SA, since both ISP and SA are known to be fully charged in this pH range (Gaginella et al., 1973; Shim, 1983).

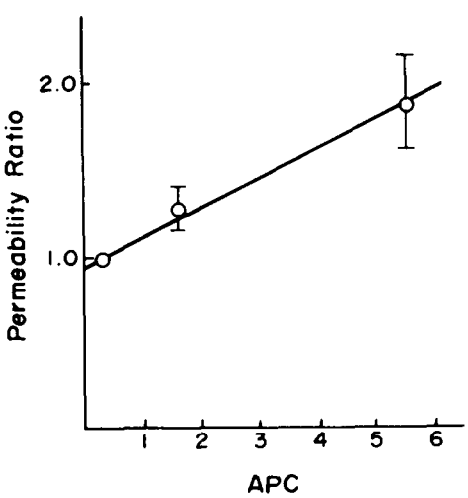

Fig. 8. Correlation between APC and permeability of ISP at 37 °C ($r = 0.999$, $P < 0.01$). Permeability ratio was calculated by [permeability constant of ISP in the presence of SA]/[permeability of ISP without SA] and expressed as the mean ± S.D. of 4 experiments.

The increment of APC of ISP by coexistence of SA may explain the permeability increase (Nishihata et al., 1980). The correlation between APC and permeability constant (Fig. 8) supports it. But the data presented here does not clearly explain the role of ion-pair formation in the penetration of ISP through the skin since gastrointestinal or rectal absorption of some drugs that do not form the ion-pair complexes with SA have been reported to be increased in the presence of SA (Nishihata et al., 1980). Additional studies are needed to elucidate the role of SA in the penetration of ISP through the skin. Although the role of ion-pair formation in drug penetration through the skin is not clarified, the idea of increasing the permeability of a fully ionized, water-soluble drug by forming a lipid-soluble complex with a counter-ion seems to be intriguing.

Conclusion

The permeability of ISP through the mouse skin increased as the temperature of the diffusion cell or hydration increased, but was independent of ISP concentration studied (1.5×10^{-2}–3.0×10^{-2} M). APC of ISP increased as the concentration of SA added increased. It was not due to direct breakdown of the skin barrier by SA since the effect of SA pretreatment disappeared after washing the pretreated skin. Correlation between APC and the permeability of ISP in the presence of SA implies the role of ion-pair complexation in the skin penetration of ISP. The exact mechanism of the increased permeability of ISP by SA needs further study to be elucidated.

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