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Changes in site and size of application of indomethacin ointment and percutaneous absorption in rabbits

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Summary

The percutaneous absorption of indomethacin from ointment applied to the skin surface was influenced by the anatomical site of skin treated and the size of the application area. Dorsal sites led to higher levels than abdominal sites which in turn led to higher levels than application to the thigh areas. The absorption increased in direct proportion to the size of the application area in the abdomen. The effects of different pH values of indomethacin solution in the ointment on the percutaneous absorption were examined. At a lower pH (7.07), there was good absorption.

Introduction

The influence of the characteristics of ointment bases on the percutaneous absorption of indomethacin has been described previously (Naito and Tsai, 1980). It was demonstrated that the percutaneous absorption of the drug from various ointment bases occurred by passive diffusion according to a first-order process, and good percutaneous absorption of the drug was found with an absorption ointment containing 0.5% urea as an additive. However, the factors which influence the penetration of the skin barrier are essentially similar to those which influence G-I absorption. These factors can be divided into physicochemical and physiological variables (Idson, 1971; Grasso and Lansdown, 1972; Idson, 1975). In addition to the physicochemical factors, the rate of absorption of substances through the skin is

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dependent on the condition of the skin, the area of skin treated, the location of the skin treated, species variations, etc. Although the anatomical site of application has been shown to affect the percutaneous absorption of drugs in man (Maibach et al., 1971; Hansen, 1978), in rats (Horbota and Fung, 1978), and in the rhesus monkey (Noonan and Wester, 1980), much of the data are conflicting. Exact information on this point is important in the case of indomethacin since systemic availability is the primary goal of topical administration.

The purpose of the present study was to examine the effects of the anatomical site of skin treated and the size of the application area on the percutaneous absorption of indomethacin in rabbits. The results may have an important bearing on the most effective use of topical administration to produce systemic effects. In addition, the effects of the pH value of the water phase of the absorption ointment on the percutaneous absorption of indomethacin were investigated.

Materials and Methods

Materials

The following reagents were used: indomethacin[1], acetonitrile, acetic acid, ethyl ether, citric acid, sodium phosphate dibasic 12 hydrate, *p*-hydroxybenzoic acid, sodium bicarbonate, urea, Brij-35, cetyl alcohol[2], sorbitan sesquioleate[3] and white vaselin (JP)[4].

Solution-type absorption ointment

The water phase of the absorption ointment was prepared by dissolving 200 mg of indomethacin in 4 ml of distilled water containing 120 mg of sodium bicarbonate and the additive of 0.5% urea was then dissolved into it. The oil phase contained 26.7% white vaselin, 12% cetyl alcohol, 4% sorbitan sesquioleate, and 0.4% Brij-35. The aqueous and oil phases were heated separately to about 75°C in a water bath and the aqueous phase was then added to the oil phase with appropriate stirring. After formation of an emulsion, the stirring was continued until the temperature of the cream reached 30°C.

Absorption ointment bases of various pH values were prepared by substituting the amounts of sodium bicarbonate shown in Table 1 for the same weight of water in the original formula mentioned above. The pH was determined with a Horiba pH meter (F7AD)[5] for the aqueous phase of each ointment base.

Adhesive test of the ointment on rabbit skin

White male rabbits, 1.8–2.2 kg, were used. The hair was removed with electric

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[1] Sumitomo Chemicals, Osaka, Japan.
[2] Nakarai Chemicals, Kyoto, Japan.
[3] Tokyo Chemicals , Tokyo, Japan.
[4] Maruishi Pharmaceuticals, Osaka, Japan.
[5] Horiba, Kyoto, Japan.

TABLE 1

AMOUNTS OF SODIUM BICARBONATE AND THE CORRESPONDING pH VALUES FOR THE AQUEOUS PHASE, AND PERCENTAGES OF UNDISSOCIATED INDOMETHACIN [a] IN THE AQUEOUS PHASE AT EACH pH

Sodium bicarbonate (mg/7 g of ointment)	Aqueous phase pH	Percent of indomethacin in the aqueous phase at 25°C [b]
60	7.07	0.28
120	7.73	0.03

[a] The pK_a values of indomethacin were obtained from Fuwa et al. (1971).
[b] The values were determined from the Henderson-Hasselbach buffer equation.

hair clippers from the skin of the intended dosing region, such as the abdomen and dorsal surface of thigh, 24 h prior to application of the ointment. Seven grams of ointment was spread uniformly over the surface of a sheet of cloth, 5×6, 6×10 or 7.5×12 cm^2, and this was applied to the shaved surface of the rabbit. To employ occlusive dressing techniques (ODT) and ensure adequate contact between the ointment and the skin, the cloth was covered with a thin plastic film and fastened with the aid of adhesive tape around the edges.

Analytical method

The method for the analysis of indomethacin was that described previously (Tsai and Naito, 1980).

Results and Discussion

The effects of anatomical site of treated skin on the percutaneous absorption of indomethacin in rabbits were studied using an absorption ointment base containing 0.5% urea. The results of these experiments are summarized in Fig. 1. Application of indomethacin absorption ointment to the shaved dorsal surface showed rapid drug absorption into the systemic circulation, with peak plasma levels ranging between 2.5 and 3.5 μg/ml after 5 h. For comparison, the relationship between the area under the curve by the graphpaper weight method for plasma and the change in dosing site is shown in Fig. 2. The relative weight ratios were 1.43 (dorsal surface), 1 (abdomen) and 0.41 (thigh). The present data strongly suggest the presence of a site dependence for the topical absorption of indomethacin in rabbits (i.e. dorsal surface > abdomen > thigh). Cronin and Stoughton (1962), using the erythema reaction induced by vasodilators such as ethyl nicotinate and histamine, demonstrated that the forehead and dorsal surface showed a greater response than the limbs. They concluded that the presence of more hair follicles in the forehead and dorsal surface indicated increased penetration through the sebaceous glands. The hair and hair follicles at the dorsal site are thicker than those of the abdomen and thigh. This may provide a partial explanation for the differences in absorption. Other factors, such as

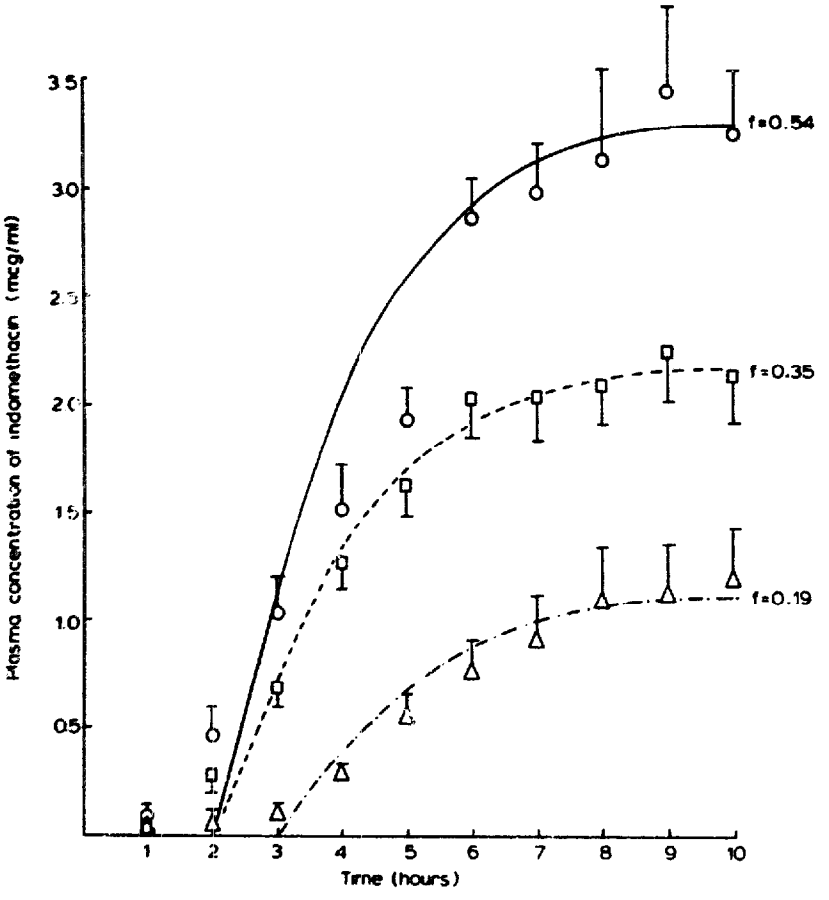

Fig. 1. Effect of treated skin site (60 cm^2) on the percutaneous absorption of indomethacin from absorption ointment base containing 0.5% urea. Key: O——O, dorsal surface; □------□, abdomen; △·–·–·△, thigh. All curves (solid and broken lines) for indomethacin were calculated from Eqn. 3 of Scheme III (Naito and Tsai, 1981). Each point represents the mean of 4 rabbits with the standard error; f = fraction of drug absorbed to the total drug in the ointment base.

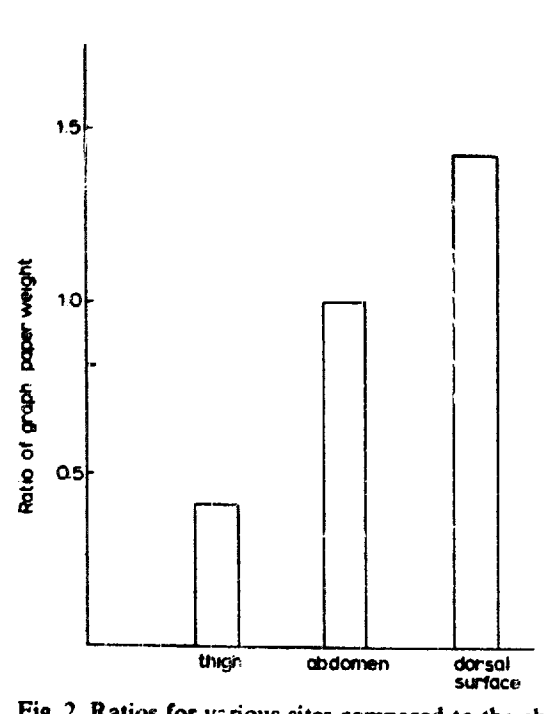

Fig. 2. Ratios for various sites compared to the abdomen of the graphpaper weight of the area under the curve of plasma concentration of indomethacin.

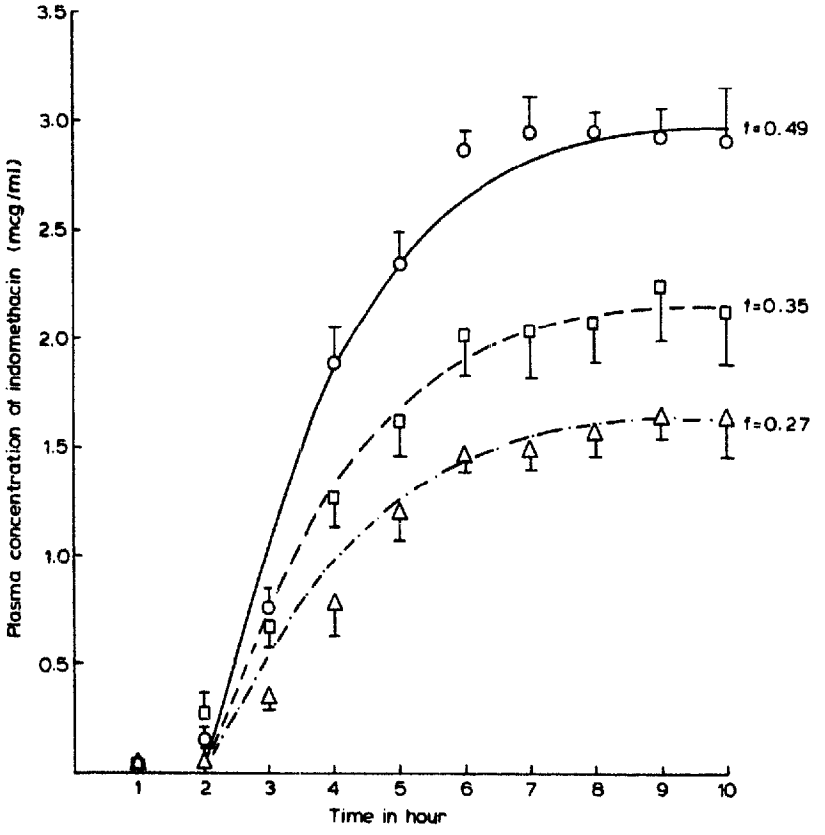

Fig. 3. Effect of the size of the application area in the abdomen on the percutaneous absorption of indomethacin from absorption ointment base containing 0.5% urea. Key: O——O, 90 cm^2; □------□, 60 cm^2; △·-·-·△, 30 cm^2. All curves (solid and broken lines) for indomethacin were calculated from Eqn. 3 of Scheme III (Naito and Tsai, 1981). Each point represents the mean of 4 rabbits with the standard error; f = fraction of drug absorbed to the total drug in the ointment base.

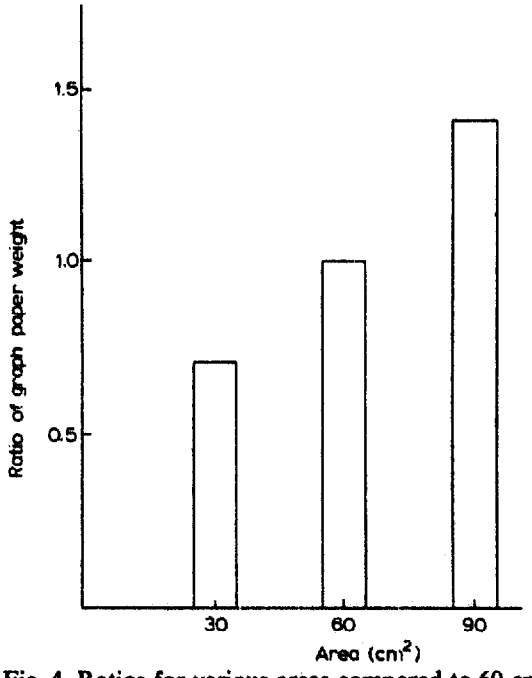

Fig. 4. Ratios for various areas compared to 60 cm^2, by weighing the graphpaper of area under the curve of plasma concentration of indomethacin.

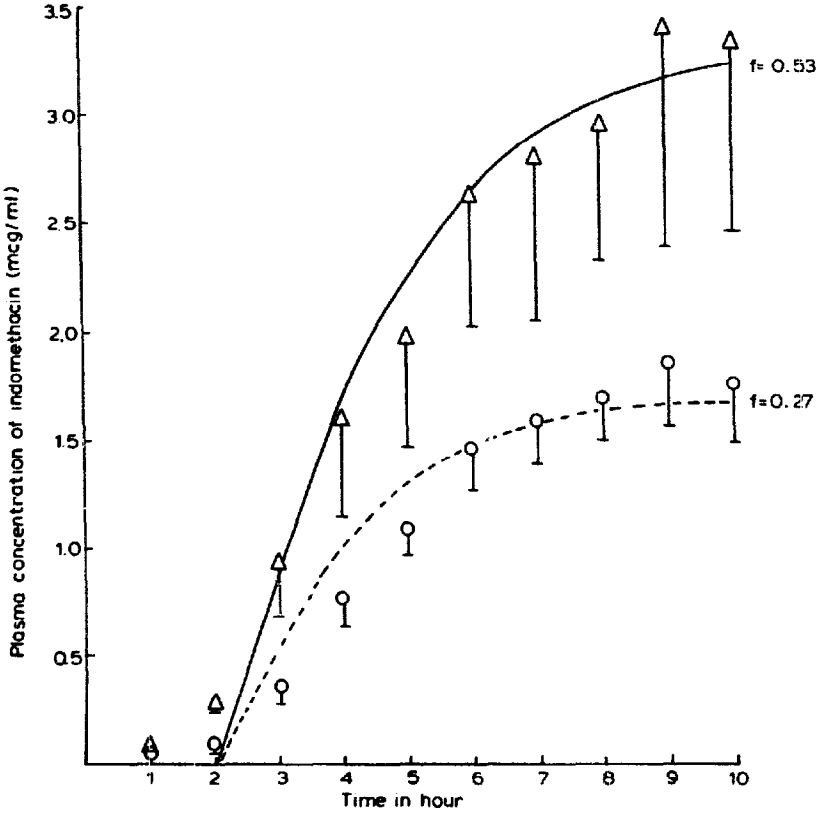

Fig. 5. Effect of pH on the percutaneous absorption of indomethacin from absorption ointment base. Key: O------O, pH = 7.73; △——△, pH = 7.07. All curves (solid and broken lines) for indomethacin were calculated from Eqn. 3 of Scheme III (Naito and Tsai, 1981). Each point represents the mean of 4 rabbits with the standard error; f = fraction of drug absorbed to the total drug in the ointment base.

variations in local blood flow, may also play a role in the observed site dependency. Site variations in blood perfusion have been suggested to explain differences in absorption after intramuscular injection (Cohen et al., 1972). In the present experiments, there may have been intrinsic differences in blood perfusion at the 3 sites.

The relationships between plasma concentration, time course of indomethacin and changes in size of the application area in the abdomen are shown in Fig. 3. Increasing the size of the skin surface area increased the percutaneous absorption of indomethacin. As shown in Fig. 4, the relative weight ratios were 1.41 (90 cm^2), 1 (60 cm^2) and 0.71 (30 cm^2). These results confirm the observations of Noonan and Wester (1980).

The effect of the pH value of the aqueous phase of the absorption ointment base on the percutaneous absorption is shown in Fig. 5. The results indicate that at pH 7.07 the blood levels of indomethacin obtained from the absorption ointment base exceeded those obtainable at pH 7.73. As Higuchi (1960) theorized, decreasing the pH of the vehicle would be expected to increase the thermodynamic activity of the undissociated form of a weakly acidic drug like indomethacin. As the data in Table 1 demonstrate, the higher concentration of undissociated indomethacin was present at

pH 7.07. The lower concentration of the undissociated species at pH 7.73 probably accounts for the lower levels observed. These findings agree with those of Marcus et al. (1970) who noted an increased percutaneous absorption of salicylic acid solution at pH values where undissociated salicylic acid was present in relatively greater concentrations. This suggests that preferential absorption of the unionized form of the drug is remarkable. This phenomenon may therefore explain the present finding that the optimal percutaneous absorption of indomethacin from the absorption ointment base occurred at the lower pH (7.07).

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