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Site Dependence for Topical Absorption of Nitroglycerin in Rats

Keyphrases □ Nitroglycerin—percutaneous absorption in rats, effect of application site □ Absorption, percutaneous—nitroglycerin in rats, effect of application site □ Vasodilators, coronary—nitroglycerin, percutaneous absorption in rats, effect of application site

To the Editor:

Topical nitroglycerin ointment produces clinically significant reductions in the frequency and severity of exercise-induced angina attacks (1, 2) and reductions in heart workload and determinants of myocardial oxygen consumption (3). This dosage form enjoys a growing popularity because it provides sustained hemodynamic effects (4), its administration is noninvasive, and unabsorbed drug can be removed conveniently.

Little is known about the physical and physiological factors that influence percutaneous nitroglycerin absorption. Such information is important with nitroglycerin since systemic availability is the primary goal of topical application. Furthermore, because of the relatively short nitroglycerin elimination half-life (5), the percutaneous absorption rate becomes the limiting kinetic factor and essentially determines the plasma concentration–time profile after topical drug application ["flip-flop pharmacokinetics" (6)]. The application mode and the topical preparation vehicle are important factors to consider with nitroglycerin ointment.

This communication describes the effect of the ointment application site on nitroglycerin absorption in the rat. The results may have important bearing not only on the most effective use of nitroglycerin ointment for systemic effects but also on the choice of proper animal models for screening topically delivered drugs.

Male Sprague-Dawley rats[1], 280–390 g, were used. Fifteen to eighteen hours prior to an experiment, the intended dosing site was clipped and shaved with an electric razor[2]. The animal was returned to a cage with free access to water only. The skin was examined under low power magnification for damage resulting from shaving or scratching, and the animal was not used if the skin barrier was disrupted.

A cannula[3] was implanted in the right jugular vein under ether anesthesia and kept patent with heparin sodium (20 U/ml) in normal saline. The animal was loosely restrained to expose the dosing site. Light ether anesthesia was maintained for the remainder of the 4-hr experiment to preclude movements that could result in removal or contamination of the applied dose. Experiments were conducted at ambient temperatures (21–24°). Doses were applied to a 3 × 3-cm area centered midline and midway between the sternum and penis (abdominal site) or midline and 6 cm up from the tail connection (back site).

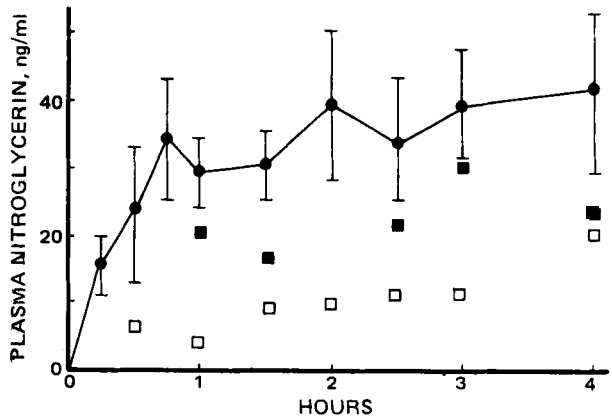

Figure 1—*Plasma nitroglycerin concentrations in rats after application of 2% nitroglycerin ointment. Key: ●, shaved abdominal surface (mean ± SEM, n = 6, 20 mg of nitroglycerin/kg); and ■ and □, back area after stripping with adhesive tape (results from two animals, 14 mg of nitroglycerin/kg).*

At appropriate intervals, 0.5 ml of blood was sampled *via* the implanted cannula. Plasma (200 μl) was stabilized against rapid degradation of nitroglycerin by the addition of 10 μl of 1.0 *N* $AgNO_3$ and assayed for intact nitroglycerin by the specific electron-capture GLC procedure of Yap *et al.* (7). The lower limit of quantitation in this experiment was 1.0 ng of nitroglycerin/ml of plasma.

Nitroglycerin was applied to the shaved back in a variety of doses and dosage forms. Plasma nitroglycerin levels could not be detected after topical application of a 2% nitroglycerin commercial ointment[4] (7 and 14 mg/kg) or a 6.9% nitroglycerin alcohol solution (8, 15, and 20 mg/kg). Each test group consisted of at least two animals. Oral dosing of rats with nitroglycerin at 7 mg/kg gives peak plasma concentrations up to about 10 ng/ml (8).

The tissue on the underside of the animal was of distinctly different character and strength than the dorsal region. Application of 2% nitroglycerin ointment to the shaved abdominal surface showed rapid drug absorption into the systemic circulation, with peak plasma levels ranging between 30 and 40 ng/ml (Fig. 1).

Considerable documentation is available describing the role of the stratum corneum as a drug penetration barrier (9). Comparative photomicrographs of histologic preparations[5] of back and abdominal tissue sections from a rat show a marked difference in the epidermis between the two sites (Fig. 2). The number of layers and relative depth of cornified tissue on the back area are significantly greater than on the abdomen.

If the stratum corneum represents a barrier to nitro-

[1] Blue Spruce Farms, Altamont, N.Y.
[2] Lady Remington MS-120, Sperry Remington, Bridgeport, CT 06602.
[3] Intramedic 7410, Clay-Adam, Parsippany, NJ 07054.
[4] Lot T4808 Nitro-bid Ointment, Marion Laboratories, Kansas City, MO 64137.
[5] Sections taken from a 295-g animal, fixed in formalin, and stained with eosin.

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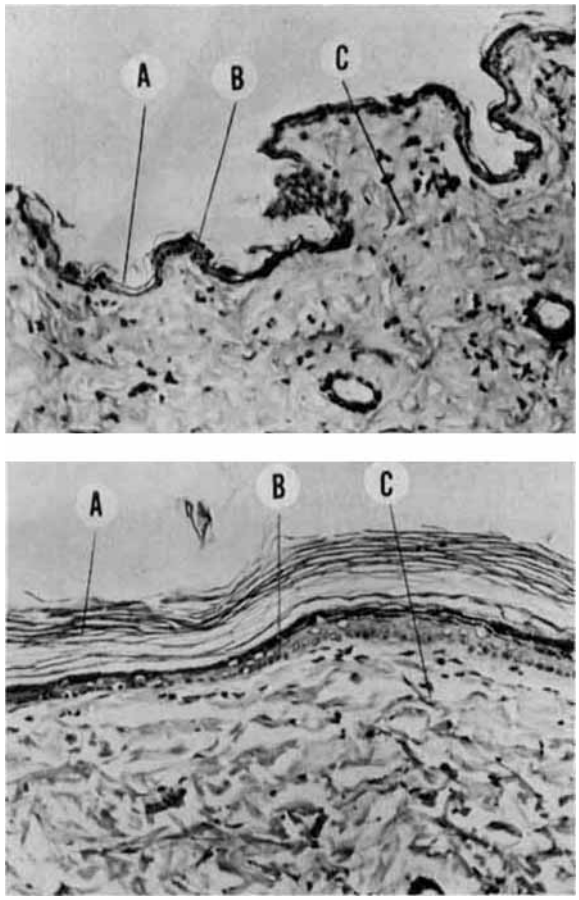

Figure 2—*Photomicrographs of rat skin tissue sections. Key: upper panel, abdominal area; lower panel, back area; A, cornified tissue; B, viable epidermis; and C, dermis.*

glycerin transdermal absorption, then removal of a portion of the barrier should increase nitroglycerin absorption. Classic stripping technique with adhesive tape (10) was used to eliminate the thick, brown, desquamating skin layer at the dorsal site. Care was taken to prevent exposure of the underlying viable epidermis or dermal capillaries. Application of the 2% nitroglycerin ointment then yielded significant drug absorption (Fig. 1).

The present data strongly suggest the presence of a site dependence for topical absorption of nitroglycerin in the rat. The evidence also implicates the stratum corneum as a barrier contributing to the lack of systemic availability from the back. Other factors, such as variations in local blood flow, may play a role in the observed site dependency. Site variations in blood perfusion have been suggested to explain differences in absorption after intramuscular injection (11). Craig *et al.* (12) showed recently that the percutaneous absorption of a cholinesterase inhibitor was a function of skin temperature. This effect was rationalized on the basis of temperature-dependent changes in skin blood flow. In the present experiment, there might have been intrinsic differences in blood perfusion at the two sites. Local changes in skin perfusion might have been induced by the stripping maneuver, thereby increasing drug absorption.

For practical reasons, many drug screening studies of topical absorption utilize the back of the rat as the primary testing site (13–16). This study shows that drug absorption from this location may be poor, even for a relatively nonpolar compound such as nitroglycerin. Lack of penetration of a drug from the back does not necessarily imply poor absorption from topical application when the drug is delivered through other skin areas.

Variations in penetration rates were demonstrated for full thickness human cadaver skin isolated from different sites (17). Regional variations in percutaneous penetration of hydrocortisone (18) and pesticides (19) in humans were reported. No reports document relationships between plasma concentrations and changes in dosing site for topically applied nitroglycerin in humans. The clinical implications of the present laboratory findings are unknown.

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