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Report

Cutaneous Pharmacodynamics of Transdermally Delivered Isosorbide Dinitrate

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Laser doppler velocimetry (LDV) has been used to assess the cutaneous pharmacodynamics of isosorbide dinitrate (ISDN) following transdermal delivery of the drug from prototypal patches. The delivery systems, which were saturated with ISDN, (a) produced various degrees of skin occlusion and (b) spanned a six-fold range of adhesiveness. The patches were applied to the ventral forearm skin of 10 healthy volunteers and the local ISDN-induced increase in local skin blood flow was determined using LDV by locating the probe in a central hole in the delivery system. Measurements were made for 1.5 hr and the pharmacodynamics were quantified by (i) the maximum LDV response and (ii) the area under the LDV response versus time curve. These parameters were not sensitive to patch occlusivity. They were significantly ($P < 0.01$) dependent on patch adhesiveness, though, and decreased with increasing adhesion. Although this observation suggested that ISDN diffusion through the adhesive could determine, at least in part, the rate of drug delivery, it was subsequently demonstrated that ISDN release (*in vitro*, into a perfect "sink") was unaffected by the level of cross-linking in the adhesive polymer. Because the drug was present in all systems at unit thermodynamic activity, these results cannot be explained on the basis of altered ISDN partitioning at the device–stratum corneum interface. We speculate that the *in vivo–in vitro* discrepancy may be due to the efficiency of skin contact achieved by different adhesives: that is, the more adhesive, less flexible systems make poorer contact with the skin surface, thereby decreasing the effective surface area of drug delivery. These results indicate that the noninvasive procedure of LDV contributes to the screening of transdermal formulations.

KEY WORDS: isosorbide dinitrate; skin permeation; laser doppler velocimetry; skin blood flow; vasodilation

INTRODUCTION

Isosorbide dinitrate (ISDN), the vasodilative action of which resembles that of nitroglycerin (NTG), has been extensively used to treat cardiovascular disorders (1,2). There has been interest in the development of transdermal delivery systems for ISDN. Indeed, in Japan, a topical ISDN formulation (Frandol tape-S, Toa Eiyo Ltd., Tokyo) is used to provide prophylactive therapy for patients suffering from angina pectoris.

An important component of the evaluation of a transdermal drug delivery system is the performance of the formulation *in vivo* in man, and detailed clinical trials are necessary. Once the basic safety and feasibility have been established, however, it would be convenient to have available a relatively facile formulation screening procedure. For vasodilative drugs, such as ISDN and NTG, laser doppler velocimetry (LDV) is a technique (3) applicable to this objective. LDV is a noninvasive, optical procedure allowing continuous, real-time monitoring of skin blood flow; the method is particularly suitable for the assessment of local pharmacodynamic changes induced by the topical administration of vasoactive chemicals. For example, the LDV technique has been extensively used to examine the vasoresponse in man to topically applied esters of nicotinic acid (which are potent, local vasodilators) (4). Furthermore, these experiments have been analyzed to provide (a) insight to the percutaneous absorption process itself (5,6) and (b) an understanding of the effect of formulation on the topical bioavailability of the applied drug (7–9). Recently, LDV was used to monitor the response of the cutaneous microvasculature to transdermally absorbed NTG (4). Despite considerable variation in the time and frequency of topical dosing, a relatively constant response to the drug was observed, indicating that the presentation of NTG at the site of action was controlled by the skin and not by the vehicle.

The aim of the study reported here was to use LDV to evaluate the cutaneous pharmocodynamics of ISDN follow-

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ing transdermal administration of the drug from a series of model delivery systems.

MATERIALS AND METHODS

ISDN Transdermal Delivery Systems

The basic formulation design was a drug-saturated layer of adhesive (1.6 mg/cm^2) cast on a drug-free backing membrane. The system variables were (a) backing membrane permeability to water (9–351 g/m^2/24 hr) and (b) patch adhesiveness (varied by a factor of 6). The range of backing membrane water permeabilities was achieved by using different backing membranes. Patch adhesiveness was controlled by varying the degree of polymerization with monomers to synthesize the acrylate copolymer. In order that LDV observations of skin blood flow changes beneath the delivery systems could be made during the application period, small holes were punched in the centers of the patches. Typically, the patch areas were 1.27 cm^2, and the observation "port" occupied less than 10% of this area.

Measurements of Delivery System Characteristics

The permeability of water through the ISDN patches and the adhesion of the delivery systems were measured according to Japanese Industrial Standard procedures (Fig. 1) (11,12). ISDN release from the patches into an aqueous receptor phase was determined by the dissolution test of Japanese Pharmacopeia (13). The delivery system was rotated (50 rpm) in 800 ml of water at 37°C for 90 min. Samples of the aqueous phase were withdrawn periodically and assayed for ISDN by uv spectrophotometry.

LDV Experiments

The human volunteers had no history of dermatologic disease. The subjects (n = 10; age range, 22–65 years) were receiving no prescription medication during their participation in the study and they refrained from caffeine and hot beverages for at least 5 hr prior to LDV measurements. The delivery systems were applied to the ventral forearm and remained in place for 90 min. Immediately after the patches were positioned, the LDV probe was located centrally over the "porthole," which had been created during the fabrication process. Output from the laser doppler velocimeter (MedPacific Corp., Seattle, WA) was recorded every 5 min as a voltage deflection. This deflection has been shown (3) to be proportional to the flux (number multiplied by velocity) of red blood cells within the observation area. The velocimeter provides, therefore, a relative assessment of skin blood flow in response to a perturbation (in this case, the delivery of the vasodilator, ISDN). In preliminary control experiments, we demonstrated [as has been previously reported in different circumstances, for shorter periods (9,10)] that 90-min occlusion of the skin by patches containing no ISDN causes no significant changes in LDV-assessed skin blood flow. The results presented below were therefore corrected for baseline (i.e., pretreatment) flow; the latter was determined at zero time (immediately after the patch was applied to the skin). The testing environment maintained reasonably constant conditions (temperature, 22 + 2°C; RH, 50–70%). All transdermal systems were tested on each of the volunteers in a randomized pattern. The LDV response was characterized by (a) the maximum change from baseline signal and (b) the area under the LDV signal versus time curve from 0 to 90 min.

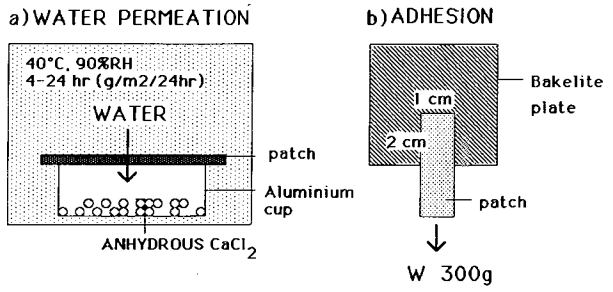

Fig. 1. Schematic illustrations of the procedures used to determine (a) water permeability through the transdermal delivery systems and (b) the adhesiveness of adhesive. (a) The patch was used to seal an aluminum cup (25-cm^2 area) containing anhydrous calcium chloride. The cup was then maintained at 40°C for 4–24 hr in an environment at 90% relative humidity. Periodic assessment of water uptake by the $CaCl_2$ enabled the steady-state flux of water through the patch to be determined. (b) A 2 × 1-cm area of the patch was adhered to a vertically suspended Bakelite plate. A 300-g weight was then attached to the lower portion of the patch and the time of retention (until the patch broke contact with the bakelite) was measured.

Delivery System-Induced Skin Hydration

To determine the extent to which the different patches caused occlusion of the skin, transepidermal water loss (TEWL) measurements were made at the system application site (i) prior to administration and (ii) immediately after a 2-hr contact period. TEWL was measured using an evaporimeter (ServoMed AB, Stockholm, Sweden). These measurements were performed on five of the subjects (ages, 18–40 years).

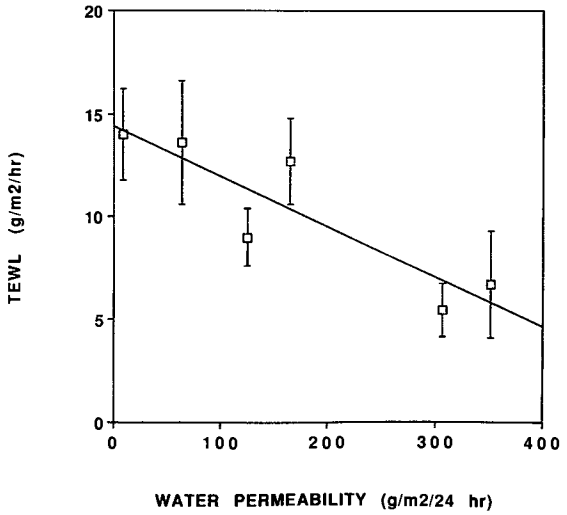

Fig. 2. Correlation (r = 0.89) between *in vitro* transdermal patch permeability to water and *in vivo* TEWL (mean ± SE; n = 5) measured immediately after patch removal following a 2-hr application period.

Table I. The Effect of System Occlusivity (as Measured by Water Permeability) on the LDV Response to Transdermally Delivered ISDN (Mean ± SE; n = 9–10)

Patch No.	Permeability to water ($g/m^2/24$ hr)	Maximum response (mV)[b]	AUC[a] (mV × min × 100)[b]
1	64	28.6 ± 6.5	24.4 ± 5.5
2	9	22.9 ± 3.5	18.6 ± 3.3
3	351	18.2 ± 2.9	13.5 ± 2.9

[a] Area under the baseline-corrected LDV response versus time curve.
[b] ANOVA shows no significant differences ($\alpha > 0.05$) among the three systems.

Table II. The Effect of System Adhesiveness on the LDV-Assessed Response to Transdermally Delivered ISDN (Mean ± SE; n = 9–10)

Patch No.	Adhesiveness (min)[a]	Maximum response (mV)*	AUC[b] (mV × min × 100)*
1	40	28.6 ± 6.5	24.4 ± 5.5
4	58	18.2 ± 2.9	9.8 ± 2.8
5	10	38.3 ± 4.9	32.4 ± 5.0

[a] Measured as described in the legend to Fig. 1.
[b] Area under the baseline-corrected LDV response versus time curve.
* Value for patch 4 significantly ($P < 0.01$) less than those for patches 1 and 5.

Statistics

Differences in the measured parameters due to formulation changes were examined first by ANOVA and then, when appropriate, by the Newman Keuls multiple-range test.

RESULTS AND DISCUSSION

The effect of patch occlusivity of ISDN delivery was initially considered. First, six patches [with identical drug loading (1.6 mg/cm^2) and adhesiveness] were applied for 2 hr, then removed, and TEWL at the administration site was immediately measured. Figure 2 correlates these results with the *in vitro* determinations of water permeation through the patches themselves (using the technique illustrated in Fig. 1). As one might expect, an inverse correlation is found: the lower the transdermal delivery system's permeability to water, the more occlusive its effect on skin. Second, the LDV responses to three of these systems were evaluated (Table I). The time course of the baseline-corrected LDV response to ISDN released from the three systems is shown in Fig. 3. This progressively increasing perfusion was observed for all the patches tested. Over the period of observation, the ISDN-provoked vasodilatation was not significantly dependent upon patch occlusivity. It is possible that during steady-state delivery of the drug (i.e., when the application time is extended), distinctions between systems will become more obviously apparent. Furthermore, the LDV response to ISDN [and to NTG (4)] is relatively modest compared to that elicited, for example, by esters of nicotinic acid (4,6–9).

Second, the adhesion variable was addressed (Table II) using systems with the same backing membrane and (again) common drug loading. There was an inverse correlation between the LDV response and the system adhesiveness (Fig. 4). Indeed, it was found that the strongest adhesive (i.e., the "stickiest" patch) significantly attenuated the response to (and, by implication, the delivery of) ISDN. Furthermore, a preliminary *in vivo* study in rabbits (data not shown) has confirmed an inverse relationship between delivery system adhesiveness and systemic bioavailability. It is tempting to conclude from these observations that, in the patch with the strongest adhesive, ISDN diffusion is slowed to an extent that drug transport to the stratum corneum surface now contributes to the determination of delivery rate. To test this hypothesis, we then measured ISDN release from systems 1,

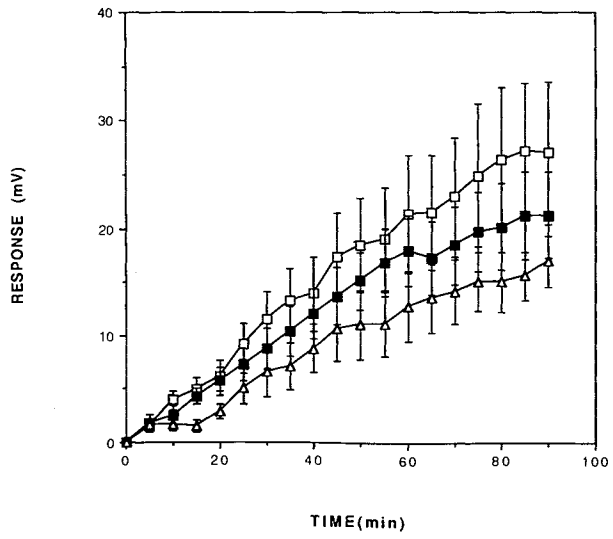

Fig. 3. Baseline-corrected LDV response (mean ± SE; n = 9–10) to ISDN delivered transdermally from three prototypal patches (□, No. 1; ■, No. 2; △, No. 3) as a function of time post-application.

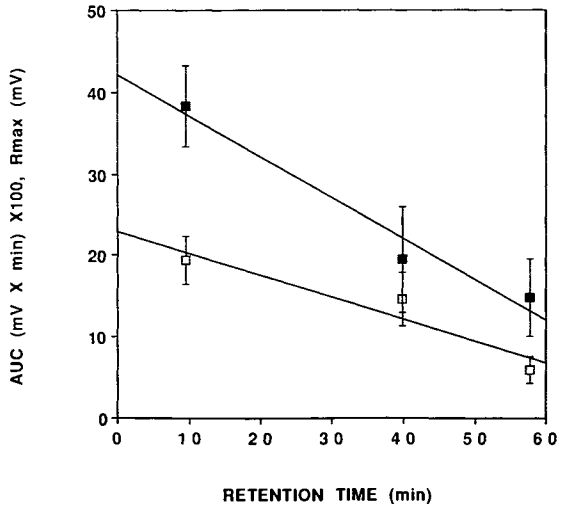

Fig. 4. maximum LDV response (■; mV) and LDV response versus time AUC (□; mV · hr) (mean ± SE; n = 9–10) plotted as a function of delivery system adhesion time (measured as described in the legend to Fig. 1). The correlation coeficients for the two lines are greater than 0.95.

4, and 5 into an aqueous receptor "sink." The results in Fig. 5, however, are contrary to the above argument and suggest that the diffusion coefficient of ISDN in the three different adhesives are essentially similar. What can account, then, for the differential LDV responses, i.e., the diminished response with increasing adhesion? One possibility, which warrants further study, is that the less adhesive, more flexible systems achieve better contact with the skin surface, thereby increasing the effective surface of drug delivery. As ISDN is present in all three systems at the same thermodynamic activity, an explanation in terms of altered drug partitioning at the device–skin interface cannot be accepted.

In summary, these experiments have been designed to demonstrate that the simple, *in vivo*, noninvasive procedure of LDV can contribute usefully to the screening of transdermal formulations and may allow resolution and comprehension of conflicting *in vitro* and *in vivo* observations. The scope and limitations of the approach need to be fully defined in further research.

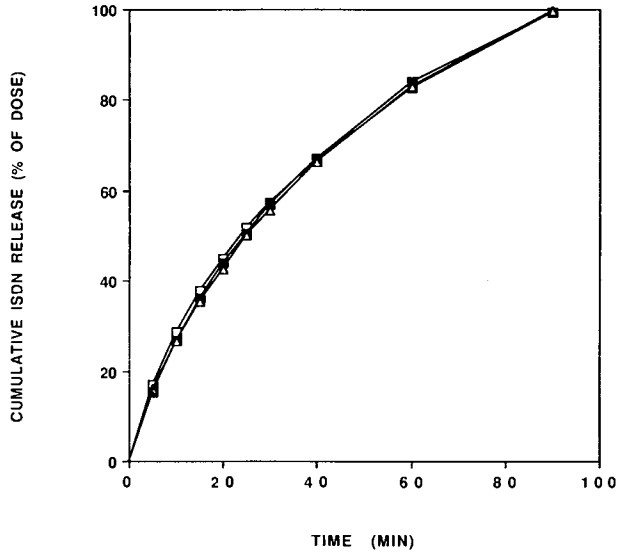

Fig. 5. Cumulative ISDN release (% dose) as a function of time into an aqueous receptor phase entry as a sink (average of two replicates) (□, No. 1; ■, No. 4; △, No. 5).

ACKNOWLEDGMENTS

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