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Research Article

Percutaneous Penetration Kinetics of Nitroglycerin and Its Dinitrate Metabolites Across Hairless Mouse Skin *in Vitro*

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The percutaneous penetration kinetics of the antianginal, nitroglycerin (GTN), and its primary metabolites, 1,2- and 1,3-glyceryl dinitrate (1,2- and 1,3-GDN), were evaluated *in vitro,* using full-thickness hairless mouse skin. GTN and the 1,2- and 1,3-GDNs were applied (a) in aqueous solution as pH 7.4 phosphate-buffered saline (PBS) and (b) incorporated into lipophilic ointment formulations. The cutaneous transformation of GTN to its dinitrate metabolites was detected, but no interconversion between 1,2-GDN and 1,3-GDN was observed. Following application of the nitrates in PBS solution, all three compounds exhibited steady-state transport kinetics. The steady-state flux of GTN (8.9 ± 1.5 nmol cm^{-2} hr^{-1}) was significantly greater ($P < 0.05$) than those of 1,2-GDN (0.81 ± 0.54 nmol cm^{-2} hr^{-1}) and 1,3-GDN (0.72 ± 0.20 nmol cm^{-2} hr^{-1}). The corresponding permeability coefficient (ρ) for GTN ($20 \pm 3 \times 10^{-3}$ cm hr^{-1}) was significantly larger than the corresponding values for 1,2-GDN ($1.4 \pm 0.9 \times 10^{-3}$ cm hr^{-1}) and 1,3-GDN ($1.2 \pm 0.4 \times 10^{-3}$ cm hr^{-1}), which were statistically indistinguishable ($P > 0.05$). Further analysis of the transport data showed that the differences between GTN and the GDNs could be explained by the relative stratum corneum/water partition coefficient (K_s) values of the compounds. The apparent partition parameters, defined as $\kappa = K_s \cdot h$ [where h is the diffusion path length through stratum corneum (SC)] were $19.8 \pm 2.5 \times 10^{-2}$ cm for GTN and $1.91 \pm 1.07 \times 10^{-2}$ and $1.81 \pm 0.91 \times 10^{-2}$ cm for 1,2- and 1,3-GDN, respectively. However, when the nitrates were administered in an ointment base, the apparent partition parameter (κ') and permeability coefficient (ρ') of GTN markedly decreased, to $2.51 \pm 0.75 \times 10^{-2}$ cm and $1.6 \pm 0.3 \times 10^{-3}$ cm hr^{-1}, respectively. In contrast, the κ' and ρ' results for 1,2- and 1,3-GDN were not significantly different ($P > 0.05$) from the corresponding κ and ρ values, which were measured following dosing as aqueous solutions. As a result, the steady-state fluxes of all three nitrates from the ointment formulation were comparable (GTN, 154 ± 28 nmol cm^{-2} hr^{-1}; 1,2-GDN, 162 ± 22 nmol cm^{-2} hr^{-1}; 1,3-GDN, 162 ± 34 nmol cm^{-2} hr^{-1}). It follows that the dinitrates can be as efficiently delivered across the skin as GTN when a suitable formulation is employed. This finding may support transdermal therapy using 1,2- or 1,3-GDN if, indeed, they are found to be pharmacologically effective.

KEY WORDS: glyceryl trinitrate; nitroglycerin; glyceryl dinitrates; percutaneous; transdermal; hairless mouse skin.

INTRODUCTION

The extremely short residence time of nitroglycerin (GTN) in the systemic circulation has necessitated the use of sustained-release GTN delivery systems in the prophylactic management of angina. As a result of GTN's almost-complete first-pass metabolism following oral administration (1), the transdermal route for GTN delivery has engendered much recent interest. However, the percutaneous first-pass metabolism of GTN to its primary dinitrate metabolites, i.e., 1,2- and 1,3-glyceryl dinitrate (1,2- and 1,3-GDN) has been reported (2–4), with the extent of GTN metabolism increasing as GTN flux through skin decreases (4).

Despite studies in animals (5,6), demonstrating that the GDNs are less "potent" vasodilators than GTN, and hence the suggestion that they contribute little to GTN therapeutic efficacy, we hypothesize that the GDNs play a significant role in GTN therapeutic effect. Recently we have demonstrated that the GDNs have an appreciable effect upon peripheral vascular resistance in man (7). Following oral administration of 1,2- or 1,3-GDN to healthy volunteers, significant decreases in diastolic blood pressure were observed, with an apparent correlation between the GDN plasma concentration–time profiles and the diastolic blood pressure–time profiles (7). To assess the contribution of the GDNs to the therapeutic efficacy of GTN requires not only pharma-

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codynamic evaluation, but also consideration of their comparative pharmacokinetic characteristics. Pharmacokinetic studies performed in this laboratory (8–10) have demonstrated that the 1,2- and 1,3-dinitrate metabolites of GTN have a much longer residence time in the systemic circulation than the parent compound. Thus, upon GTN dosing, the 1,2- and 1,3-GDN metabolites would be expected to accumulate. Indeed, Noonan and Benet (11) reported that following topical application of GTN ointment, combined 1,2- and 1,3-GDN steady-state plasma concentrations can be six- to sevenfold higher than the steady-state level of GTN.

To examine the relationship between the clinical pharmacodynamics and pharmacokinetics of GTN and those of its 1,2- and 1,3-dinitrate metabolites will ultimately require administration of the GDNs to stable angina subjects, via various routes of administration. This present investigation of the *in vitro* percutaneous transport kinetics of GTN and its dinitrate metabolites is one component of this complex matrix of work. We present here comparative skin penetration data for GTN and its GDN metabolites and correlate these observations with simple physicochemical parameters. In addition, we examine how formulation of the nitrates with simple ointment bases can influence their transdermal flux. Although a recent study (12) has examined the percutaneous penetration characteristics of 1,3-GDN across shaved rat skin, the investigation described below represents, we believe, the first comprehensive and comparative examination of dinitrate transdermal delivery.

MATERIALS AND METHODS

Chemicals. All chemicals used in this study were of reagent grade and were purchased, unless otherwise stated, from either Aldrich Chemical Co., Inc. (Milwaukee, WI), or Fisher Scientific (Santa Clara, CA).

GTN and GDN Aqueous Solution and Ointment Formulations. The 1,2- and 1,3-GDNs, supplied as a mixture with GTN (ICI Americas Inc., Specialty Chemicals, Wilmington, DE), were separated, purified, and characterized (>99% purity) as previously described (13). To examine nitrate percutaneous penetration kinetics from aqueous solutions, a commercial GTN formulation (Tridil, 5 mg/ml; American Critical Care, McGaw Park, IL) was diluted to a final concentration of 0.1 mg/ml using pH 7.4 phosphate-buffered saline (PBS), and 1,2- and 1,3-GDN solutions (also at 0.1 mg/ml) were prepared by dissolving the appropriate amount of the respective GDN in PBS. Although the commercial GTN solution (Tridil) contains 30% ethanol and 30% propylene glycol, the final concentration of these solvents in the donor phase of the diffusion cell is 0.6%. At these concentrations it is unlikely that the nonaqueous solvents will have any influence on the transdermal GTN permeability. To examine percutaneous penetration kinetics from an ointment base, 2% (w/w) GTN, and 2% (w/w) 1,2- and 1,3-GDN ointments were prepared by direct incorporation of the nitrates into a commercially available base (Aquaphor, Beiersdorf Ind., Norwalk, CT). An FDA-approved generic GTN ointment [2% (w/w) GTN; Fougera & Co., Melville, NY] was also investigated.

Analysis of GTN and 1,2- and 1,3-GDN. Nitrate assay was performed using a specific and sensitive capillary column gas chromatography procedure (14), with slight modifications. The nitrates from 1 ml of the skin diffusion cell perfusate were extracted by the triplicate addition of 10 ml of a solvent mixture consisting of 80% *n*-pentane (Burdick & Jackson, Muskegon, MI) and 20% methyl-t-butyl ether (Omnisolve, EM Science, Gibbstown, NJ). Recoveries of GTN, 1,2- and 1,3-GDN, and the internal standard, *o*-iodobenzyl alcohol (Aldrich Chemical Co., WI), were greater than 90%. The combined organic solvent fractions were evaporated under nitrogen and the residue was reconstituted with *n*-butyl acetate (Burdick & Jackson, Muskegon, MI). Standard curves were linear for all three nitrates over the concentration range 0.1–15.0 ng/ml. Coefficients of variation associated with intra- and interday assay precision and accuracy were less than 8%. Analyses were conducted on Varian 6000 and 6500 gas chromatographs (Varian Associates, Walnut Creek, CA), equipped with HP-1 fused-silica capillary columns (25 m × 0.32-mm I.D., 1-μm film thickness; Hewlett Packard, Palo Alto, CA).

Determination of GTN and 1,2- and 1,3-GDN Water Solubility and Octanol/Water Partition Coefficient. To measure the solubility of the nitrates in water, 5 mg of GTN oil (extracted with *n*-pentane from 10% GTN adsorbed lactose; Schering-Plough Research, Miami, FL) or 150 mg of 1,2- or 1,3-GDN oil was added to 500 μl of purified water and shaken vigorously at room temperature for 2 hr. The water and oil layers were separated by centrifugation at 1500*g* for 10 min, and the nitrate concentration in the water (upper) layer was determined as described above. To measure octanol/water partition coefficients, 3 mg of GTN (incorporated into the octanol phase) or 3 mg of 1,2- or 1,3-GDN (incorporated into the aqueous phase) was allowed to equilibrate between equal volumes (3 ml) of octanol and purified water. To achieve equilibration, the phases were vigorously shaken for 1 hr at room temperature. After separation, the concentrations of nitrate in the aqueous and organic phases were determined as described above, and the partition coefficient was calculated.

Percutaneous Transport Studies. The transdermal penetration of GTN, 1,2-GDN, and 1,3-GDN was measured across full-thickness hairless mouse (SKH: HR-1) skin *in vitro*. Dorsal skin from 10- to 15-week-old mice (Skin Cancer Hospital, Philadelphia, PA) was excised at sacrifice and used immediately. The skin separated the donor and receptor chambers of glass flow-through diffusion cells (15) (Laboratory Glass Apparatus, Berkeley, CA), which were maintained at 32°C throughout the experiment. The perfusate (i.e., PBS) was delivered to, and carried from, the receptor chamber by Tygon tubing (Norton Co., Akron, OH). The receptor phase of the diffusion cell was continually stirred and perfused at a rate of 5 ml/hr, sufficient to exchange the entire receptor compartment contents within 60 min. At the start of the experiment, a 1-ml volume of GTN, 1,2-GDN, or 1,3-GDN aqueous solution or a 0.5-g quantity of GTN, 1,2-GDN, or 1,3-GDN ointment was applied to the exposed epidermal skin surface (area, 0.95 cm^2) in the donor phase of the diffusion cell. Subsequently, hourly samples of the receptor perfusate were collected onto a fraction collector (Gilson FC-220, Gilson Co., Middleton, WI).

Data Analysis. Statistical evaluation of the data used either Student's *t* test (where comparisons were made between two groups) or ANOVA and Duncan's multiple-range

Table I. Water Solubility and Octanol/Water Partition Coefficients (K_0) of GTN, 1,2-GDN, and 1,3-GDNs (Mean ± SD; n = 3)

	Water solubility*		
	mg/ml	μmol/ml	log K_0**
GTN	1.63 ± 0.07	7.18 ± 0.30	2.21 ± 0.02
1,2-GDN	29.5 ± 2.5	162 ± 14	0.83 ± 0.04
1,3-GDN	28.0 ± 7.1	154 ± 39	0.71 ± 0.03

* GTN value significantly ($P < 0.05$) smaller than those for the GDNs (which are not significantly different).

** GTN value significantly ($P < 0.05$) greater than those for the GDNs (which are not significantly different).

test (16) (for comparisons between more than two groups). The nitrate transport data across the skin were expressed as plots of the cumulative amount (M_t) penetrating to the receptor phase of the diffusion cell as a function of time (t). These graphs exhibited a classic non-steady-state lag phase followed by a linear (quasi-steady-state) portion. Assuming that the stratum corneum (SC) was the rate-limiting membrane for nitrate permeation through full-thickness skin [as we recently demonstrated (22)], the M_t versus t results were fitted by nonlinear regression to the full solution of the diffusion equation (Fick's second law), using the boundary conditions that (i) an infinite reservoir of nitrate existed in the donor compartment and (ii) the receiver chamber was a perfect sink (17). Nonlinear regressions (weighted as M_t^{-1}) were performed using PCNONLIN (SCI Software, Lexington, KY). For the aqueous solutions, all data collected to 10 hr postdosing were used; for the ointments, data to 8 hr were employed. Following Okamoto *et al.* (18), the regressions yielded a characteristic diffusion parameter [δ (hr^{-1})] for both solution and ointment experiments, and an apparent partitioning parameter [κ (cm) for solution experiments and κ′ (cm) from the ointment studies]. The parameters δ, κ, and κ′ are defined as follows:

$$\delta = D_s/h^2 \quad (1)$$

$$\kappa = K_s h \quad (2)$$

$$\kappa' = K_v h \quad (3)$$

where D_s is the diffusion coefficient of nitrate in the SC, h is the diffusion path length through the SC, and K_s and K_v are the nitrate's SC/water and SC/ointment partition coefficients, respectively. Further parameters defined by Eqs. (4)–(6) are useful because they depend exclusively upon either D_s or the partition coefficient (K_s or K_v). Additionally, these parameters require no assumption about the absolute value of h, requiring only that h be independent of the applied phase and be the same value for each of the three nitrates. Equations (4)–(7) were used to determine the classic descriptors of the M_t versus time profiles, namely, the lag times (T_L), the respective permeability coefficients (ρ and ρ′), and the steady-state fluxes (J_{ss}):

$$T_L = h^2/(6D_s) = 1/(6\delta) \quad (4)$$

$$\rho = K_s D_s/h = \delta \cdot \kappa \quad (5)$$

$$\rho' = K_v D_s/h = \delta \cdot \kappa' \quad (6)$$

$$J_{ss} = \rho C_w \text{ or } \rho' C_v \quad (7)$$

where C_w and C_v are the applied nitrate concentrations in the aqueous solutions and in the ointments, respectively. The calculated J_{ss} values using Eq. (7) were compared with the mean observed J_{ss} results.

RESULTS

Physicochemical Properties. The water solubilities and octanol/water partition coefficients (K_0) of GTN and 1,2- and 1,3-GDN are presented in Table I. The water solubilities of the GDNs were similar ($P > 0.05$) and about 18 times greater than that of GTN. Similarly, K_0 values of 1,2- and 1,3-GDN were essentially identical and significantly less than the K_0 of GTN. The GTN water solubility and K_0 are in good agreement with previously published values (19,20).

Cutaneous Transformation. The interconversion of 1,2-GDN to 1,3-GDN is known to occur in aqueous solutions of high pH (21). However, no interconversion was observed during GDN penetration through the skin under the conditions of this study.

Percutaneous Penetration Kinetics. The *in vitro* transdermal kinetic parameters for GTN and 1,2- and 1,3-GDN are presented in Tables II and III, following application of the aqueous solution and ointment preparations, respectively. The parameters for GTN were derived utilizing total nitrate (GTN and 1,2- and 1,3-GDN) flux into the receptor chamber and, thus, reflect penetration of GTN across the stratum corneum, i.e., assuming GTN transport through the rate-limiting barrier preceded biotransformation in the viable

Table II. Percutaneous Penetration Parameters for GTN[a], and 1,2- and 1,3-GDNs Following Their Application as Aqueous Solutions (Mean ± SD)

Parameter	GTN (n = 5)	1,2-GDN (n = 6)	1,3-GDN (n = 6)	Statistical comparisons[b]
δ($\times 10^{-2}$ hr^{-1})	10.4 ± 2.3	8.27 ± 2.83	7.52 ± 2.45	1,3-GDN 1,2-GDN GTN
κ($\times 10^{-2}$ cm)	19.8 ± 2.5	1.91 ± 1.07	1.81 ± 0.91	1,3-GDN 1,2-GDN GTN
ρ($\times 10^{-3}$ cm hr^{-1})	20 ± 3	1.4 ± 0.9	1.2 ± 0.4	1,3-GDN 1,2-GDN GTN
T_L (hr)	1.66 ± 0.35	2.35 ± 1.23	2.42 ± 0.76	GTN 1,2-GDN 1,3-GDN
J_{ss} (nmol cm^{-2} hr^{-1})				
Obs	8.9 ± 1.5	0.81 ± 0.54	0.72 ± 0.20	1,3-GDN 1,2-GDN GTN
Calc	8.9 ± 1.5	0.80 ± 0.48	0.68 ± 0.22	1,3-GDN 1,2-GDN GTN

[a] GTN parameters were calculated from total nitrate (GTN + GDNs) penetration.

[b] ANOVA and Duncan's multiple-range test: groups are arranged left to right in the order of ascending magnitude for each parameter. Underlined nitrates do not differ significantly ($P > 0.05$) from each other.

Table III. Percutaneous Penetration Parameters (Mean ± SD; $n = 5$) for GTN[a] and 1,2- and 1,3-GDNs Following Their Application in Ointment Formulations (F, Fougera; A, Aquaphor)

Parameter	GTN(F)	GTN(A)	1,2-GDN(A)	1,3-GDN(A)	Statistical comparisons[b] Nitrates			GTN ointments
$\delta(\times 10^{-2}\ hr^{-1})$	6.98 ± 2.13	6.91 ± 2.19	8.33 ± 2.13	10.9 ± 6.6	GTN(A)	1,2-GDN	1,3-GDN	F = A
$\kappa'(\times 10^{-2}\ cm)$	1.44 ± 0.69	2.51 ± 0.75	2.93 ± 1.91	3.23 ± 1.34	GTN(A)	1,2-GDN	1,3-GDN	F < A
$\rho'(\times 10^{-3}\ cm\ hr^{-1})$	0.9 ± 0.3	1.6 ± 0.3	2.1 ± 0.6	2.8 ± 0.6	GTN(A)	1,2-GDN	1,3-GDN	F < A
T_L (hr)	2.53 ± 0.62	2.58 ± 0.68	2.17 ± 0.83	1.97 ± 0.98	1,3-GDN	1,2-GDN	GTN(A)	F = A
J_{ss} (nmol $cm^{-2}\ hr^{-1}$)								
Obs	106 ± 14	158 ± 23	162 ± 22	162 ± 34	GTN(A)	1,2-GDN	1,3-GDN	F < A
Calc	107 ± 39	161 ± 33	185 ± 49	182 ± 38	GTN(A)	1,3-GDN	1,2-GDN	F < A

[a] GTN parameters were calculated from total nitrate (GTN + GDNs) penetration.

[b] Two comparisons were performed. (1) For the three nitrates (GTN, 1,2-GDN, and 1,3-GDN) delivered from Aquaphor, ANOVA and Duncan's multiple-range test were used. Groups are arranged left to right in the order of ascending magnitude for each parameter. Underlined nitrates do not differ significantly ($P > 0.05$) from each other. (2) For GTN delivered from the two ointments (F and A), an unpaired t test was used to compare the parameters. F < A means that the A value was significantly ($P < 0.05$) greater than the F value; F = A means no significant difference.

layers of the skin. It was further assumed that the GDNs were not metabolized significantly during their transdermal passage. In support of this assumption we have shown that GTN is metabolized to GDN in homogenized hairless mouse skin (23): GTN decreases and 1,2- and 1,3-GDNs increase with time, but the total molar concentration of GTN, 1,2-GDN, and 1,3-GDN did not change.

Figure 1 shows the temporal pattern of GTN metabolism following application of this compound in aqueous solution and in two ointment formulations. As previously noted (23), we observed that the cutaneous metabolism of GTN diminished with time and that the percentage metabolites formed was greatest when GTN flux was least.

Nitrate Transport from Aqueous Solution. Figure 2 shows the flux of nitrates into the receptor chamber as a function of time. For GTN, results are presented both for GTN flux across the SC (i.e., total nitrate flux) and for GTN penetration across the whole skin (i.e., GTN flux). The cumulative permeation curves (M_t versus t) are presented in Fig. 3; the considerably greater nitrate flux following GTN administration is clear, and the degree of difference between the GTN and the GDN curves supports the assumption that GTN metabolism takes place predominantly after the SC has been traversed. The penetration parameters are summarized in Table II. The diffusion coefficient-dependent parameters (T_L and δ) are similar for all three nitrates. On the other hand, the apparent partitioning parameter (κ) for GTN is significantly greater than those for the GDNs (the values of which are comparable). This distinction in κ values, therefore, explains the larger J_{ss} and ρ values observed for GTN compared to the GDNs. In approximate terms, κ, J_{ss}, and ρ for GTN exceed the corresponding GDN values by an order of magnitude.

Nitrate Transport from Ointment Bases. Figure 4 shows the flux of nitrates into the receptor phase following application of the ointment formulations. The corresponding M_t versus t curves are in Fig. 5. As above, for GTN the total

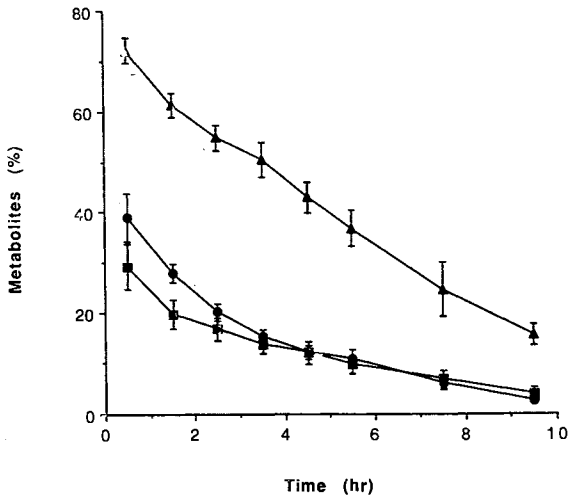

Fig. 1. Ratios (±SE) of metabolites to total nitrate following topical applications of GTN preparations. (▲) GTN solution; (●) GTN ointment (Fougera); (■) GTN ointment (Aquaphor).

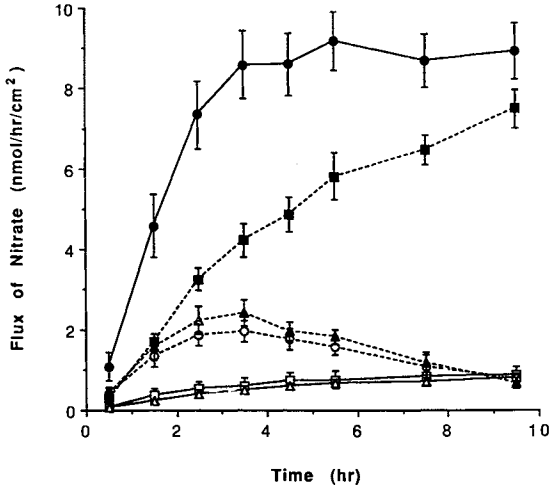

Fig. 2. Fluxes (±SE) of nitrates following topical applications of GTN, 1,2-GDN, and 1,3-GDN solutions. GTN application: (○) 1,2-GDN; (▲) 1,3-GDN; (■) GTN; (●) total nitrate (GTN + GDNs). GDN application: (□) 1,2-GDN; (△) 1,3-GDN.

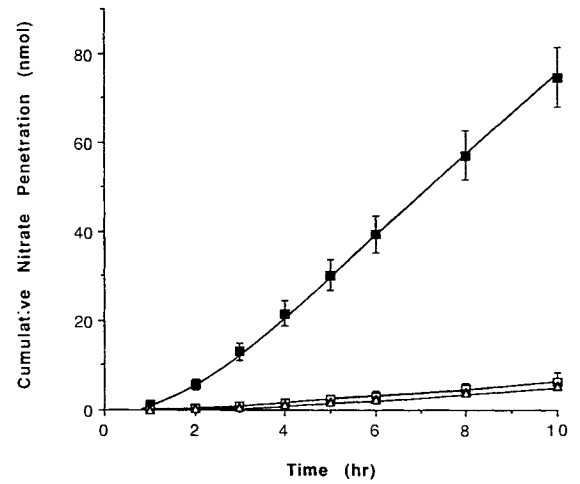

Fig. 3. Cumulative nitrate penetration (±SE) following topical applications of GTN, 1,2-GDN, and 1,3-GDN solutions. GTN application: (■) total nitrate (GTN + GDNs). GDN application: (□) 1,2-GDN; (△) 1,3-GDN. The curves denote calculated values.

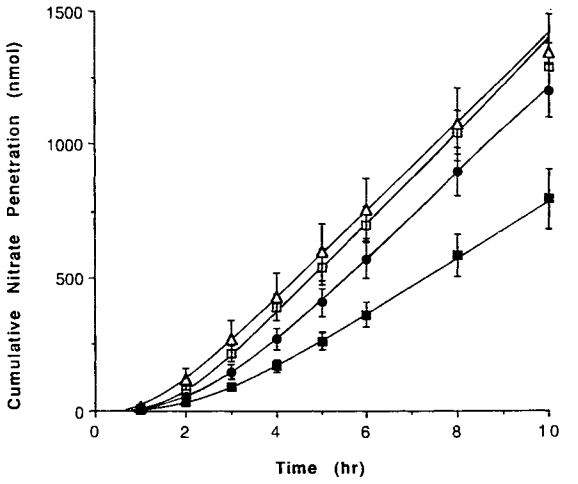

Fig. 5. Cumulative nitrate penetration (±SE) following topical applications of GTN, 1,2-GDN, and 1,3-GDN ointments. GTN application: (■) total nitrate (GTN + GDNs) (Fougera); (●) total nitrate (GTN + GDNs) (Aquaphor). GDN application: (□) 1,2-GDN; (△) 1,3-GDN (Aquaphor). The curves denote calculated values.

nitrate transport (GTN + GDNs) is shown. We observe that (a) GTN delivery from Aquaphor is about 50% greater than that from the commercially available Fougera ointment and (b) delivery of GDNs from the topical ointment formulation is as efficient (in terms of moles delivered per unit time per unit area) as that of GTN. The penetration parameters are summarized in Table III. Similar to the results from the aqueous solution experiments, there were no significant differences in the calculated values of T_L and δ (between GTN formulations or between nitrates). There were, however, some important differences in the parameters J_{ss}, ρ', and κ'. (i) J_{ss} of GTN applied as the Fougera ointment was significantly less than that following administration of the Aquaphor formulation. (ii) J_{ss} of 1,2- and 1,3-GDNs (which were essentially identical) were not significantly different from

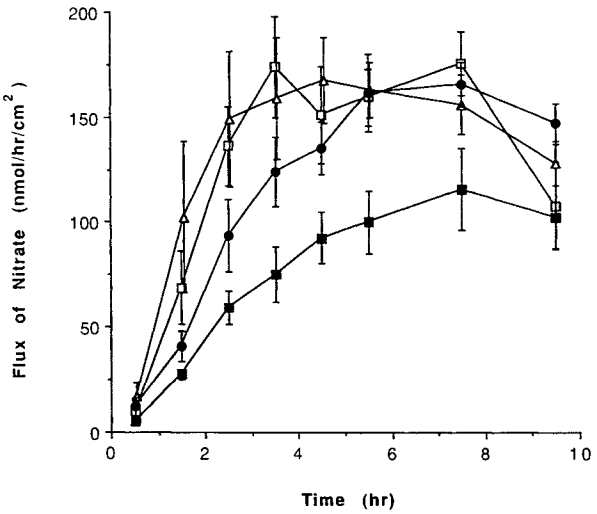

Fig. 4. Fluxes (±SE) of nitrates following topical applications of GTN, 1,2-GDN, and 1,3-GDN ointments. GTN application: (■) total nitrate (GTN + GDNs) (Fougera); (●) total nitrate (GTN + GDNs) (Aquaphor). GDN application: (□) 1,2-GDN; (△) 1,3-GDN (Aquaphor).

that of GTN (Aquaphor ointment); this is in contrast to the results observed following application of the aqueous solutions. (iii) The ρ' and κ' parameters showed very similar trends. The values for GTN post-Fougera treatment were significantly less than that when the vehicle was Aquaphor.

DISCUSSION

Consistent with previous reports (2–4,23), significant metabolism of GTN to its 1,2- and 1,3-GDN metabolites was observed in this study. Furthermore, although GTN flux across the stratum corneum attained an apparent steady state within a few hours, the level of metabolite formation diminished with time. This behavior, as previously observed, has been attributed to a progressive loss of enzymatic viability (23), signifying that GTN metabolism takes place in viable tissue. If the extent of metabolism, observed in the first hour of the *in vitro* GTN ointment experiments reported here, was duplicated *in vivo*, then a cutaneous first-pass effect of 30–40% could be expected. Although this seems to be a rather high estimate, it is consistent with several other relevant findings, including: (a) the calculations of Nakashima *et al.* (3), who formulated a physiological flow model using data from both animals and man and predicted the cutaneous metabolism of GTN to be 24–32%; (b) the *in vivo* experiments of Wester *et al.* (2), who measured a cutaneous first-pass effect for GTN in the rhesus monkey of 16–21%; and (c) the *in vitro* data of Santus *et al.* (4) using human (breast) skin, in which GDN metabolite formation was very similar to that in hairless mouse skin.

The percutaneous penetration kinetics of 1,2-GDN and 1,3-GDN were very similar. Given the considerable overlap in their physicochemical properties (Table I), this similarity in membrane transport behavior is expected. Compared to GTN, the greater water solubility of the GDNs explains the greater permeation of GTN (relative to the GDNs), from aqueous solution. At the fixed donor concentration of 0.1 mg/ml, the "escaping tendency" of GTN (i.e., its thermodynamic activity) is about 20-fold greater than that of the

dinitrates. In other words, the partitioning of GTN from aqueous solution into the stratum corneum is favored, relative to that of the dinitrates, by at least an order of magnitude. This is reflected in the transport parameters in Table II: the partitioning parameter (κ) and the permeability coefficient (ρ) for GTN are about 10 and 15 times greater than the corresponding GDN results, respectively. This interpretation is confirmed by the lag time and characteristic diffusion parameter (δ) values, which are insensitive to the nature of the nitrate species; that is, the diffusion coefficients of GTN and the GDNs across the stratum corneum are, within experimental error, identical. Differences in ρ and J_{ss}, therefore, are completely explained by differences in skin/vehicle partitioning. The coincidence in the δ parameters for GTN and for the GDNs means that if these compounds were applied as saturated solutions (i.e., at the same thermodynamic activity), then similar maximum fluxes (J_{ss}^{max}) should result, since

$$J_{ss}^{max} = K_s D_s C_w^{sat}/h = \rho C_w^{sat} \quad (8)$$

$$= D_s C_s^{sat}/h \quad (9)$$

where C_w^{sat} and C_s^{sat} are the saturation solubilities of the permeant in water and in the stratum corneum, respectively, and we note that, by definition, $K_s = C_s^{sat}/C_w^{sat}$. Using the C_w^{sat} values in Table I and the data for ρ in Table II, we may calculate the following J_{ss}^{max} values (units, nmol/cm^2/hr): GTN, 144; 1,2-GDN, 227; and 1,3-GDN, 185. As expected, the results are of comparable magnitude, differing by less than a factor of two.

The relative permeation of the nitrates changes when the vehicle is a common ointment (Aquaphor). First, much higher steady-state fluxes are observed (Table III). These reflect, in part, the higher nitrate concentrations (approx. 2%, w/w, = 20 mg/ml) present in the ointment. Furthermore, from the discussion in the preceding paragraph, we can deduce that the magnitudes of the J_{ss} imply that the nitrates (at 2%, w/w) are close to their saturation solubilities in Aquaphor. The partitioning parameters (κ') and ρ' values for GTN, 1,2-GDN, and 1,3-GDN delivered from Aquaphor are similar, implying that the leaving tendency of the three nitrates from the ointment base is of the same order. Comparison of the κ' parameters with the κ values (Table II) associated with nitrate delivery from aqueous solution reveals that the GDN partition coefficients between SC and water, and between SC and Aquaphor, are similar. In contrast, for GTN, $\kappa' \simeq 0.1\ \kappa$, showing that the affinity of GTN for Aquaphor (a relatively lipophilic ointment containing white petrolatum, mineral oil, mineral wax, and lanolin alcohol) is approximately 10 times greater than its affinity for water. As previously noted for the aqueous delivery data, the δ values are independent of nitrate species and are not influenced significantly by changing the formulation to an ointment. Again, therefore, we deduce that the vehicle-to-skin partitioning process (as opposed to SC diffusion) provides the key influence on the relative magnitude of nitrate flux.

When GTN delivery from Aquaphor was compared to that from the commercial formulation (Fougera ointment, which is composed of lanolin and white petrolatum), no significant differences in δ and T_L values were found. However, κ', ρ', and J_{ss} were all significantly less when the vehicle was the Fougera product. This difference may be due to the fact that the GTN incorporated into the commercial formulation is adsorbed onto lactose, whereas the Aquaphor vehicle was prepared with GTN that contained no lactose. We may speculate that the adsorption of GTN onto lactose lowers the effective skin/vehicle partition coefficient (K_v) of the penetrant and, hence, causes κ', ρ', and J_{ss} to be reduced.

Recently, Ogiso *et al.* (12) examined the *in vitro* percutaneous penetration of 1,3-GDN across shaved rat skin. The transdermal kinetic parameters they reported differ markedly from our results, reflecting a study design that included (i) the skin soaked in a buffer solution for 14 hr prior to absorption experiments, (ii) a high concentration of ethanol contained in the ointment formulations, and (iii) the use of shaved skin from a different animal.

In summary, the results presented here lead to the following conclusions. (i) The percutaneous penetration kinetics of 1,2- and 1,3-GDNs are comparable, reflecting their similar physicochemical properties. (ii) Significant biotransformation of GTN to its 1,2- and 1,3-GDN metabolites occurs during transdermal delivery, but the metabolism occurs predominantly after the rate-limiting barrier (i.e., the stratum corneum) has been transversed. (iii) When delivered from aqueous solution at the same concentration, GTN flux is about 10 times higher than that of the GDNs. However, comparable fluxes are predicted when the nitrates are administered at equivalent thermodynamic activities (e.g., as saturated solutions). (iv) Delivery of the nitrates from Aquaphor ointment (2%, w/w) supports the above hypothesis. Similar fluxes for GTN, 1,2-GDN, and 1,3-GDN are observed. It follows that the future use of 1,2- and/or 1,3-GDN in topical delivery systems may be supported, if these metabolites are shown to be therapeutically effective.

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