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 mmol 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 (p) for GTN (2.0 ± 3.0 x 10⁻⁵ cm hr⁻¹) was significantly larger than the corresponding values for 1,2-GDN (1.4 ± 0.9 x 10⁻⁵ cm hr⁻¹) and 1,3-GDN (1.2 ± 0.4 x 10⁻⁵ 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 (Kₚ) values of the compounds. The apparent partition parameters, defined as α = Kₛ · h [where h is the diffusion path length through stratum corneum (SC)], were 19.8 ± 2.5 x 10⁻² cm for GTN and 1.91 ± 1.07 x 10⁻² cm and 1.81 ± 0.91 x 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 (α) and permeability coefficient (p) of GTN markedly decreased, to 2.51 ± 0.75 x 10⁻⁵ cm and 1.6 ± 0.3 x 10⁻⁵ cm hr⁻¹, respectively. In contrast, the α and p results for 1,2- and 1,3-GDN were not significantly different (P > 0.05) from the corresponding α and p 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-
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 (Burick & 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, α-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 (Burick & 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 1500g 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 cm²) 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