Dose Proportionality of Transdermal Nitroglycerin

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The FDA Cooperative Efficacy Study of transdermal nitroglycerin utilized a combination of marketed products over a wide dose range. Unfortunately, plasma nitroglycerin concentrations were not determined. The current study was conducted to assess plasma nitrate concentrations after transdermal doses of 15, 30, 60, and 105 mg/24 hr employing the FDA Cooperative Study design. Plasma concentrations of nitroglycerin, 1,3-glyceryl dinitrate, and 1,2-glyceryl dinitrate were determined during the 24 hr of application and for 1 hr after transdermal system removal. Dose proportionality was assessed after normalizing the data by theoretical dose. For nitroglycerin, dose-normalized $AUC_{(0-\infty)}$ and C_{max} were higher for the 105 mg/24 hr dose than for the other doses. For the metabolites, 1,3-glyceryl dinitrate and 1,2-glyceryl dinitrate, there were no differences in dose-normalized $AUC_{(0-\infty)}$ and dose-normalized C_{max} between the dose levels. No differences were seen in T_{max} between the dose levels for all three species. Based on the dinitrate metabolites, dose proportionality was seen over the 15 to 105 mg/24 hr dose range. Nitroglycerin, however, was found to be linear only between 15 and 60 mg/24 hr.

KEY WORDS: nitroglycerin; transdermal administration; dose proportionality; dinitrate metabolites.

INTRODUCTION

Some clinical studies have suggested that large doses of transdermal nitroglycerin may be necessary to demonstrate efficacy (1). In the late 1980s, the Food and Drug Administration, in collaboration with three pharmaceutical companies, conducted a transdermal nitroglycerin cooperative efficacy study (2). An objective of the FDA Cooperative Study was to evaluate the antianginal activity of transdermally delivered nitroglycerin at doses larger than those currently used in clinical practice; the doses studied ranged from 15 to 105 mg/24 hr. The FDA Cooperative Study, however, included no determinations of plasma nitroglycerin concentrations.

The present work was undertaken to assess the plasma concentrations of nitroglycerin and its dinitrate metabolites after transdermal nitroglycerin doses of 15, 30, 60, and 105 mg/24 hr employing the FDA Cooperative Study design and to evaluate the dose proportionality of nitroglycerin after transdermal delivery.

MATERIALS AND METHODS

Subjects

One hundred twenty (120) healthy male and female volunteers between 19 and 70 years of age and within 30% of ideal body weight were selected for the study. Demographic information for the 119 subjects who completed the study is presented in Table I; analysis of variance did not demonstrate differences between the groups in age and weight values. Voluntary and informed consent was obtained prior to study initiation. All participants were determined to be in good health and were free from acute or chronic diseases as documented by a prestudy medical history, physical examination, and standard biochemical screens for renal, hepatic, hematological, and electrolyte abnormalities.

Study Conduct

The study was an open-label, parallel design with 30 subjects enrolled at each of four dose levels: 15, 30, 60, and 105 mg/24 hr. All subjects completed the study except one at the 105 mg/24 hr dose. The transdermal units utilized in this study included first-generation Nitro-Dur (5 and 10 mg/24 hr; Key Pharmaceuticals, Inc.), Nitrodisc (5 and 10 mg/24 hr; G. D. Searle & Co.), and Transderm-Nitro (5, 10, and 15 mg/24 hr; Ciba-Geigy Pharmaceutical Co.); Table I lists the units utilized in the various treatments.

The transdermal devices were applied to a nonhairy area of the chest or alternate nonhairy area of the trunk. Blood samples were collected immediately before treatment (0 hr) and at 0.5, 1, 2, 4, 6, 8, 12, 20, 22, and 24 hr after dose administration. The transdermal units were checked for adhesion at each blood sampling time. In order to ensure that meaningful nitroglycerin plasma concentration measurements would be obtained, the units were secured with tape if they became detached. After 24 hr, the transdermal units were removed. Two additional blood samples were collected at 0.5 and 1 hr after removal of the transdermal units.

Collection of Samples

Blood samples of 10 ml each were taken via Vacutainer tubes using heparin as the anticoagulant. The tubes were chilled in an ice bath for at least 30 min prior to use. Once the blood was collected, the tubes were inverted a single time for mixing and chilled in a shaking ice bath for 5 min. The samples were centrifuged at 3000 rpm for 10 min in a refrigerated centrifuge at 4°C, then placed in an ice bath during the plasma transfer process. The plasma was transferred into vials, which were immediately placed on dry ice to freeze the samples. Samples were stored at −70°C until analysis.

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Table I. Transdermal Units Utilized in the Various Treatments and Summary of Demographic Information[a]

Dose (mg/24 hr)	Transdermal units (mg/24 hr)	M	F	Age (yr)	Weight (lb)
15	Nitro-Dur, 5 Nitrodisc, 5 Transderm-Nitro, 5	20	10	43.3 ± 17.8 (21–70)	161.7 ± 29.6 (100–214)
30	Nitro-Dur, 10 Nitrodisc, 10 Transderm-Nitro, 10	20	10	39.7 ± 14.8 (20–70)	155.9 ± 33.3 (102–263)
60	Nitro-Dur, 5 Nitrodisc, 5 Transderm-Nitro, 5 Nitro-Dur, 10 Nitrodisc, 10 Transderm-Nitro, 10 Transderm-Nitro, 15	19	11	45.6 ± 13.5 (19–70)	156.7 ± 18.5 (128–195)
105	Nitro-Dur, 5 Nitrodisc, 5 Transderm-Nitro, 5 Nitro-Dur, 10 Nitrodisc, 10 Transderm-Nitro, 10 Transderm-Nitro, 15 Transderm-Nitro, 15 Transderm-Nitro, 15 Transderm-Nitro, 15	27	2	36.8 ± 13.6 (21–70)	167.9 ± 29.1 (111–231)

[a] Nitro-Dur, Key Pharmaceuticals, Inc.; Nitrodisc, G.D. Searle & Co.; Transderm-Nitro, Ciba-Geigy Pharmaceutical Co. M, number of males; F, number of females. Numbers in parentheses are ranges of ages and weights.

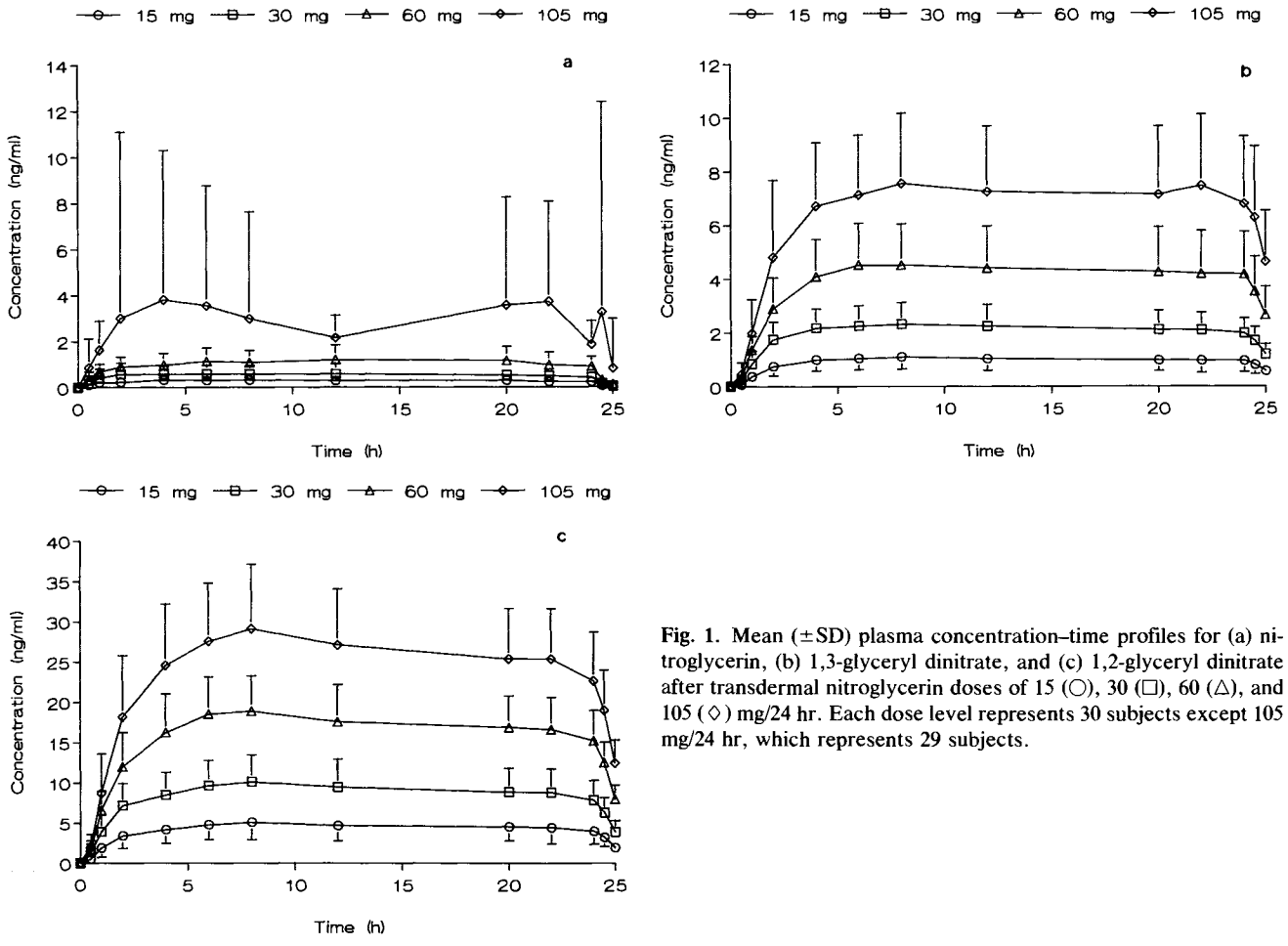

Fig. 1. Mean (±SD) plasma concentration–time profiles for (a) nitroglycerin, (b) 1,3-glyceryl dinitrate, and (c) 1,2-glyceryl dinitrate after transdermal nitroglycerin doses of 15 (○), 30 (□), 60 (△), and 105 (◇) mg/24 hr. Each dose level represents 30 subjects except 105 mg/24 hr, which represents 29 subjects.

Table II. Mean Pharmacokinetic Parameters

	Dose (mg/24 h)			
	15	30	60	105
Nitroglycerin				
T_{max} (hr)	11.9	9.4	10.7	13.4
	(57.9%)[a]	(78.9%)	(61.6%)	(63.4%)
C_{max} (pg/ml)	431.4	824.3	1616.2	9244.5
	(63.4%)	(64.2%)	(50.9%)	(133.9%)
$AUC_{(0-\infty)}$ (pg · hr/ml)	6792.1	13056.7	25874.0	72521.5
	(51.3%)	(56.4%)	(41.8%)	(76.2%)
1,3-Glyceryl dinitrate				
T_{max} (hr)	11.1	11.3	10.9	14.3
	(54.3%)	(53.7%)	(58.8%)	(53.0%)
C_{max} (ng/ml)	1.18	2.47	4.86	8.65
	(41.2%)	(33.5%)	(33.5%)	(36.1%)
$AUC_{(0-\infty)}$ (ng · hr/ml)	24.79	53.43	106.36	175.20
	(40.4%)	(32.8%)	(35.5%)	(33.9%)
1,2-Glyceryl dinitrate				
T_{max} (hr)	11.1	10.7	9.8	10.4
	(50.3%)	(48.8%)	(56.8%)	(53.8%)
C_{max} (ng/ml)	5.40	10.49	19.96	30.10
	(40.1%)	(32.1%)	(23.9%)	(24.7%)
$AUC_{(0-\infty)}$ (ng · hr/ml)	109.37	218.64	412.16	625.76
	(39.8%)	(32.8%)	(23.1%)	(25.7%)

[a] Numbers in parentheses are coefficients of variation.

Analytical Methodology

The plasma samples were assayed for nitroglycerin and its dinitrate metabolites using validated capillary gas chromatography/negative ion chemical ionization mass spectrometry at Pharmaco Analytical Laboratories, Richmond, VA (3). N^{15}-Nitroglycerin and N^{15}-1,3-glyceryl dinitrate were used as internal standards for nitroglycerin and the dinitrate metabolites, respectively. The method was validated over the range of 26.0 to 5000 pg/ml for nitroglycerin, 0.1 to 10 ng/ml for 1,3-glyceryl dinitrate, and 0.2 to 10 ng/ml for 1,2-glyceryl dinitrate. Samples with concentrations above the standard curve were diluted appropriately. The inter- and intraday estimates of variability for the method based on spiked plasma containing nitroglycerin and its dinitrate metabolites were <10% in every case. Concentrations below the limits of quantification were assigned a value of zero.

Data Analysis

The maximum plasma concentration, C_{max}, and time of maximal concentration, T_{max}, were determined directly from each data set. The elimination rate constant, k_{el}, was determined by least-squares linear regression analysis of the terminal portion of the log-linear concentration–time profile for each subject. The area under the plasma concentration–time curve from 0 hr to infinity, $AUC_{(0-\infty)}$, was calculated by the linear trapezoidal rule with extrapolation to infinity by dividing the final measurable plasma concentration by $k_{el}C_{max}$ and $AUC_{(0-\infty)}$ data were normalized to the lowest theoretical dose of 15 mg/24 hr before statistical analysis.

Normality testing [Martinez and Iglewicz test (4)] indicated that the C_{max} and $AUC_{(0-\infty)}$ data were not normally distributed; after log transformation, the data were normally distributed. In order to satisfy the underlying assumption of data normality in the statistical tests, the log-transformed C_{max} and $AUC_{(0-\infty)}$ data were used for all testing procedures. T_{max} data were normally distributed without transformation.

The test employed for determining dose proportionality was the protected least significant difference test (PSD), which consists of an analysis of variance (ANOVA) followed by a least significant difference test (LSD) *if and only if* the ANOVA indicates that a difference exists (5). The level of significance (α) was selected to be 0.05.

RESULTS

Figures 1a–c depict the mean plasma concentration profiles for nitroglycerin, 1,3-glyceryl dinitrate, and 1,2-glyceryl dinitrate after transdermal administration of nitroglycerin. Mean pharmacokinetic parameters at the four dose levels are included in Table II.

Figures 2a–c show the relationship between C_{max} and theoretical nitroglycerin dose for nitroglycerin, 1,3-glyceryl dinitrate, and 1,2-glyceryl dinitrate. The solid lines are the results of linear regression analyses; the linear regression for nitroglycerin omitted the 105 mg/24 hr data. ANOVA demonstrated a difference between the dose levels for the dose-normalized and log-transformed C_{max} data for nitroglycerin ($P = 0.0001$); the dose-normalized nitroglycerin C_{max} was higher for the 105 mg/24 hr dose than for the other three doses, which were not significantly different from one another. The ANOVA procedures detected no significant differences between the various dose levels in dose-normalized

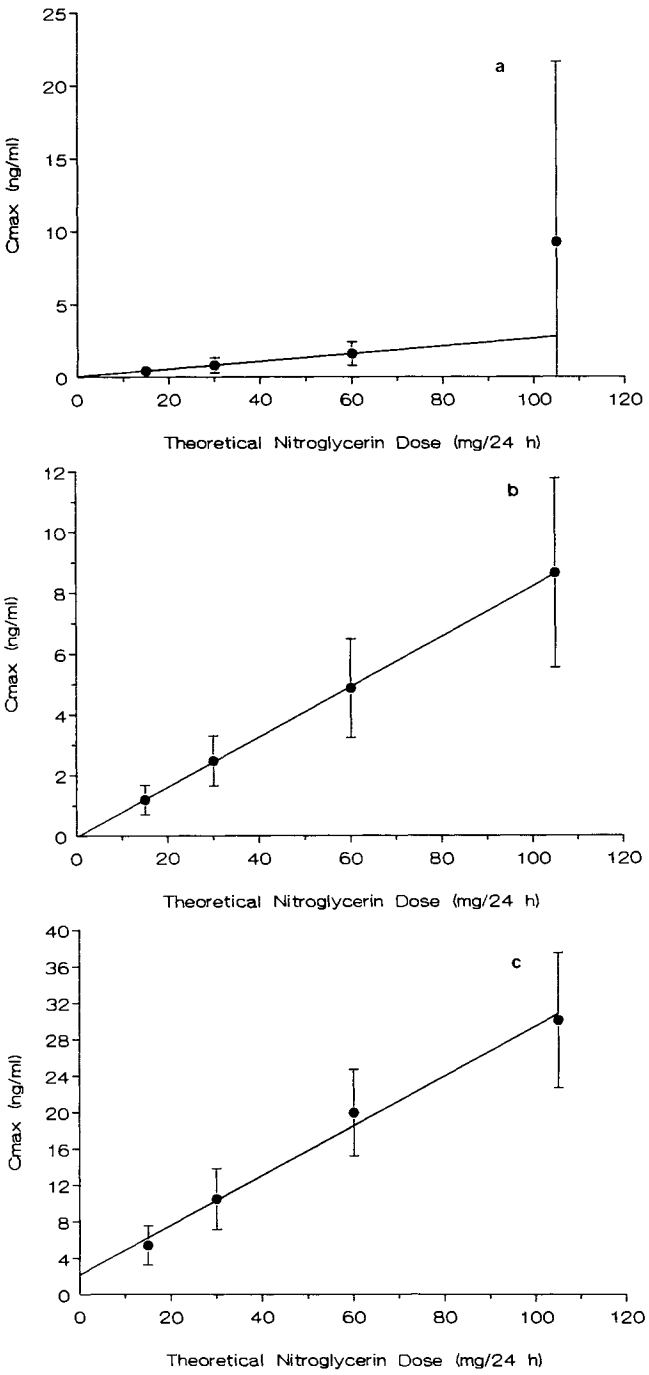

Fig. 2. Relationship between C_{max} and theoretical transdermal nitroglycerin dose for (a) nitroglycerin, (b) 1,3-glyceryl dinitrate, and (c) 1,2-glyceryl dinitrate. The solid lines are the results of linear regression analyses; the linear regression for nitroglycerin omitted the 105 mg/24 hr data. Each dose level represents 30 subjects except 105 mg/24 hr, which represents 29 subjects.

C_{max} for 1,3-glyceryl dinitrate ($P = 0.814$) and 1,2-glyceryl dinitrate ($P = 0.062$).

Figures 3a–c illustrate the relationship between $AUC_{(0-\infty)}$ and theoretical nitroglycerin dose for nitroglycerin, 1,3-glyceryl dinitrate, and 1,2-glyceryl dinitrate. The solid lines are the results of linear regression analyses; the linear regression for nitroglycerin excluded the 105 mg/24 hr data. ANOVA showed a difference between the dose levels for the dose-normalized and log-transformed $AUC_{(0-\infty)}$ data for nitroglycerin ($P = 0.0295$); the dose-normalized $AUC_{(0-\infty)}$ was higher after the 105 mg/24 hr dose than after the other three doses, which were not significantly different from one another. The ANOVA procedures detected no significant dif-

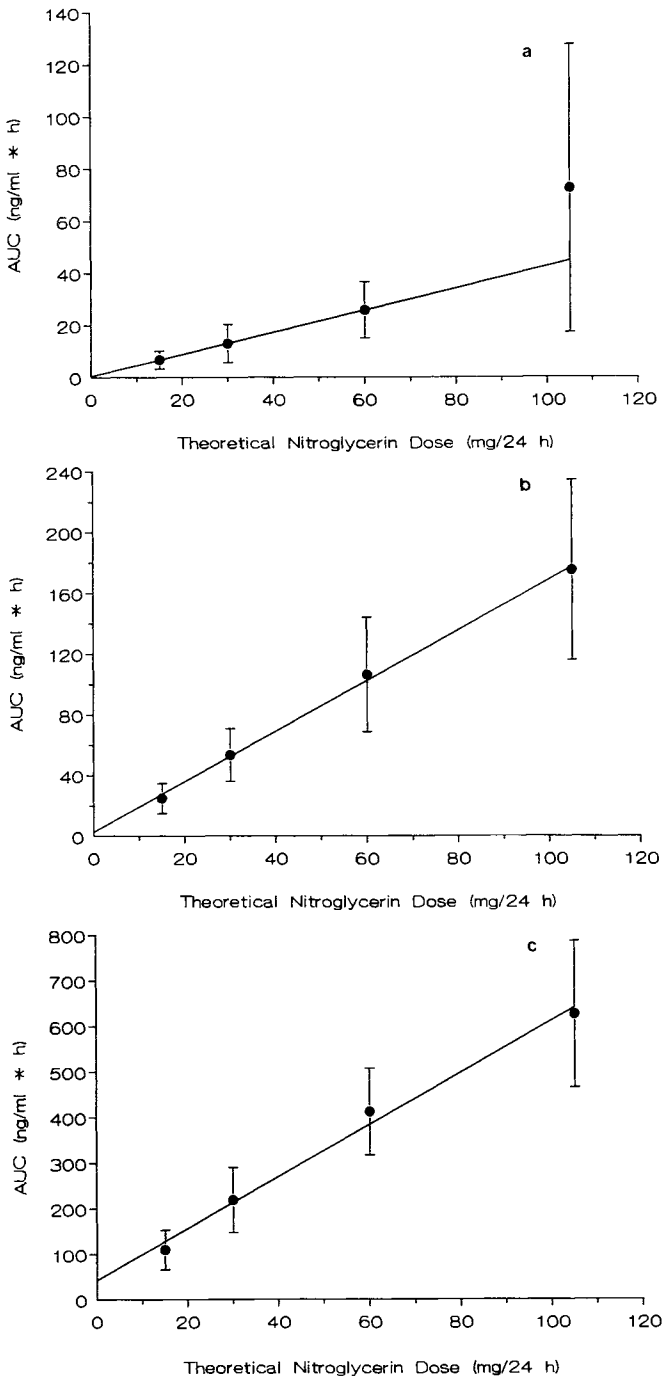

Fig. 3. Relationship between $AUC_{(0-\infty)}$ and theoretical transdermal nitroglycerin dose for (a) nitroglycerin, (b) 1,3-glyceryl dinitrate, and (c) 1,2-glyceryl dinitrate. The solid lines are the results of linear regression analyses; the linear regression for nitroglycerin omitted the 105 mg/24 hr data. Each dose level represents 30 subjects except 105 mg/24 hr, which represents 29 subjects.

ferences between the various dose levels in dose-normalized $AUC_{(0-\infty)}$ for 1,3-glyceryl dinitrate ($P = 0.579$) and 1,2-glyceryl dinitrate ($P = 0.084$).

ANOVA detected no differences between the dose levels in T_{max} for nitroglycerin ($P = 0.196$), 1,3-glyceryl dinitrate ($P = 0.150$), and 1,2-glyceryl dinitrate ($P = 0.817$).

DISCUSSION

The degree of variability observed in nitroglycerin plasma concentrations was greater than that observed for the dinitrate metabolites, 1,3-glyceryl dinitrate and 1,2-glyceryl dinitrate; this can be clearly seen in Figures 1a–c. The average coefficients of variation for the mean plasma concentrations from 2 to 24 hr were 62, 63, 52, and 136% for nitroglycerin at the 15, 30, 60, and 105 mg/24 hr doses, respectively; for 1,3-glyceryl dinitrate, they were 43, 34, 37, and 38%, while for 1,2-glyceryl dinitrate, they were 42, 34, 27, and 29%. The differing pharmacokinetic behavior of nitroglycerin and its dinitrate metabolites may explain the difference in the degree of plasma concentration variability for the three compounds. The clearance of nitroglycerin is very rapid and highly variable between subjects; the elimination half-life of nitroglycerin after intravenous dosing has been reported as 1–4 min (6). In contrast, the half-lives of 1,3-glyceryl dinitrate and 1,2-glyceryl dinitrate are closer to 1 hr (6). The slower elimination of the metabolites may contribute to the lower variability seen in their plasma concentrations.

The slower elimination of the dinitrate metabolites also causes their plasma concentrations to be much greater (4 to 20-fold) than those of nitroglycerin after transdermal delivery. Furthermore, a recent report demonstrated that both 1,3-glyceryl dinitrate and 1,2-glyceryl dinitrate possess pharmacological activity in humans (7). The combination of decreased variability of plasma concentration, much higher plasma concentrations, and pharmacological activity for the dinitrate metabolites indicates that they should be included in any assessment of nitroglycerin pharmacokinetics.

Of the pharmacokinetic parameters evaluated (T_{max}, C_{max}, and $AUC_{(0-\infty)}$ for nitroglycerin, 1,3-glyceryl dinitrate, and 1,2-glyceryl dinitrate), only nitroglycerin C_{max} and $AUC_{(0-\infty)}$ failed to show dose proportionality over the entire dose range. Dose proportionality for nitroglycerin was demonstrated for transdermal doses of 15 to 60 mg/24 hr. The remaining pharmacokinetic determinations support a conclusion of dose proportionality for the dinitrate metabolites over a dose range of 15 to 105 mg/24 hr.

A survey of the literature concerning the dose proportionality of nitroglycerin after different routes of administration found conflicting results. Mean plasma concentrations and clearances have been estimated after intravenous infusion of nitroglycerin, and dose dependency has been described for this compound (6,8–10). Based on these reports, dose proportionality of nitroglycerin after intravenous infusion cannot be concluded; there is at least a suggestion of some nonlinear pharmacokinetic behavior at higher infusion rates. However, the dose dependency of nitroglycerin after intravenous infusion in all of these cases was observed under nonequilibrium conditions (e.g., short-term infusion). When the intravenous infusion was extended beyond 2 hr, this nonlinearity disappeared (6,11).

The issue of dose proportionality of nitroglycerin after transdermal delivery has been examined in two reports. In a limited number of subjects, Imhof *et al.* (10) reported a 66% increase in plasma nitroglycerin concentrations when the surface area (and thus the dose) doubled. Riess *et al.* (12) concluded that nitroglycerin $AUC_{(0-24)}$ was proportional to the surface area of the transdermal unit using 10- and 20-cm^2 units (labeled doses of 5 and 10 mg/24 hr); in addition, an examination of the nitroglycerin plasma concentration 24 hr after application of one or more transdermal units to a total 10, 20, 30, 40, and 50 cm^2 (labeled doses of 5 to 25 mg/24 hr) showed a linear relationship between plasma concentration and surface area.

The current findings significantly add to the previously published indications of dose proportionality after transdermal nitroglycerin delivery. Over the dose range of 15 to 105 mg/24 hr, the dinitrate metabolites were dose proportional after transdermal delivery of nitroglycerin. Nitroglycerin itself demonstrated dose proportionality from 15 to 60 mg/24 hr, but the $AUC_{(0-\infty)}$ and C_{max} deviated from linearity at the 105 mg/24 hr dose. In addition, since the dinitrate metabolites are present at much higher plasma concentrations than nitroglycerin and possess pharmacological activity, the measurement of the dinitrate metabolites should be included when examining nitroglycerin pharmacokinetics.

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