Nitroglycerin Absorption from Transdermal Systems: Formulation Effects and Metabolite Concentrations

Roger L. Williams,[1,3] Kamlesh M. Thakker,[2] Vivian John,[2] Emil T. Lin,[1] Winnie Liang-Gee,[1] and Leslie Z. Benet[1]

Received February 5, 1990; accepted January 15, 1991

We recently compared plasma concentrations of nitroglycerin and its two dinitrate metabolites in 16 healthy male subjects after application of two controlled-release transdermal formulations of the drug. Analysis of the resulting plasma concentration–time curves indicated that the two formulations did not produce equivalent concentrations of parent drug or either of the dinitrate metabolites during the initial period of dosing. In addition, both formulations produced concentrations of the two dinitrate metabolites that exceeded the concentration of the parent drug by severalfold. Even if the pharmacologic effect of the dinitrate metabolites is low compared to that of nitroglycerin, these higher concentrations may contribute to the effect of nitroglycerin. Scrutiny of the ratio of 1,2-glyceryl dinitrate to 1,3-glyceryl dinitrate in the 16 subjects confirmed previous observations that preferential formation of the 1,2-glycerol dinitrate metabolite may occur depending on the route of administration. This ratio may thus be indicative of the bioavailability of nitroglycerin following transdermal application. Additional data suggesting racial differences in nitroglycerin absorption after transdermal application are presented.

KEY WORDS: nitroglycerin; nitroglycerin metabolites; bioequivalence; transdermal drug delivery.

INTRODUCTION

Nitroglycerin is a potent vasodilator that is used in the treatment of angina pectoris, congestive heart failure, and myocardial infarction (1,2). The drug can be administered orally, sublingually, intravenously, and transdermally. There are now at least five controlled-release transdermal systems and four ointment formulations that can deliver the drug topically over a 24-hr period. We recently compared plasma concentrations of nitroglycerin and its two dinitrate metabolites in healthy male subjects after application of two of the transdermal systems. The primary purpose of the study was to assess the bioequivalence of the two formulations. Because we were able to measure metabolite concentrations in addition to concentrations of parent drug, the study also permitted evaluation of the metabolism of nitroglycerin after topical application.

[1] The Drug Studies Unit, Departments of Medicine, Pharmacy and Pharmaceutical Chemistry, University of California, San Francisco, California 94143.

[2] Pharmaceuticals Division, Ciba-Geigy Corporation, Ardsley, New York 10502.

[3] To whom correspondence should be addressed at FDA HFD 600, 5600 Fisher Lane, Rockville, Maryland 20857.

SUBJECTS AND METHODS

Subjects. The study was conducted in the Drug Studies Unit at the University of California, San Francisco. The clinical protocol and consent form were approved by the Committee on Human Research at the University. Sixteen nonsmoking subjects between 21 and 35 years of age completed both phases of the two-period crossover study. Six subjects discontinued the study because of intolerance to the study medication manifested primarily by headache, and one subject discontinued for personal reasons. Subjects were determined to be in good health prior to entry into the study on the basis of standard clinical evaluations and were of normal weight for height and frame. All medications, including over-the-counter products, were prohibited for at least 2 weeks prior to the first dose in the study.

Study Procedures. The study compared the following two formulations of nitroglycerin: Formulation I—Nitroglycerin Transdermal System (NTS, Bolar Pharmaceuticals), 5 mg/24 hr; and Formulation II—Nitroglycerin Therapeutic System (Transderm-Nitro, Ciba-Geigy Corp.), 5 mg/24 hr. A treatment consisted of the application of two patches (total labeled dose, 10 mg/24 hr) of one of the transdermal systems to intact skin of a relatively hairless area of the anterior right shoulder. Subjects were randomly assigned to the two possible treatment sequences, with at least 1 week separating each treatment.

After fasting overnight, subjects entered the clinical study site at 7 AM on the day of each treatment and remained until approximately 10 AM the following morning. Between 7 and 8 AM on the day of entry, vital signs were measured, an indwelling, nonheparin-requiring catheter/obturator (Angiocath 20-gauge Teflon catheter and obturator, Deseret Medical, Inc., Becton Dickinson and Company, Sandy, Utah) was placed in a forearm antecubital vein, and leads for continuous EKG monitoring were placed. The two transdermal systems of Formulation I or II were applied at approximately 8 AM with removal 24 hr later. Sitting blood pressure and pulse were measured at 0, 1, 2, 4, 8, 12, and 24 hr relative to the start of dosing. At 9 AM, 12 noon and 6 PM, subjects received a standard breakfast, lunch, and dinner, respectively. Subjects were allowed to become ambulatory 4 hr after the onset of dosing. The transdermal systems were removed at 24 hr, followed by washing of the application site with soap and water.

Sample Collection and Processing. Relative to the start of dosing, blood samples (5 ml) were collected at the following times: 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 14, 16, 20, 22, 24, and 24.5 hr. Immediately after collection, blood samples were placed into capped polypropylene centrifuge tubes (VWR Scientific, Inc.) and centrifuged at high speed (12,000*g*, Beckman Microcentrifuge 12) for 1 min. The resulting plasma samples were transferred to a labeled glass dram vial and flash-frozen in a dry ice/acetone bath. Samples were stored briefly in a −20°C freezer, then transferred to a −70°C freezer until assay. This method yields frozen plasma within 2 min of blood collection to prevent significant nitroglycerin metabolism within the sample following collection.

Analytical Methods. Nitroglycerin (GTN) and 1,2- and 1,3-glyceryl dinitrate (1,2-GDN and 1,3-GDN) in plasma

0724-8741/91/0600-0744$06.50/0

were measured simultaneously using a sensitive and specific capillary gas–liquid chromatographic method based on electron capture detection (3). The interday coefficient of variation was 6.13% for GTN (concentration range, 0.050–7.50 ng/ml), 5.13% for 1,2-GDN (range 0.20–7.50 ng/ml), and 7.16% for 1,3-GDN (range, 0.200–7.50 ng/ml). Using the same range, the intraday coefficient of variation was 6.34% for GTN, 6.29% for 1,2-GDN, and 5.78% for 1,3-GDN. The lower limit of quantitation was 0.05 ng/ml for GTN and 0.1 ng/ml for both metabolites.

Data Analysis. Maximum plasma concentrations (C_{max}) of GTN and 1,2- and 1,3-GDN and times of these concentrations (T_{max}) were measured as the highest observed concentration in the collection interval and time of this concentration without interpolation. $AUC_{0-24.5\ hr}$ values for GTN and each metabolite were calculated by linear trapezoidal rule for ascending concentrations to C_{max} and by log-linear trapezoidal rule for descending concentrations beginning at C_{max}. Extrapolation was not performed. Analysis of variance was used to detect statistically significant treatment, period, and sequence differences for C_{max}, T_{max}, and $AUC_{0-24.5\ hr}$ and for drug and metabolite ratios at each sampling point. Correlations were performed using the Pearson correlation coefficient. Data are presented as mean ± SD.

RESULTS

Standard bioequivalence comparisons (C_{max}, T_{max}, $AUC_{0-24.5\ hr}$) between Formulation I (Bolar NTS) and Formulation II (Ciba-Geigy TTS) are summarized in Table I. No statistically significant differences between these variables for the two formulations were observed, with the exception of a longer nitroglycerin T_{max} for Formulation I ($P < 0.0379$). Comparison of the mean plasma concentration–time curves for GTN and its two metabolites (Fig. 1) suggested, however, that Formulation II produced higher concentrations of drug and metabolites during approximately the first half of the 24.5-hr sampling interval. Statistical comparisons (ANOVA) at each sampling point confirmed this observation (Fig. 1). When AUCs for GTN and the two metabolites were calculated from 0 to 14 hr and compared statistically, significant differences between the two formulations were demonstrated for GTN and the 1,2-GDN metabolite (Table I). $AUC_{0-14\ hr}$ was chosen for these comparisons because it most closely approximated the duration of the greatest AUC discrepancies between the two formulations (Fig. 1).

Mean ratios of 1,2-GDN/GTN, 1,3-GDN/GTN, and 1,2-GDN/1,3-GDN were calculated at each sampling time after application of Formulations I and II (Fig. 2). With the exception of a significantly higher 1,2-GDN/GTN ratio for Formulation II at 5 hr, no statistically significant differences between the two formulations were observed for either the 1,2-GDN/GTN or the 1,3-GDN/GTN ratios. The most consistent observation was a persistently higher 1,2-GDN/1,3-GDN ratio for Formulation II in comparison to

Table I. Mean ± SD Peak Concentration (C_{max}), Time of This Concentration (T_{max}), and Unextrapolated AUC ($AUC_{0-24.5\ hr}$ or $AUC_{0-14\ hr}$) for Nitroglycerin, 1,2-Glyceryl Dinitrate, and 1,3-Glyceryl Dinitrate After Application of Two 5-mg/24-hr Transdermal Systems (Formulation I, Bolar; Formulation II, Ciba–Geigy) in 16 Healthy Subjects

Variable	Formulation I	Formulation II	*P*
Nitroglycerin			
C_{max} (ng/ml)	0.453 ± 0.294	0.472 ± 0.259	NS
T_{max} (hr)	13.2 ± 7.32	7.08 ± 6.76	0.0379
$AUC_{0-24.5\ hr}$ (ng/ml × hr)	5.46 ± 3.58	6.03 ± 3.89	NS
$AUC_{0-14\ hr}$ (ng/ml × hr)	2.59 ± 1.35	3.41 ± 2.10	0.0112
1,2-Glyceryl dinitrate			
C_{max} (ng/ml)	2.47 ± 0.897	2.59 ± 0.902	NS
T_{max} (hr)	19.5 ± 4.47	15.0 ± 7.14	NS
$AUC_{0-24.5\ hr}$ (ng/ml × hr)	42.0 ± 17.2	47.1 ± 17.4	NS
$AUC_{0-14\ hr}$ (ng/ml × hr)	20.3 ± 9.2	26.4 ± 10.4	0.0037
1,3-Glyceryl dinitrate			
C_{max} (ng/ml)	0.687 ± 0.311	0.675 ± 0.272	NS
T_{max} (hr)	14.9 ± 7.08	12.4 ± 7.47	NS
$AUC_{0-24.5\ hr}$ (ng/ml × hr)	11.5 ± 5.13	11.7 ± 5.13	NS
$AUC_{0-14\ hr}$ (ng/ml × hr)	5.79 ± 2.50	6.53 ± 2.61	NS

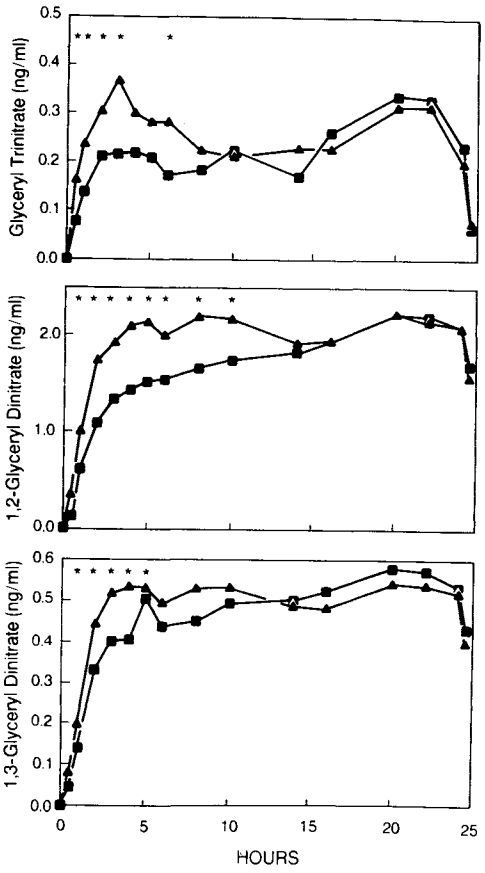

Fig. 1. Mean plasma concentrations of glyceryl trinitrate (GTN), 1,2-glyceryl dinitrate (1,2-GDN) and 1,3-glyceryl dinitrate (1,3-GDN) 0–24.5 hr after application of Formulation I (Bolar, squares) and Formulation II (Ciba-Geigy, triangles) transdermal nitroglycerin systems (10 mg/24 hr) in 16 healthy male subjects. Statistically significant differences ($P < 0.05$) are noted with an asterisk.

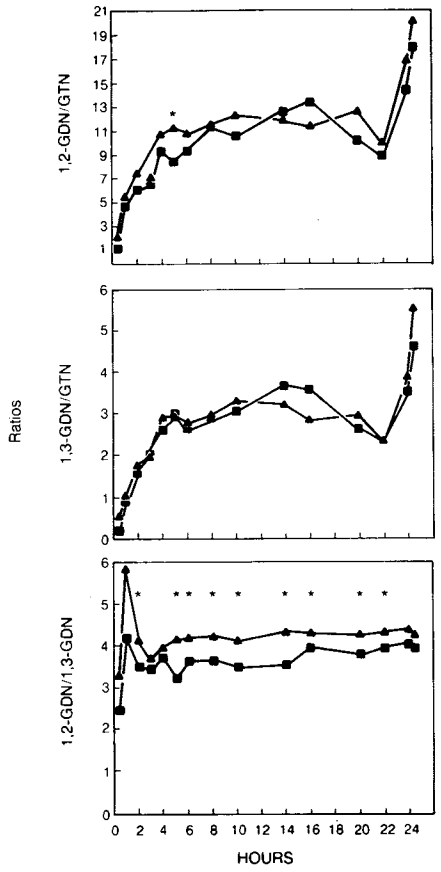

Fig. 2. Mean ratios at each plasma sampling point of 1,2-GDN/GTN, 1,3-GDN/GTN, and 1,2-GDN/1,3-GDN after transdermal administration of Formulation I (squares) and Formulation II (triangles). Statistically significant differences ($P < 0.05$) are noted with an asterisk.

Table II. Ratios of 1,2-GDN to GTN, 1,3-GDN to GTN, and 1,2-GDN to 1,3-GDN by Subject Averaged Over All Samples Within a Treatment

Subject No.	Ratio					
	1,2-GDN/GTN		1,3-GDN/GTN		1,2-GDN/1,3-GDN	
	I[a]	II	I	II	I	II
1	11.1	15.2	2.72	3.75	3.90	4.14
2	11.1	12.4	2.76	2.92	3.93	4.14
3	10.8	8.54	3.81	2.14	2.65	3.92
4	10.1	9.37	2.21	1.75	4.62	5.35
5	5.69	4.26	1.11	0.98	4.60	4.50
6	18.6	17.9	3.21	2.49	5.86	7.05
7	8.50	11.8	2.27	2.33	3.59	5.11
8	12.5	12.6	2.81	2.46	4.47	5.19
9	7.58	10.6	2.20	2.83	3.53	3.72
10	5.40	5.96	2.83	3.39	1.93	1.83
11	6.58	11.0	1.86	2.75	3.62	4.04
12	12.0	14.1	4.29	4.60	2.85	2.87
13	8.58	12.3	4.74	5.17	1.65	2.36
14	7.57	9.72	2.78	2.88	2.67	3.32
15	11.1	9.80	2.23	1.72	4.93	5.68
16	5.34	4.64	1.19	1.12	4.49	4.95
Mean	9.53	10.6	2.69	2.71	3.71	4.26
SD	3.41	3.68	0.99	1.13	1.14	1.31
P	NS		NS		0.0004	

[a] Formulation.

Formulation I, which achieved statistical significance at 2, 5, 6, 8, 10, 14, 16, 20, and 22 hr (Fig. 2).

The 1,2-GDN/GTN, 1,3-GDN/GTN, and 1,2-GDN/1,3-GDN ratios were also averaged within each individual and each treatment over all sampling points after each treatment, with further calculation of group means and statistical comparisons (Table II). No statistically significant formulation differences were observed for the overall mean 1,2-GDN/GTN or 1,3-GDN/GTN ratios. The overall mean 1,2-GDN/1,3-GDN ratio was significantly higher after application of Formulation II in comparison to Formulation I.

DISCUSSION

Administered acutely into the buccal cavity, nitroglycerin has long been used to treat angina pectoris (4). When given via this route, the drug has a rapid onset and offset of action. In the last decade, interest in nitroglycerin has focused on its effects when administered over a prolonged period of time, either in the prophylaxis of angina (5–8) or in the reduction of cardiac pre- and afterload in congestive heart failure and myocardial infarction (9,10). Chronic administration of nitroglycerin has been achieved through intravenous administration, oral dosing, and application of the drug to the skin. Only the latter two routes are acceptable for outpatient use. While oral dosing of the drug is not commonly employed, topical application of nitroglycerin has achieved widespread use, despite concern that tolerance to its beneficial effects occurs (2). Improved methods of assay have provided a better understanding of the complex pharmacokinetics of nitroglycerin and its two primary glyceryl dinitrate metabolites. Several authors have suggested that the two dinitrate metabolites of nitroglycerin may be responsible for at least some of the therapeutic effects of nitroglycerin, particularly when given chronically (11,12). Evidence for nonlinear pharmacokinetics of nitroglycerin has been presented (13).

Various nitroglycerin ointment formulations and transdermal reservoir systems are available to deliver the drug to the skin over a specified period. Most transdermal formulations [Nitrodisc, Searle; Nitro-Dur, Schering; Deponit, Wyeth-Ayerst; Nitrocine, Kremers-Urban; Nitroglycerin Transdermal System, Bolar (Formulation I in this study)] rely on matrices of varying composition in which the nitroglycerin is suspended. The resulting material is applied directly to the skin and serves as a reservoir of the drug over the period of application. One of the transdermal systems (Transderm-Nitro, Ciba-Geigy; Formulation II in this study) utilizes an ethylene/vinyl acetate membrane to separate a nitroglycerin-containing fluid from the skin surface. For all formulations, the absorption of nitroglycerin through the skin is a significant factor in determining the overall rate of delivery to the circulation. Skin appears to absorb only about 0.5 mg nitroglycerin/cm^2/24 hr (14).

McAllister *et al.* (15) compared the plasma concentrations of three transdermal systems (Nitrodisc, Nitro-Dur,

and Transderm-Nitro) to an ointment formulation (Nitrobid) in 24 healthy males. The transdermal systems were designed to deliver 10 mg of the drug to the body over 24 hr. All three transdermal systems produced steady-state nitroglycerin concentrations between 100 and 200 pg/ml within a few hours of application. In contrast, the ointment formulation releasing 15 mg nitroglycerin (1 in.) produced markedly higher plasma concentrations (1.2 ng/ml) that peaked approximately 1 hr and then declined to undetectable values by about 12 hr after application. Two of the transdermal formulations (Nitrodisc and Nitro-Dur) demonstrated moderately reduced plasma concentration–time curves in comparison to the third formulation (Transderm-Nitro): the ratio of the means of Nitrodisc $AUC_{0-24\ hr}$ to Formulation II $AUC_{0-24\ hr}$ equaled 0.822 (range, 0.649–1.042) and the ratio of the means of Nitro-Dur $AUC_{0-24\ hr}$ to Formulation II $AUC_{0-24\ hr}$ equaled 0.747 (range, 0.589–0.947). More recently, Noonan *et al.* (16) demonstrated that Nitro-Dur and the new formulation Nitro-Dur II also produced concentrations of nitroglycerin of about 200 pg/ml consistently over a 24-hr period when given in a dose of 10 mg/24 hr.

In this report, the time-averaged nitroglycerin concentration ($AUC_{0-24.5\ hr}$/24.5 hr) for Formulation I was 223 ± 146 pg/ml and that for Formulation II was 246 ± 159 pg/ml. The values for Formulation II are thus moderately higher than those reported by McAllister *et al.* (15) (160 ± 84 pg/ml) for the same formulation and about the same as those reported by Noonan *et al.* for the two Nitro-Dur formulations (about 200 pg/ml) (16).

Unlike the reports of McAllister *et al.* and Noonan *et al.*, the analytical data in this report included measurement of the plasma dinitrate metabolites of nitroglycerin. The time-averaged concentration of 1,2-glyceryl dinitrate was 1.71 ± 0.70 ng/ml after application of Formulation I and 1.92 ± 0.71 ng/ml after application of Formulation II. For 1,3-glyceryl dinitrate, the corresponding values are 0.469 ± 0.209 (Formulation I) and 0.477 ± 0.209 ng/ml (Formulation II). Thus the concentrations of the 1,2- and the 1,3-dinitrate metabolites of nitroglycerin are approximately seven and two times greater, respectively, than those of the parent drug after transdermal dosing. Concentrations of nitroglycerin and its two dinitrate metabolites achieved steady-state concentrations 2 to 5 hr after application of the transdermal systems (Fig. 1), an observation that confirms the relatively short half-lives of all three species. Although the observed plasma half-life of nitroglycerin is extremely short (a few minutes), the time to reach steady state for this compound was about the same as that observed for the two dinitrate metabolites, each of which possess a half-life of about an hour.

Using human cadaver skin, Berner and Mazzenga (17) observed that although mean steady-state fluxes between Formulation I and Formulation II were not significantly different, the time lag to steady state was longer for Formulation I in comparison to Formulation II (I, 3.0 ± 0.8 hr; II, 1.3 ± 0.2 hr, $P < 0.02$). Estimates of time to attain 95% of steady-state flux were 5.5–8.0 hr for Formulation I and 0.8–3.5 hr for Formulation II, and variances of steady-state fluxes of the two formulations were significantly different ($P < 0.05$). In light of these *in vitro* data, it was thus not surprising to find in the present study that the early concentrations of nitroglycerin and its two dinitrate metabolites after application of Formulation II were about 20% higher than after Formulation I (Fig. 1) during the first 14 hr of application. Time-averaged concentrations of nitroglycerin during this period were 185 ± 96.6 (Formulation I) and 244 ± 150 pg/ml (Formulation II). Corresponding values for the 1,2-dinitrate metabolite were 1.45 ± 0.66 (Formulation I) and 1.89 ± 0.74 ng/ml (Formulation II), and those for the 1,3-dinitrate metabolite were 0.414 ± 0.178 (Formulation I) and 0.466 ± 0.186 (Formulation II).

The metabolites of nitroglycerin are of interest because *in vivo* animal data (18,19) suggest that they possess between 2 and 10% of the activity of the parent compound. In addition, clinical investigations (20) have documented the efficacy of nitroglycerin given orally, even though concentrations of the parent compound after oral administration are essentially zero, while the concentrations of the two dinitrate metabolites are high (21,22). Assuming equipotency between the 1,2- and the 1,3-dinitrate metabolites and also that they possess about one-tenth of the activity of the parent compound based on animal data, the approximate ratio of 10:1 for the sum of the two dinitrate metabolites to nitroglycerin in this study (derived from the AUC data in Table I) suggests that about half the effect of nitroglycerin after transdermal application may be attributable to the dinitrate metabolites.

Noonan and Benet (12) recently commented on the variable ratio of 1,2-GDN to 1,3-GDN after different routes of nitroglycerin administration. Their data indicated a ratio of approximately seven after intravenous administration, two after oral administration, and four after transdermal (ointment) and buccal administration. Assuming nonspecific metabolism, the expected ratio of 1,2-GDN to 1,3-GDN after nitroglycerin dosing is two, because the 1,2-GDN metabolite can be formed by reduction of the nitrate ester at either the 1- or the 3-glyceryl position, while the 1,3-GDN metabolite can be formed only by reduction of the nitrate ester at the 2 position. Noonan and Benet postulated that 1,2-GDN was more likely to be formed after intravenous, transdermal and buccal administration because the enzyme(s) responsible for the metabolism of nitroglycerin at these sites preferentially reacts with primary nitrate esters (1 or 3 position).

The data in this report also indicate a 1,2-GDN/1,3-GDN ratio of about four after application of the two transdermal formulations of nitroglycerin (3.71 for Formulation I and 4.26 for Formulation II; Table II). Nakashima *et al.* (23) used a modified physiologic model to predict transdermal bioavailability and skin first-pass metabolism of GTN and the ratio of 1-2-GDN to 1,3-GDN following transdermal dosing. Their model suggests transdermal first-pass metabolism of 24–32% (i.e., GTN bioavailability of 68–76%), with skin metabolism favoring 1,3-GDN formation over the 1,2-metabolite. The complicated model of Nakashima *et al.*, which predicts the time course of GTN and GDN concentrations following ointment dosing, may be simplified to suggest that the observed GDN ratio after transdermal dosing is a combination of preferential metabolism to 1,3-GDN in the skin and to 1,2-GDN in the systemic circulation. An increase in transdermal bioavailability should lead to an increase in the 1,2-GDN/1,3-GDN ratio if bioavailability differences relate to differences in the amount of drug metabolized in the

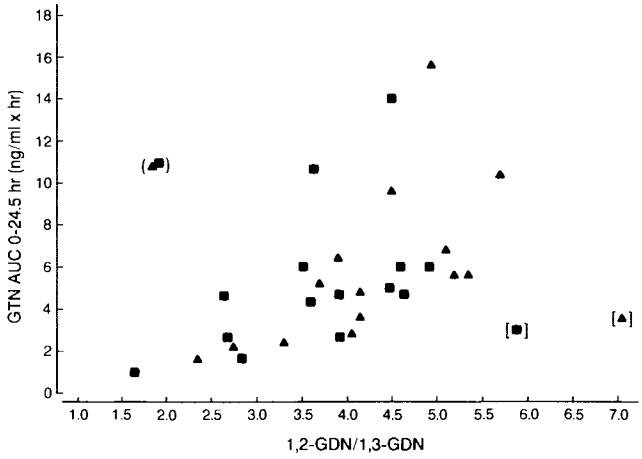

Fig. 3. Correlation between 1,2-GDN/1,3-GDN ratio and GTN $AUC_{0-24.5\ hr}$ after administration of Formulations I (squares) and II (triangles). Pearson correlation coefficient is 0.104 ($P = 0.7007$) for Formulation I and 0.179 ($P = 0.5069$) for Formulation II. Excluding Subjects 6 (brackets) and 10 (parentheses), the corresponding value is 0.505 ($P = 0.0656$) for Formulation I and 0.666 ($P = 0.0093$) for Formulation II.

skin. If this hypothesis is correct, the 1,2-GDN/1,3-GDN ratio may reflect the systemic availability of nitroglycerin. To test this hypothesis, the individual 1,2-GDN/1,3-GTN ratio and GTN AUC pairs from the 16 subjects in the study after dosing with each formulation were correlated (Fig. 3). The results do not indicate a high degree of correlation between the two variables, although the value of the correlation increases if the data for Subjects 6 and 10, which appear to be outliers, are excluded. Another explanation for the observed 1,2-GDN/1,3-GTN ratio difference between the two study formulations (Table II) is a differential effect of a formulation excipient on dermal nitrate metabolism.

Four of the sixteen subjects in this study were black (Nos. 4, 5, 12, and 13), and the remaining twelve were either Caucasian or Asian. Two of the black subjects demonstrated GTN and dinitrate metabolite AUCs that were substantially below the mean value for either formulation. These values were sufficiently low so that when the group of 4 black subjects was separated from the group of 12 Caucasian/Asian subjects, statistically significant differences between the two groups were noted (Table III). These post hoc analyses suggest altered transdermal availability of nitroglycerin in some black subjects, a possibility that merits further investigation.

SUMMARY

This report summarizes several observations regarding transdermal application of nitroglycerin: (*i*) different transdermal formulations produce different concentrations of nitroglycerin and its dinitrate metabolites; (*ii*) the ratio of 1,2-GDN to 1,3-GDN varies depending on the route of administration and may be indicative of specificity in the metabolic transformation of nitroglycerin; (*iii*) the ratio of 1,2-GDN to 1,3-GDN may be reflective of differences in bioavailability

Table III. Nitroglycerin and Metabolite Data for Formulation I (Bolar) or Formulation II (Ciba-Geigy) in Black ($n = 4$) and Caucasian/Asian ($n = 12$) Subjects

Variable	Formulation I		Formulation II	
	Black	Caucasian/Asian	Black	Caucasian/Asian
Nitroglycerin				
C_{max} (ng/ml)	0.28 ± 0.22	0.51 ± 0.30	0.38 ± 0.25	0.52 ± 0.26
T_{max} (hr)	9.76 ± 3.68	14.3 ± 8.00	7.76 ± 6.02	6.85 ± 7.20
$AUC_{0-14\ hr}$ (ng/ml × hr)	1.87 ± 1.42	2.82 ± 1.30	2.47 ± 2.11	3.73 ± 2.09
$AUC_{0-24.5\ hr}$ (ng/ml × hr)	3.24 ± 2.35	6.19 ± 3.69	4.52 ± 3.80	6.53 ± 3.95
1,2-Glyceryl dinitrate				
C_{max} (ng/ml)	1.69 ± 0.96	2.72 ± 0.74	1.66 ± 0.76	2.90 ± 0.70
	$P < 0.05$		$P < 0.05$	
T_{max} (hr)	20.0 ± 4.00	19.3 ± 4.70	18.0 ± 6.70	14.0 ± 7.30
$AUC_{0-14\ hr}$ (ng/ml × hr)	11.4 ± 7.76	23.3 ± 7.80	30.7 ± 14.5	29.1 ± 9.32
	$P < 0.05$		$P < 0.05$	
$AUC_{0-24.5\ hr}$ (ng/ml × hr)	25.0 ± 14.7	47.6 ± 14.3	30.7 ± 14.5	52.6 ± 14.9
	$P < 0.05$		$P < 0.05$	
1,3-Glyceryl dinitrate				
C_{max} (ng/ml)	0.41 ± 0.13	0.78 ± 0.30	0.46 ± 0.09	0.75 ± 0.27
	$P < 0.05$		$P < 0.05$	
T_{max} (hr)	12.8 ± 7.20	15.6 ± 7.20	12.8 ± 7.20	12.3 ± 7.90
$AUC_{0-14\ hr}$ (ng/ml × hr)	3.55 ± 1.18	6.55 ± 2.39	4.45 ± 0.93	7.22 ± 2.64
	$P < 0.05$		$P < 0.05$	
$AUC_{0-24.5\ hr}$ (ng/ml × hr)	7.00 ± 2.22	13.0 ± 4.97	7.90 ± 1.33	12.9 ± 5.34
	$P < 0.05$		$P < 0.05$	

via a particular route of administration; and (iv) racial differences in transdermal absorption of nitroglycerin may exist. Given the apparent differences in potency between nitroglycerin and its metabolites and the wide variability in ratios between the metabolites either to each other or to nitroglycerin itself, the selection of route of administration becomes an important variable in determining the pharmacologic effect of the drug. Recent clinical data suggest that tolerance to nitroglycerin may occur with continuous transdermal application, which has led to dosing schedules in which the transdermal system is removed for up to 12 hr in a 24-hr dosing interval. If this schedule is followed, the formulation differences noted in Fig. 1, which were observed primarily during the first 12 hr of application, may be important. However, no clinical data now suggest that formulation differences in GTN and metabolite concentrations as described in this report are clinically significant. Studies to assess this possibility are likely to benefit from measurement of both parent drug and its dinitrate metabolites.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Ciba-Geigy Corporation and in part by NIH Grant HL 32243.

REFERENCES

1. W. Aronow. Medical treatment of angina pectoris. IV. Nitroglycerin as an antianginal drug. *Am. Heart J.* 84:415 (1972).
2. S. H. Taylor. Scientific and clinical experience with transdermal nitroglycerin. *Am. Heart J.* 112:197–207 (1986).
3. F. W. Lee, N. Watari, J. Rigod, and L. Z. Benet. Simultaneous determination of nitroglycerin and its dinitrate metabolites by capillary gas chromatography with electron-capture detection. *J. Chromatogr.* 426:259–266 (1988).
4. W. Murrell. Nitroglycerine as a remedy for angina pectoris. *Lancet* 1:80–81, 113–115, 151–152, 225–227 (1879).
5. R. K. Mahapatra, D. Mahapatra, and S. Vadan. Clinical experience with a transdermal nitroglycerin system. *Angiology* 4:277–286 (1987).
6. R. Greco, D. D'Alterio, M. Schiattarella, A. Boccia, L. Greco, and F. Marsico. Efficacy of a new transdermal nitroglycerin patch (Deponit 10) for stable angina pectoris. *Am. J. Cardiol.* 61:44E–51E (1988).
7. H. Dogar, D. Guzelsoy, N. Guitekin, N. Gurses, and C. Demiroglu. Acute and sustained antianginal effects of nitroglycerin patches: Objective evaluation with training-effect-eliminated exercise performance index. *Am. J. Cardiol.* 61:39E–43E (1988).
8. D. E. Rezokovic, L. Pavicic, and M. Majacic. A randomized placebo controlled, double-blind crossover trial of transdermal nitroglycerin in stable angina pectoris. *Eur. Heart J.* 9(Suppl A):73–81 (1988).
9. K. Lindvall, S. V. Eriksson, A. Lagerstrand, and A. Sjogren. Efficacy and tolerability of transdermal nitroglycerin in heart failure—a noninvasive placebo controlled double-blind crossover study. *Eur. Heart J.* 4:373–379 (1988).
10. L. Bolognese, G. Sarasso, G. Rognoni, J. Makmur, G. Fornaro, A. Perucca, and P. Rossi. Sustained beneficial hemodynamic effects of low-transdermal nitroglycerin doses compared with placebo in patients with congestive heart failure. *Clin. Cardiol.* 11:79–82 (1988).
11. H. R. Ochs, V. Verburg-Ochs, and N. J. Greenblatt. Kinetics of 1,2-dinitroglycerin following sustained release nitroglycerin: Influence of propranolol and metoprolol. *Klin. Wochenschr.* 63:1170–1173 (1985).
12. P. K. Noonan and L. Z. Benet. Variable glyceryl dinitrate formation as a function of route of nitroglycerin administration. *Clin. Pharmacol. Ther.* 42:273–277 (1987).
13. P. K. Noonan, R. L. Williams, and L. Z. Benet. Dose dependent pharmacokinetics of nitroglycerin after multiple intravenous infusions in healthy volunteers. *J. Pharmacokin. Biopharm.* 13:143–157 (1985).
14. W. R. Good. Transderm^{R}-Nitro controlled delivery of nitroglycerin via the transdermal route. *Drug Dev. Ind. Pharm.* 9:647–670 (1983).
15. A. McAllister, H. Mosberg, J. A. Settlage, and J. A. Steiner. Plasma levels of nitroglycerin generated by three nitroglycerin patch preparations, Nitradisc, Transderm-Nitro and Nitro-Dur and one ointment formulation, Nitrobid. *Br. J. Clin. Pharmacol* 21:365–369 (1986).
16. P. K. Noonan, M. A. Gonzalez, D. Ruggirello, J. Tomlinson, E. Babcock-Atkinson, M. Ray, A. Golub, and A. Cohen. Relative bioavailability of a new transdermal nitroglycerin delivery system. *J. Pharm. Sci.* 7:688–691 (1986).
17. B. Berner and G. C. Mazzenga. Ciba-Geigy Report BPR 86149.
18. P. Needleman, D. J. Blehm, and K. S. Rotskoff. Relationship between glutathione-dependent denitration and the vasodilator effectiveness of organic nitrates. *J. Pharmacol. Exp. Ther.* 165:286–288 (1969).
19. M. G. Bogaert, M. T. Rosseel, and A. F. DeSchaepdryver. Cardiovascular effects of glyceryl dinitrates as compared to glyceryl trinitrates. *Arch. Int. Pharmacodyn.* 176:458–460 (1968).
20. T. Winsor and J. H. J. Berger. Oral nitroglycerin as a prophylactic antianginal drug: Clinical physiologic, and statistical evidence of efficacy based on a three phase experimental design. *Am. Heart J.* 90:611–626 (1975).
21. P. J. Noonan and L. Z. Benet. The bioavailability of oral nitroglycerin. *J. Pharm. Sci.* 75:241–243 (1986).
22. D. K. Yu, R. L. Williams, L. Z. Benet, E. T. Lin, and D. H. Giesing: Pharmacokinetics of nitroglycerin and metabolites in humans following oral dosing. *Biopharm. Drug Dispos.* 9:557–565 (1988).
23. E. Nakashima, P. K. Noonan, and L. Z. Benet. Transdermal bioavailability and first-pass skin metabolism: A preliminary evaluation with nitroglycerin. *J. Pharmacokin. Biopharm.* 15:423–438 (1987).