amiodarone analysis using this procedure. The assay has been used for patients receiving amiodarone in combination with drugs such as procainamide, quinidine, lidocaine, disopyramide, digoxin, prednisone, furosemide, and other thiazide diuretics.

REFERENCES

(1) M. B. Rosenbaum, P. A. Chiale, D. Ryba, and M. V. Elizari, *Am. J. Cardiol.*, **34**, 215 (1974).

(2) M. B. Rosenbaum, P. A. Chiale, M. S. Halpern, G. J. Nau, J. Przybylski, R. J. Levi, J. O. Latzari, and M. V. Elizari, *ibid.*, **38**, 935 (1976).

(3) J. Broekhuysen, R. Laruel, and R. Sion, *Arch. Int. Pharmacodyn.*, **177**, 340 (1969).

(4) R. J. Flanagan, G. C. A. Storey, and D. W. Holt, *J. Chromatogr.*, **187**, 391 (1980).

ACKNOWLEDGMENTS

Presented at the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, San Antonio meeting, November 1980.

The authors gratefully acknowledge the assistance of J. Broekhuysen (Labaz) in the development of the assay and for supplying amiodarone and internal standard powders and thank P. Norkin (Labaz) for his helpful review of the manuscript. The technical assistance of A. Conlan, C. Oliver, J. Ericson, and M. Karcasinas in the preparation of the manuscript is appreciated.

Influence of the Method of Application on Pharmacokinetics of Nitroglycerin from Ointment in Humans

S. SVED *x, W. M. McLEAN ‡, and I. J. McGILVERAY *

Received April 6, 1981, from the **Bureau of Drug Research, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada K1A 0L2*, and the ‡*Drug Consultation Services, Ottawa General Hospital, and Department of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada K1H 8L6.* Accepted for publication June 2, 1981.

Abstract □ A study was designed to test the influence of surface area on the percutaneous absorption of nitroglycerin from a commercial ointment formulation, using a simple crossover design. On separate occasions, three volunteers were given 16 mg of nitroglycerin (2%) over a 25- and 100-cm^2 area. Plasma nitroglycerin concentration was measured at 30, 45, 60, and 90 min using a sensitive capillary GLC–electron-capture detection method capable of quantitating to 150 pg/ml. Plasma concentrations at all times increased at least twofold with the increased surface area; highest observed concentrations were 0.17 and 0.41 ng/ml, respectively. A fourth volunteer received 16 and 32 mg of nitroglycerin over 100 cm^2. Doubling the dose increased the 0–90-min *AUC* by only 76% but caused a 3.5-fold increase in the 90-min plasma concentration. These results suggest that the surface area of application significantly influences the pharmacokinetics of nitroglycerin ointment.

Keyphrases □ Nitroglycerin—ointments, effect of method of application on pharmacokinetics, humans □ Ointments—nitroglycerin, effect of method of application on pharmacokinetics, humans □ Pharmacokinetics—effect of method of application on nitroglycerin ointments, humans □ Vasodilators—nitroglycerin ointments, effect of method of application on pharmacokinetics, humans

Nitroglycerin (glyceryl trinitrate), a vasodilator, is used extensively in the treatment of angina, cardiac infarction, and other circulatory conditions (1). However, because of its rapid elimination (2, 3), sustained-release or percutaneous dosage forms were developed. It was recently shown (4) that the ointment dosage form is more bioavailable than a sustained-action oral capsule formulation, and evidence was presented (5) that blood concentrations obtained after ointment application were dose dependent. However, neither study examined the influence of surface area on percutaneous absorption. It was also demonstrated (6) that the amount of nitroglycerin absorbed through the skin of a rhesus monkey was dependent on the surface area (but not the anatomical area) of application; however, the importance of this finding may not be generally recognized in the everyday use of this dosage form.

The present study investigated the influence of the surface area of application of the ointment on nitroglycerin bioavailability.

EXPERIMENTAL

Three healthy adult male volunteers were each given 650 mg of acetaminophen orally (to control possible side effects), followed by 16 mg of nitroglycerin as a 2% ointment[1]. In the first part of the experiment ("small area"), the ointment was spread on paper[2] cut to 25 cm^2, and the paper was applied over the chest near the sternum and covered with an adhesive bandage. In the second part ("large area"), performed 2 months later on the same volunteers, an identical amount of nitroglycerin ointment was spread over 100 cm^2 in the same region of the chest, outlined on the skin with the help of a template. The ointment was applied as uniformly as possible with a stainless steel spatula and immediately occluded with a sheet of aluminum foil attached with adhesive tape. A fourth volunteer received 16 and 32 mg of nitroglycerin ointment (2%) spread over 100 cm^2 as described for the large area experiment.

Blood (10 ml) was collected by venipuncture, using an all-glass syringe, at the times indicated in Tables I and II, and dispensed at once into a chilled glass tube containing 0.3 ml of heparin sodium (300 U/per tube) and 50 μl of 0.002 *M* $AgNO_3$ (7). The contents were mixed by inversion, centrifuged immediately in the cold (5 min, 2000 rpm, 4°), and the plasma was separated for the nitroglycerin assay.

The assay method[3] consisted of mixing 1 or 2 ml of plasma with 100 μl of 1 *M* $AgNO_3$ (8), extracting with 10 ml of redistilled pentane containing 3 ng of *o*-dinitrobenzene[4], and evaporating the solvent in an ice bath with a gentle nitrogen stream. The residue was redissolved in 25 μl of redistilled hexane, and 4 μl of this solution was chromatographed on a capillary GLC system[5] equipped with an electron-capture detector[6], using a 25-m methyl silicone gum-coated open tubular column[7]. The chromatographic conditions were carrier gas, helium (6-ml/min septum purge, 2-ml/min column flow); makeup gas, argon–methane (95:5, 30 ml/min); injection, splitless, at 200°, 30-sec delay; oven, programmed temperature from 80 to 130° at 30°/min; detector, 150°; and retention

[1] Nitrol, Kramer-Urban, Milwaukee, Wis.
[2] Appli-Ruler, provided by the manufacturer as package insert.
[3] To be described in detail elsewhere.
[4] RotoRack, Fisher Scientific, Ottawa, Canada. Extraction at 15 rpm, 20 min.
[5] Hewlett-Packard model 5730A with model 18740B capillary inlet system.
[6] Hewlett-Packard model 18713A linear electron-capture detector.
[7] Hewlett-Packard model SP 2100.

0022-3549/81/1200-1368$01.00/0

Table I—Effect of Surface Area on Nitroglycerin Absorption

Area, cm^2	Minutes after Application	Blood Concentration, ng/ml (16-mg dose) Subject MT	Subject WN	Subject RL	Mean
25	30	0.17	0.15	0.18	0.17
	60	0.13	ND[a]	0.16	0.14
	90	ND[a]	ND[a]	ND[a]	—
100	30	[b]	0.32	0.32	0.32
	45	0.47	0.36	0.39	0.41
	60	0.44	0.21	0.29	0.31
	90	0.32	0.16	0.26	0.25
	AUC (0–90)	0.48	0.33	0.39	0.40

[a] None detected (<0.05 ng/ml). [b] Sample unavailable.

Table II—Effect of Dose on the Nitroglycerin Absorption

Area, cm^2	Minutes after Application	Blood Concentration, ng/ml 16-mg Dose	32-mg Dose
100	30	0.31	0.20
	45	0.36	0.44
	60	0.37	0.88
	90	0.24	0.83
	AUC (0–90)	0.41	0.72

times, 3.8 min for nitroglycerin and 5.5 min for *o*-dinitrobenzene. Quantitation was by the peak height ratio method.

A two-compartment open model without lag time [$C_t = Ae^{-\alpha t} + Be^{-\beta t} - (A + B)e^{-k_a t}$] was used with an iterative computer program to fit a weighted ($1/y$) least-squares nonlinear regression curve to the experimental points. Goodness of fit was estimated as $r^2 = 1 - (\Sigma \text{ deviation}^2/\Sigma \text{ observation}^2)$.

RESULTS AND DISCUSSION

The described method was specific for the unchanged drug and quantitative to ~0.15 ng/ml, and a detection limit of ~0.05 ng/ml. A standard curve, consisting of 20 determinations from 0.2 to 5.0 ng/ml, gave a mean relative standard deviation of 7.8% (range 2.5–12.3%) and a coefficient of determination (r^2) of 0.987. The regression of peak height ratio on concentration gave a slope of 0.483 ml/ng and an intercept of −0.004.

Table I shows the results obtained in three volunteers. After the application of 16 mg of nitroglycerin over 25 cm^2, the highest blood concentrations were observed at 30 min, with values of 0.15–0.18 ng/ml, close to the quantitation limit of the method, followed by a rapid decline to below the detection limits at 90 min. Because of these low concentrations, no meaningful area under the curve (*AUC*) values could be calculated. When the same amount of ointment was applied to the same volunteers but over a 100-cm^2 area, peak plasma concentrations occurred at 45 min and the decline was much slower. All concentrations were at least double the corresponding concentrations in the small area experiment.

For certain drugs, percutaneous absorption is known to be influenced by occluding the skin. However, this does not appear to be the case with nitroglycerin (9). In addition, it seems unlikely that the differences in the vapor barrier characteristics of the wax-impregnated paper used in the small area experiment and the aluminum foil used in all subsequent experiments would account for the differences obtained.

Table II shows the effect of dose on plasma nitroglycerin levels in one volunteer with the area held constant. The 16-mg dose over the 100-cm^2 area gave results very similar to those obtained for the three previous volunteers, although the relative absorption rate appeared to be marginally slower. With double the dose over the same surface area, absorption continued for a substantially longer time, as shown by the 3.5-fold increase in the 90-min blood concentration. However, the *AUC* (0–90 min) increased by only 76%, suggesting a nonlinear transfer process.

Preliminary pharmacokinetic studies for one experiment (Table II, 16 mg) prolonged for 6.5 hr (Fig. 1) suggest a two-compartment open model, with a distribution phase (α) lasting for ~2 hr and a terminal

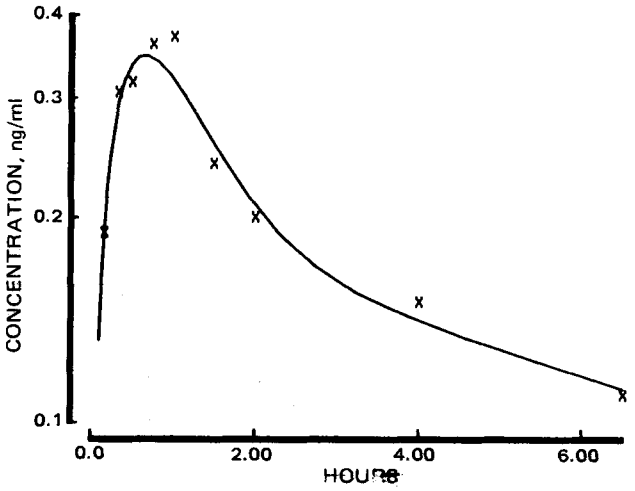

Figure 1—*Plasma profile of nitroglycerin following ointment application with a 16-mg dose in 2% ointment spread over 100 cm^2. The solid line indicates least-squares nonlinear regression to a two-compartment open model without lag time ($C_t = 28.1e^{-1.94t} + 0.197e^{-0.086t} - 28.3e^{-1.98t}$). Goodness of fit ($r^2$) = 0.994.*

decay (β) with a half-life of 3.5 hr. Nitroglycerin elimination from the blood is known to be rapid, with a half-life of 4–8 min after sublingual administration (4). Therefore, the apparent long terminal half-life in the present experiment must be due to other processes, *e.g.*, prolonged absorption.

These results agree well with those of Maier-Lenz *et al.* (4) who reported venous plasma concentrations between 0.4 and 0.7 ng/ml 60 min after the application of 30.6 mg of nitroglycerin in a 2% ointment, but they differ from those reported by Armstrong *et al.* (5), who obtained ~3 ng/ml after 0.27 mg of nitroglycerin/kg over 58 cm^2. The differences may be attributed largely to experimental design since the latter workers measured the concentration in arterial rather than venous blood and also examined percutaneus absorption in patients pretreated with intravenous nitroglycerin.

While the results reported here are only preliminary, they illustrate the cardinal importance of the application method, especially surface area, in the pharmacokinetics and, possibly, clinical efficacy of nitroglycerin ointments. These results indicate that the ointment should be carefully and consistently applied.

REFERENCES

(1) Martindale, "The Extra Pharmacopoeia," 27th ed., A. Wade, Ed., Pharmaceutical Press, London, England, 1977, p. 1646.

(2) P. W. Armstrong, J. A. Armstrong, and G. S. Marks, *Circulation*, **59**, 585 (1979).

(3) *Ibid.*, **62**, 160 (1980).

(4) H. Maier-Lenz, L. Ringwelski, and A. Windorfer, *Arzneim.-Forsch.*, **30**, 320 (1980).

(5) P. W. Armstrong, J. A. Armstrong, and G. S. Marks, *Am. J. Cardiol.*, **46**, 670 (1980).

(6) P. K. Noonan and R. C. Wester, *J. Pharm. Sci.*, **69**, 365 (1980).

(7) H. Laufen, F. Scharpf, and G. Bartsch, *J. Chromatogr. Biomed. Appl.*, **146**, 457 (1978).

(8) R. A. Morrison and H.-L. Fung, "Abstracts," vol. 10, APhA Academy of Pharmaceutical Sciences, 1980, p. 126.

(9) S. T. Horhota and H.-L. Fung, *J. Pharm. Sci.*, **68**, 608 (1979).

ACKNOWLEDGMENTS

The authors thank Dr. A. Balsys, Dr. R. Lalonde, Mr. M. Tierney, and Mrs. B. Hughes for expert advice and assistance and Mr. D. L. Wilson for the analyses.