Pharmaceutical Research, Vol. 15, No. 4, 1998

Novel Pharmacokinetic Modelling of Transdermal Nitroglycerin

Barbara Auclair,[1] Gérard Sirois,[1] Anh Ho Ngoc,[1] and Murray P. Ducharme[1,2]

Received October 27, 1997; accepted January 10, 1998

Purpose. To construct a pharmacokinetic (PK) model and to determine population PK parameters of nitroglycerin (GTN), 1,2-dinitroglycerin (1,2-GDN), and 1,3-dinitroglycerin (1,3-GDN).

Methods. Data were obtained in thirty healthy volunteers following a single dose of a GTN reservoir transdermal patch. Blood samples were obtained just before and at 0.5, 1, 2, 3, 4, 6, 8, 12, 14, and 24 hours after the patch application and 1 hour after its removal. GTN, 1,2-GDN, and 1,3-GDN concentrations were determined using HPLC and simultaneously best fitted using a first-pass mixed-order release one-compartment PK model. Individual estimates (ADAPT-II) were used as priors for a population PK analysis (IT2S). Fitted parameters included the percentage (A) of the nitroglycerin dose reaching the systemic circulation that was released from the patch by a first-order process (K_1); two absorption (ka_1 and ka_2), two metabolite formation (k_{f1} and k_{f2}) and one metabolite elimination (k(m)) rate constants; and three volumes of distribution Vc/F, V_2/F and V_3/F.

Results. Nitroglycerin mean population parameter estimates and inter-individual variability (CV%) were: A 35% (65), K_1 0.06 h^{-1}(91), ka_1 5 h^{-1}(46), ka_2 0.47 h^{-1} (39), k_{f1} 11 h^{-1}(42), k_{f2} 0.6 h^{-1}(34), k(m) 1.4 h^{-1}(29), V_c/F 6 L(31), V_2/F 73 L(34), and V_3/F 23 L(29). The average elimination half-lives for GTN and the two metabolites were 5 and 32 minutes, respectively.

Conclusions. The proposed PK model fitted observed concentrations of GTN, 1,2-GDN and 1,3-GDN very well. This model should be useful to predict drug and metabolite concentrations and to assess bioequivalence of two transdermal formulations.

KEY WORDS: population pharmacokinetics; nitroglycerin; 1,2-dinitroglycerin; 1,3-dinitroglycerin; transdermal administration.

INTRODUCTION

Nitroglycerin (GTN) is a potent vasodilator used in the treatment of angina pectoris and in long-term therapy of coronary heart disease, myocardial infarction and congestive heart failure. When administered orally GTN has a very short elimination half-life, and undergoes extensive gastrointestinal and hepatic first-pass metabolism (1,2). Therefore, transdermal administration of nitroglycerin represents an interesting alternative formulation to obtain a good bioavailability and to prolong the duration of action (1). The PKs of transdermal GTN and of its two potentially active metabolites (1,2- and 1,3-GDN)) have not been extensively studied, and population PK analysis has not been reported in the literature. This study has two objectives. Firstly, we propose to construct a PK model so that observed concentrations of GTN, 1,2- and 1,3-GDN after GTN transdermal administration are well described, and secondly, we aim to determine population PK parameters using the proposed model in healthy volunteers after a single dose administration of a transdermal formulation (Transderm-Nitro®).

[1] Faculté de Pharmacie, Université de Montréal, PO Box 6128 Station centre-ville, Montréal, Qc., Canada, H3C 3J7, and Rhône-Poulenc Rorer Canada Inc., Canada.

[2] To whom correspondence should be addressed. (email:ducharmu@ere.umontreal.ca)

MATERIAL AND METHODS

Subjects

A group of thirty healthy adult male volunteers were included in the study after each of them gave informed consent. The mean ± SD age, weight and height of the subjects were 28 ± 6 years, 78 ± 10 kg and 179 ± 6 cm, respectively. One patch of a nitroglycerin transdermal system (Transderm-Nitro®, 0.4 mg/h, Ciba-Geigy Ltd.) was applied to the chest of each of the study subjects and was kept for 24 hours. Once removed, the transdermal systems were placed on a fresh release liner, enclosed in a fresh pouch and frozen for subsequent HPLC analysis. None of the subjects enrolled in the study received any medication other than the study drug.

Plasma Sampling and Analytical Assay

Blood samples were obtained just before and at 0.5, 1, 2, 3, 4, 6, 8, 12, 14, and 24 hours after the patch was applied to the skin and 1 hour after its removal. The average number of plasma samples per subject was 32. GTN, 1,2- and 1,3-GDN concentrations were determined using a sensitive and specific HPLC method (validated method - data on file). The lower limit of detection was 1 ng/L for GTN and 100 ng/L for both metabolites. The amount of nitroglycerin remaining in the transdermal systems were determined so that the administered amount received by each subject was known accurately. All systems, worn or unused, were extracted in methanol for 24 hours. The patches were first cut open in order to expose the reservoir formulation directly to the extracting solvent. Ten unused specimens of the product from the same lots as the clinical supplies were provided for comparative HPLC analysis.

Pharmacokinetic Analysis

PK analyzes were performed using compartmental PK techniques (3). Plasma concentrations of GTN, 1,2- and 1,3-GDN were simultaneously best fitted using a first-pass, mixed-order release, one-compartment PK model. This model is schematically illustrated in Figure 1. Six other different models (Figure 2) were investigated during the model discrimination process, but none of them fitted the observed data properly based on visual inspection of graphs (concentrations versus time), inspection of weighted residuals (difference between fitted and observed concentrations) versus observed concentrations, and values of Akaike's information criterion test. Briefly, model 1 consisted of a zero-order release and a similar volume of distribution for GTN and the GDN metabolites. Consecutive improvements in the model consisted of adding a different volume of distribution for the GDN metabolites (model 2), an additional skin reservoir (model 3), a first-order release from the skin reservoir (model 4), a first-pass process (model 5), a mixed zero- and first-order release rate (model 6), and a time-lag process (final model).

0724-8741/98/0400-0614$15.00/0

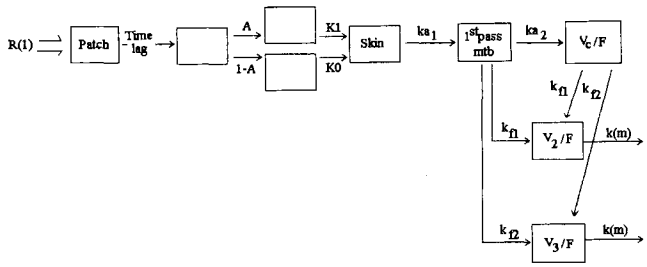

Fig. 1. Structural representation of the final PK model. A, the percentage of the delivered GTN dose reaching the systemic circulation released from the patch by a first-order process K_1; ka_1, ka_2, absorption rate constants; k_{f1}, k_{f2}, metabolites formation rate constants; k(m), metabolites elimination rate constant; V_c/F, GTN volume of distribution; V_2/F, 1,2-GDN volume of distribution; V_3/F, 1,3-GDN volume of distribution.

Fitted parameters in the final PK model included the percentage (A) of the delivered GTN dose reaching the systemic circulation released from the patch into the skin by a first-order process (K_1), two absorption rate constants (ka_1 and ka_2), two rate constants associated with the formation of the metabolites (k_{f1} and k_{f2}), a single elimination rate constant for the metabolites (k(m)), three volumes of distribution (V_c/F, V_2/F and V_3/F) into which GTN, 1,2- and 1,3-GDN appeared to be distributed into respectively, and a time-lag (Tlag). This last parameter was necessary because many patients did not have any measurable concentration in the plasma 0.5 hour after application of the transdermal patch. Estimated time-lag values were obtained during the preliminary population iterative processes. Once they were estimated with robustness, they were later fixed for each individual subjects and the population pharmacokinetic iterative process was restarted. A single elimination rate constant was used for both dinitro compounds because preliminary pharmacokinetic analysis indicated to us that the metabolites appeared to have the same elimination. Furthermore, in two studies, no difference was observed in half-life and clearance between 1,2-GDN and 1,3-GDN after oral administration of GTN and intravenous administration of the individual metabolites (4,5).

It is also suggested that the dinitrate metabolites are metabolized by the same enzyme and, contrary to their formation, it is thought that the enzyme will de-nitrate the 1,2- and 1,3-GDN in the same kinetic manner (6). The zero-order rate constant of the release of the drug from the patch was directly calculated using the fitted parameter A and the dose received by each patient (DOSE (Received)=DOSE(Initially in the Patch)-DOSE (Left in the patch)) with the following equation:

$$K0 := \frac{\text{DOSE received} \cdot (1 - A)}{24}$$

Mathematically, the PK model may be described by the following series of equations:

$Z = 0$ If Time>Tlag then $Z=1$

When patch is administered $R(2) := 1$ Else $R(2) = 0$

$$\frac{dX1}{dt} := Z \cdot (DOSE \cdot A) - Z \cdot X1$$

$$\frac{dX2}{dt} := Z \cdot (DOSE \cdot (1 - A)) - Z \cdot K0 \cdot R(2)$$

$$\frac{dX3}{dt} := Z \cdot X1 - R(2) \cdot K1 \cdot X3$$

$$\frac{dX4}{dt} = R(2) \cdot K1 \cdot X3 + R(2) \cdot Z \cdot K0 - Ka1 \cdot X4$$

$$\frac{dX5}{dt} := Ka1 \cdot X4 - Ka2 \cdot X5 - Kf1 \cdot X5 - Kf2 \cdot X5$$

$$\frac{dX6}{dt} := Ka2 \cdot X5 - Kf1 \cdot X6 - Kf2 \cdot X6$$

$$\frac{dX7}{dt} := Kf1 \cdot X5 + Kf1 \cdot X6 - K(m) \cdot X7$$

$$\frac{dX8}{dt} := Kf2 \cdot X5 + Kf2 \cdot X6 - K(m) \cdot X8$$

Where K0 is the zero-order release rate constant, Z is a "flag" enabling the calculation of Tlag, and R(2) is the time associated with the application (R(2) = 1) and the removal (R(2) = 0) of the patch. Molecular weights of GTN (227.09 ng/nmol), and of 1,2- and 1,3-GDN (182.08 ng/nmol) were used to convert

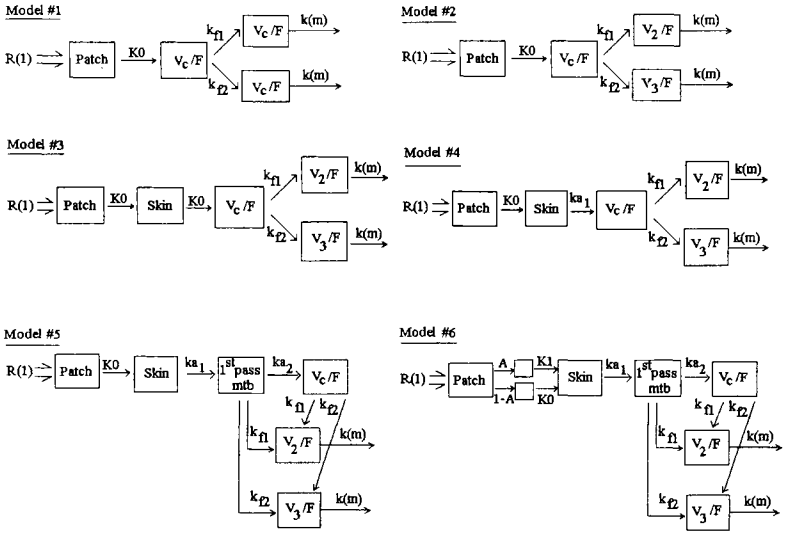

Fig. 2. Schematic representations of the rejected PK models.

the molar concentrations used by the model (nmol/L) to the observed concentrations in ng/L. The observed plasma concentrations of GTN (Y(1)), of 1,2-GDN (Y(2)) and of 1,3-GDN (Y(3)) were therefore simultaneously fitted by the model using the following output equations:

$$Y(1) := \frac{X6 \cdot 227.09}{Vc}$$

$$Y(2) := \frac{X7 \cdot 182.08}{V2}$$

$$Y(3) := \frac{X8 \cdot 182.08}{V3}$$

All concentrations were fitted with a weighting factor of $W_i = 1/S_i^2$ where the variance S_i^2 was calculated for each observations using the equation $S_i^2 = (a \times Y_i + b)^2$. The slope (a) is related to the sum of all errors associated with each concentration, and the intercept (b) is related to the limit of detection of the analytical assay. Individual PK parameters were first derived using generalized least squares analysis (ADAPT II) (7). These estimates were then used as prior values for a population PK analysis using an iterative two-stage methodology (IT2S) (8). Maximum plasma concentrations (Cmax) and times for these concentrations (Tmax) were directly obtained from the observed concentration versus time points. The area under the plasma concentration-time curve (AUC) was calculated by the linear trapezoidal methods with log-linear extrapolation to infinity. The terminal elimination half-lives ($T_{1/2}$) of the parent compound and of its two metabolites were calculated using the following formulas:

$$\text{T1/2(Trinitroglycerin)}: \frac{0.693}{(Kfl + Kf2)}$$

$$\text{T1/2(1,2-\&1,3-dinitroglycerin)}: \frac{0.693}{K(m)}$$

RESULTS

Nitroglycerin mean population PK parameter estimates from a single 24-hour transdermal administration are presented along with inter-individual variability in Table I. The mean ± SD observed maximum plasma concentrations for GTN, 1,2- and 1,3-GDN were 478 ± 500, 3011 ± 1109, and 522 ± 188 ng/L, and occurred at 8 ± 5, 11 ± 6, and 8 ± 5 hours, respectively. The average GTN AUC_{0-14}, AUC_{0-24}, and $AUC_{0-\infty}$ were 2844 ± 1719, 4766 ± 2734, and 4793 ± 2768 ng.h/L. The mean estimated time-lag was 0.24 ± 0.17 hour. The average calculated $T_{1/2}$ for GTN and for its two metabolites were 5 and 32 minutes, respectively. The average amount of GTN delivered by the patch (dose) was 10 ± 2.5 mg during the 24-hour application period, as calculated from the difference between the labeled content and the residual amount left in the patch after its application.

The proposed PK model provided a very good fit to the observed data following transdermal delivery of nitroglycerin. Population observed and fitted mean plasma concentrations versus time of GTN, 1,2-GDN, and 1,3-GDN are presented in Figures 3, 4, and 5 respectively. Goodness of fit was very good as demonstrated by the coefficients of determination (GTN $r^2 = 0.4$, 1,2-GDN $r^2 = 0.91$, and 1,3-GDN $r^2 = 0.92$), and by examination of graphs of the weighted residuals versus observed plasma concentrations which showed no systematic bias or deviation. Plasma concentrations of GTN and of its 2 metabolites as a function of time are depicted in Figure 6 for 5 subjects. These individuals were randomly selected because no subject exhibited a better or poorer fit than others as determined by Akaike criterion tests, Pearson correlation coefficient, and visual inspection of the graphs. There was substantial inter-subject variability in the GTN plasma concentration-time profiles as shown by the individual graphs (Figure 6) and by the vast deviation around the mean values (Figure 3). Single peaks were observed consistently in all subjects at times ranging from 1 to 15 hours after the patch application. The concentrations of the 1,2- and 1,3-GDN were considerably higher than those of the parent drug. Single peaks were also observed for 1,2- and 1,3-GDN. Plasma concentrations of 1,2-GDN were higher than 1,3-GDN.

Table I. Nitroglycerin mean PK parameter estimates and their interindividual variability (CV%) after a single 24-hour transdermal application to 30 healthy volunteers

PK parameters	Mean (CV%)
A (%)	35 (65)
K_1 (h^{-1})	0.06 (91)
ka_1 (h^{-1})	5 (46)
ka_2 (h^{-1})	0.47 (39)
k_{f1} (h^{-1})	11 (42)
k_{f2} (h^{-1})	0.6 (34)
k(m) (h^{-1})	1.4 (29)
V_c/F (L)	6 (31)
V_2/F (L)	73 (34)
V_3/F (L)	23 (29)

Note: A, the percentage of the delivered GTN dose reaching the systemic circulation released from the patch by a first-order process K_1; ka_1, ka_2, absorption rate constants; k_{f1}, k_{f2}, metabolites formation rate constants; k(m), metabolites elimination rate constant; V_c/F, GTN volume of distribution; V_2/F, 1,2-GDN volume of distribution; V_3/F, 1,3-GDN volume of distribution.

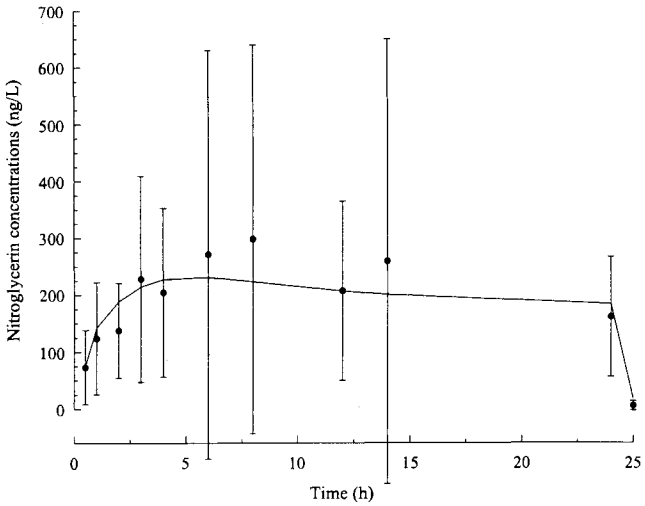

Fig. 3. Fitted (−) and observed (•, ± SD) population mean GTN plasma concentrations versus time after a single 24-hour nitroglycerin transdermal application.

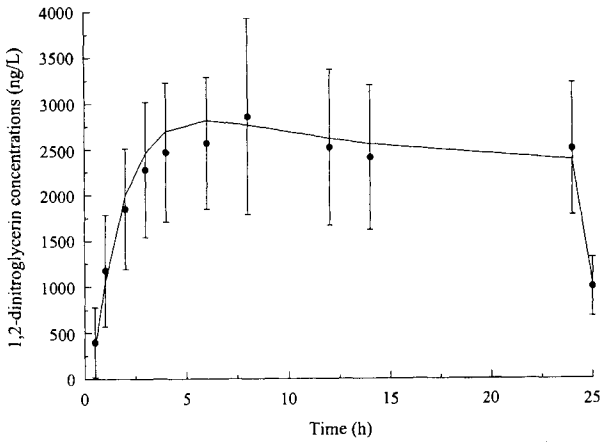

Fig. 4. Fitted (−) and observed (•, ± SD) population mean 1,2-GDN plasma concentrations versus time after a single 24-hour nitroglycerin transdermal application.

DISCUSSION

This study represents the first attempt at deriving population PK parameters of GTN and its two GDN metabolites from a 24-hour transdermal application. Our proposed PK model is an evolution of previous work from different groups of researchers who attempted to describe GTN or other drug concentrations during their transdermal administration. Chandrasekaran proposed to fit scopolamine concentrations after its transdermal application using both a constant and a "priming dose" release of the drug (9). The scopolamine transdermal system studied was a Transdermal Therapeutic System (TTS) which consists of multiple layers including an adhesive gel layer and a reservoir. The Transderm-Nitro® TTS used in the present study is a similar system. The reservoir contains the drug to be released in a constant manner, while the adhesive layer serves both as an adhesive and as an additional reservoir of the drug that provides some sort of a loading dose or "priming dose" that allow the reaching of therapeutic concentrations more rapidly. For Transderm-Nitro®, the main reservoir of the drug contains 5 times the amount that will eventually be released in order to have a driving force for drug diffusion to occur through the skin (10). This is consistent with data found in our patients, as on average, 10 mg out of the 50 mg present in the patch was released during the 24-hour administration. The adhesive layer of a reservoir patch such as Transderm-Nitro® is thought to contain up to 8% of the total amount of GTN found in the patch (10). For the Transderm-Nitro® that we studied this means that approximately 4 mg of GTN may be present in the adhesive layer. In this study we have found that on average, 35% of the available drug appeared to have been released by a first-order process (A: 35 ± 0.22). Assuming that this first-order process comes exclusively from the release of the GTN present in the adhesive layer, we can verify if our PK model estimated properly the percentage of the drug to be found there. The amount of GTN released from the patch through the skin by a first-order process can thus be estimated to be 3.5 mg (35% of the 10 mg released). It appears therefore that our PK model probably estimated accurately the percentage of GTN released by a first-order process (35%). Also, distinct peaks were seen in all individual GTN plasma concentration-time profiles. Black (10) and Curry (6) have published diagrams of individual plasma concentration curves for Transderm-Nitro® patches showing similar observations. If one pools all the data together, these peaks are minimized because they do not consistently occur at the same time. This is exemplified by the significant variation in the Tmax from this study although careful examination of the population mean concentrations shows that a majority of patients will exhibit this peak around 5 to 7 hours after the beginning of the patch administration. We believe that these peaks are mostly due to the mixed zero- and first-order release process. In the PK analysis, the PK models that incorporated only a zero-order process did not fit appropriately concentrations of GTN and of its GDN metabolites.

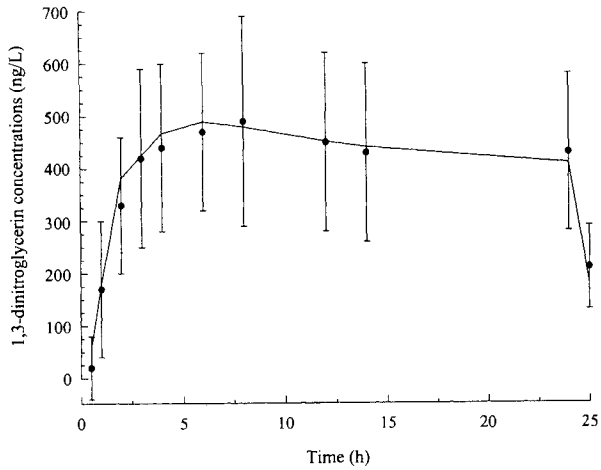

Fig. 5. Fitted (−) and observed (•, ± SD) population mean 1,3-GDN plasma concentrations versus time after a single 24-hour nitroglycerin transdermal application.

Previously proposed PK models for transdermal drug delivery have never been used in a population analysis. Many interesting models have been constructed such as the one Guy and Hadgraft have proposed incorporating both a first-order and a zero-order release of the drug (11). The ability of their PK model to describe drug disposition during transdermal application was tested using PK simulations for clonidine (12), oestradiol, hyoscine, timolol and nitroglycerin (11,13,14). PK parameters for these drugs and for GTN, however, were derived and simulated from data coming from intravenous administration and from physicochemical properties of the drugs (11). Also, their model did not appear to explain properly the early concentrations of GTN. Possible explanations for this are the absence of a time-lag and of a first-pass effect in their model. It is apparent from the data presented in this study that the metabolite concentrations appeared quicker or at the same time than the parent drug, a phenomenon consistent with first-pass metabolism of the drug in the skin. This is expected as GTN has been shown to be metabolized by cytochrome P450 enzymes (CYP) present in vascular tissue of endothelial and smooth muscle cells (15–17). Nakashima et al., when evaluating concentrations of GTN following a transdermal ointment administration, have proposed a physiological PK model that included a first-pass effect (18). Simulations were performed to demonstrate that their model fitted appropriately data coming from two patients receiving the GTN ointment. We believe, however, that their model would not appropriately fit GTN and GDN concentrations arising from administration of Transderm-Nitro® as it does not include a first-order release nor a time-

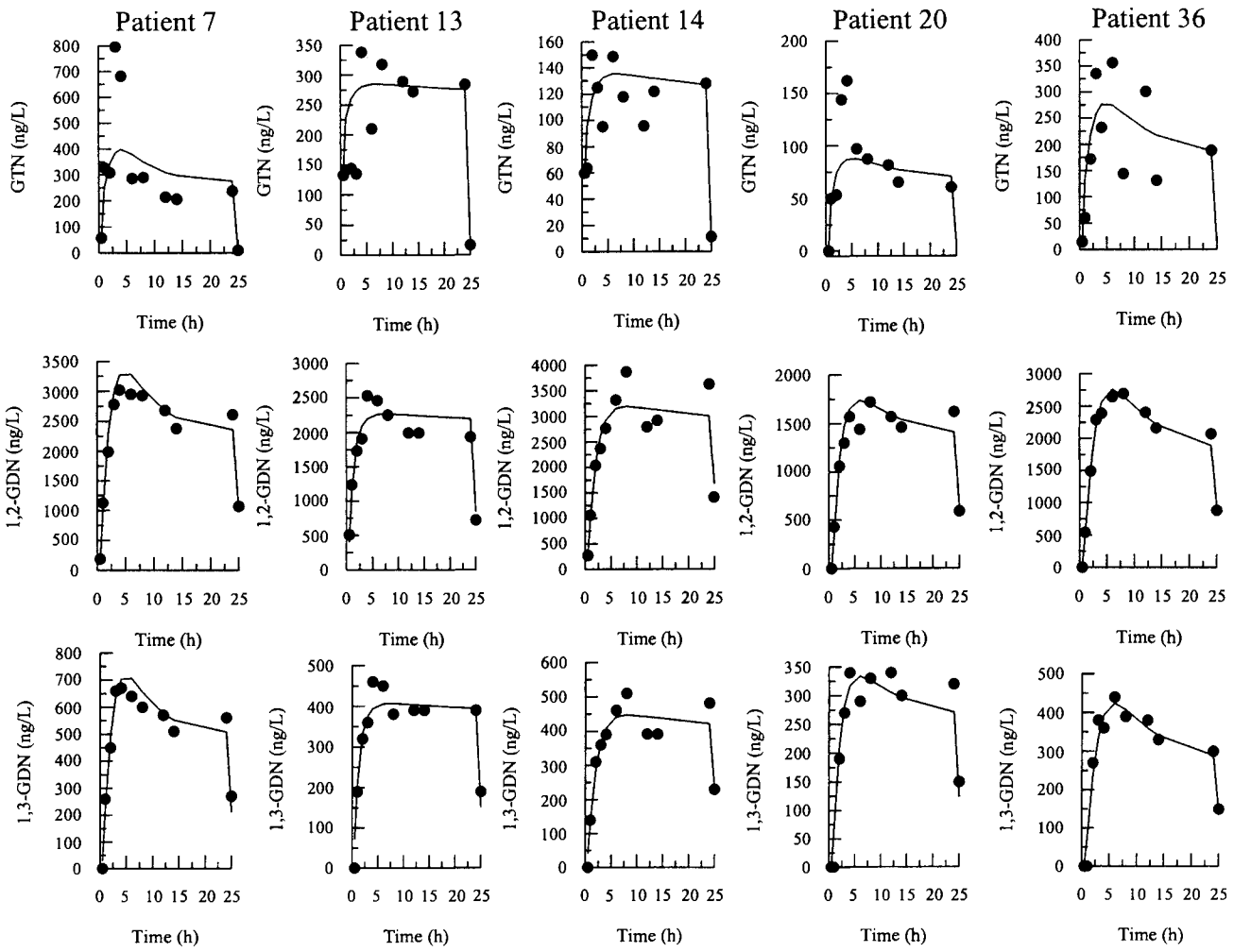

Fig. 6. Fitted (−) and observed (•) GTN, 1,2-GDN and 1,3-GDN plasma concentration-time profiles in 5 subjects.

lag process. It became necessary for us to include such a time-lag in our model as concentrations did not appear systemically in a manner consistent with instantaneous administration. This is consistent with the nature of the transdermal system, where a certain time is needed for an equilibrium to be made between the patch and the skin, and for a skin reservoir to be filled prior to the appearance of the drug in the systemic circulation (6,10).

Our model is therefore the first one to include both a first-pass effect, a mixed first- and zero-order release of the drug, as well as a time-lag. This is also the first report in which population PK parameters are calculated, not just simulated, from fitted concentrations of GTN and GDN. We believe it is extremely important to determine the PKs of GTN concomitantly with those of its GDN metabolites. Doing so provides more data to be analyzed in the same individual allowing a more robust evaluation, and helps distinguish fluctuations in GTN concentrations that could be due to "noise" or to the mixed first- and zero-order release. GTN concentrations following transdermal application are known to fluctuate widely (6,10). Heating or cooling the skin region besides the patch seems to increase or decrease GTN concentrations (19) while exercise appears to increase GTN concentrations (20,21). Some studies have shown that GTN concentrations change significantly depending if the individual rests in a sitting or a passive tilt position (22) while others have seen no difference (23). It may be reasonable to expect some difference depending on physiologic conditions (i.e., physical activity, posture, temperature, food ingestion) (6,24,25). Studies comparing the bioavailability of two formulations should therefore be very strictly controlled. Our model did not include a possibility for a change in clearance over time. However, as previously mentioned, fluctuations in GTN and/or GDN concentrations may also be caused by "noise" or from the mixed first- and zero-order release. Peaks of GTN seen concomitantly with higher concentrations of GDN are likely to be due to the mixed first- and zero-order release, while deviations in GTN only are probably the result of "noise" if they are not accompanied with either higher or lower than expected concentrations of GDN metabolites. Without a population analysis and the simultaneous fitting of GTN and its GDN metabolites, calculated area under the concentration-time curves are therefore difficult to estimate with robustness. This is problematic if one tries to compare bioavailability data between two transdermal preparations. Differences or absence of a difference may well be the result of unaccounted noise (24,26,27) or due to the mixed release of the patches.

Calculated PK parameters presented are consistent with prior knowledge. The mean GTN half-life value of 5 minutes

derived in this study is similar to values published following intravenous administration (6,25,28). Terminal elimination half-lives are independent of the method of administration and different values reported by other groups after oral or transdermal administration are probably the reflect of their PK analysis. Elimination half-life for the GDN metabolites was found to be 32 minutes. Williams et al. found a value of approximately one hour (29). Since these metabolites may contribute to the clinical activity of transdermal nitroglycerin therapy, determination of their PKs is important (25,30).

Our findings have several implications in research and clinical practice. They will allow better head to head comparison between different formulations of nitroglycerin minimizing errors resulting from the substantial fluctuations in plasma concentrations occurring during treatment with transdermal delivery systems. Not limited to comparison of transdermal nitroglycerin formulation's relative bioavailabilities, these results may also enable one to differentiate formulations in terms of onset of action and drug elimination. The PK parameter values derived from this population study will find utility in trials attempting to address the relationships between concentration and effect of nitroglycerin and its metabolites. From a clinical aspect, the PK parameters found will provide good indicators of the time required to reach steady-state after a nitroglycerin dosing regimen is initiated for a specific patient, and the expected time for the drug to be removed from the organism in problematic situations.

ACKNOWLEDGMENTS

We are grateful to Wyeth Ayerst Canada Inc. for providing Post-doctoral Fellowship assistance to Dr. B. Auclair.

REFERENCES

1. S. H. Taylor. The role of transdermal nitroglycerin in the treatment of coronary heart disease. *Am. Heart J.* **112(1)**:197–207 (1986).
2. J. Abrahams. Transdermal nitroglycerin in angina pectoris. *Eur. Heart J.* **10**(Suppl.A):11–19 (1989).
3. M. Gibaldi and D. Perrier. Pharmacokinetics, 2nd ed. New York: Marcel Dekker, 1982.
4. D. K. Yu, R. L. Williams, L. Z. Benet, E. T. Lin, D. H. Giesing. Pharmacokinetics of nitroglycerin and metabolites in humans following oral administration. *Biopharm. Drug Dispos.* **9**:557–65 (1988).
5. F. W. Lee, J. Hu, C. H. Metzler, L. Z. Benet. Nitroglycerin dinitrate metabolites do not affect the pharmacokinetics and pharmacodynamics of nitroglycerin in dog: A preliminary report. *J. Pharmacokinet. Biopharm.* **21(2)**:163–73 (1993).
6. S. H. Cury and S. M. Aburawi. Analysis, disposition and pharmacokinetics of nitroglycerin. *Biopharm. Drug Dispos.* **6**:234–80 (1985).
7. D. Z. D'Argenio and A. Schumitzky. ADAPT-II user's guide. Biomedical Simulations Resource. University of Southern California, Los Angeles: 1992.
8. D. Collins and A. Forrest. IT2S user's guide. State University of New York at Buffalo, Buffalo: 1995.
9. S. K. Chandrasekaran, W. Bayne, and J. E. Shaw. Pharmacokinetics of drug permeation through human skin. *J. Pharm. Sci.* **67**:1370–74 (1978).
10. C. D. Black. Transdermal drug delivery system. *U.S. Pharmacist* 49–75 (1982).
11. R. H. Guy, and J. Hadgraft. Kinetic analysis of transdermal nitroglycerin delivery. *Pharm. Res.* **2**:206–211 (1985).
12. R. H. Guy and J. Hadgraft. Pharmacokinetic interpretation of the plasma levels of clonidine following transdermal delivery. *J. Pharm. Sci.* **74(9)**:1016–18 (1985).
13. R. H. Guy and J. Hadgraft. The prediction of plasma levels of drugs following transdermal application. *J. Control. Rel.* **1**:177–82 (1985).
14. R. H. Guy and J. Hadgraft. Interpretation and prediction of the kinetics of transdermal drug delivery: oestradiol, hyoscine and atenolol. Int. J. Pharm. **32**:159–63 (1986).
15. B. J. McDonald and B. M. Bennett. Biotransformation of glyceryl trinitrate by rat aortic cytochrome P450. *Biochem. Pharmacol.* **45**:268–270 (1993).
16. B. J. McDonald, G. J. Monkewich, P. G. Long, and D. J. Anderson. Effect of dexamethasone treatment on the biotransformation of glyceryl trinitrate: cytochrome P450 3A1 mediated activation of rat aortic guanylyl cyclase by glyceryl trinitrate. *Can. J. Physiol. Pharmacol.* **72**:1513–1520 (1994).
17. A. A. Weber, T. Neuhaus, C. Seul, et al. Biotransformation of glyceryl trinitrate by blood platelets as compared to vascular smooth muscle cells. *Eur. J. Pharmacol.* **309**:209–213 (1996).
18. E. Nakashima, P. K. Noonan, and L. Z. Benet. Transdermal bioavailability and first-pass skin metabolism: a preliminary evaluation with nitroglycerin. *J. Pharmacokinet. Biopharm.* **15**:423–437 (1987).
19. T. O. Klemsdal, K. Gjesdal, and J. E. Bredesen. Heating and cooling of the nitroglycerin patch application area modify the plasma level of nitroglycerin. *Eur. J. Clin. Pharmacol.* **43**:625–628 (1992).
20. K. Gjesdal, T. O. Klemsdal, E. O. Rykke, and J. E. Bredesen. Transdermal nitrate therapy: bioavailability during exercise increases transiently after the daily change of patch. *Br. J. Clin. Pharmacol.* **31**:560–562 (1991).
21. R. A. Lefebvre, M. G. Bogaert, O. Teirlynck, A. Sioufi, and J. P. Dubois. Influence of exercise on nitroglycerin plasma concentrations after transdermal application. *Br. J. Clin. Pharmacol.* **30**:292–296 (1990).
22. S. H. Curry, and H. R. Kwon. Influence of posture on plasma nitroglycerin. *Br. J. Clin. Pharmacol* **19**:403–404 (1985).
23. R. Heidemann, C. Beckenbauer, and B. G. Woodcock. Effect of posture on glyceryl trinitrate plasma concentrations following transdermal application. *Br. J. Clin. Pharmacol.* **23**:246–247 (1987).
24. J. X. Sun, A. J. Piraino, J. M. Morgan, J. C. Jushi, K. Chan, V. A. John, and W. R. Good. Application of a stable isotope technique for the bioequivalence study of two transdermal nitroglycerin systems. *Amer. J. Ther.* **1**:15–21 (1994).
25. M. G. Bogaert. Clinical pharmacokinetics of nitrates. *Cardiovasc. Drugs Ther.* **8**:693–99 (1994).
26. F. D. Panti, C. Luca, F. Pamparana, L. Bianco, L. D'Angelo, M. Caravaggi, et al. Bioavailability study of three transdermal nitroglycerin preparation in normal volunteers. *Curr. Ther. Res.* **46**:111–120 (1989).
27. 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**:365–69 (1986).
28. B. Berner, and V. A. John. Pharmacokinetic characterisation of transdermal delivery system. *Clin. Pharmacokinet.* **26(2)**:121–134 (1994).
29. R. L. Williams, K. M. Thakker, V. John, E. T. Lin, W. Liang-Gee, and L. Z. Benet. Nitroglycerin absorption from transdermal systems: Formulation effects and metabolite concentrations. *Pharm. Res.* **8(6)**:744–49 (1991).
30. P. K. Noonan, M. A. Gonzald, D. Ruggirello, J. Tomlinson, E. Babcock- Atkinson, M. Ray et al.. Relative bioavailability of a new transdermal nitroglycerin delivery system. *J. Pharm. Sci.* **75**:688–91 (1986).