Application of Pharmacodynamic Modeling for Designing Time-Variant Dosing Regimens to Overcome Nitroglycerin Tolerance in Experimental Heart Failure

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Purpose. Prolonged continuous administration of nitroglycerin (NTG) leads to hemodynamic tolerance. We used a previously developed pharmacokinetic-pharmacodynamic (PK/PD) model of NTG tolerance in experimental heart failure to test whether dosage regimens, designed from this model, may allow avoidance of tolerance development upon continuous NTG infusion.

Methods. Simulation experiments (using ADAPT II) were performed to evolve a time-variant infusion regimen that would theoretically provide sustained hemodynamic effect (30% reduction in left ventricular end-diastolic pressure, LVEDP) throughout 10 hours of drug dosing. A computer controlled infusion pump was utilized to deliver this time-variant input. Infusion experiments were then conducted in CHF rats to challenge the predictability of the applied PK/PD model.

Results. Simulations showed that exponentially increasing input functions provided more sustained LVEDP effects when compared to linear or hyperbolic input functions delivering the same total NTG dose. A computer-selected infusion regimen of $6.56e^{0.00156 \times minutes}$ μg/min was anticipated to provide the desired hemodynamic profile in our animal model. Experiments conducted in rats with congestive heart failure (n = 4) confirmed the prediction of sustained hemodynamic effect without tolerance (28 ± 4% reduction in LVEDP at 10 hrs).

Conclusions. These findings support the utility of our PK/PD model of NTG tolerance in predicting NTG action, and serve as an example of therapeutic optimization through PK/PD considerations.

KEY WORDS: pharmacodynamics; nitroglycerin; nitrate; tolerance; hemodynamics.

INTRODUCTION

Nitroglycerin (NTG) is an important organic nitrate vasodilator that is commonly used to treat angina pectoris and congestive heart failure (CHF). Acute administration of NTG provides rapid relief of angina symptoms, beneficial reductions in pulmonary and venous congestion, and improvements in coronary artery blood-flow distribution (1). Despite these favorable short-term actions, prolonged continuous administration leads to the loss of hemodynamic and anti-anginal effects (2–4). This phenomenon, known as nitrate tolerance, has been commonly observed in both angina and heart failure therapy and remains a significant clinical limitation to an otherwise useful drug class. The mechanism of nitrate tolerance is incompletely understood, but likely involves physiologic counter-regulation of nitrate-induced vasodilation and/or alterations in vascular biochemical pathways (3,5,6).

We have recently developed a pharmacokinetic/pharmacodynamic (PK/PD) model that describes and predicts the development of hemodynamic tolerance in an animal CHF model (3). Here we have attempted to utilize this PK/PD model to prospectively design optimal infusion regimens that may avoid the development of NTG tolerance. Initial simulation experiments were conducted to examine the effectiveness of various time-variant infusion protocols to (theoretically) provide sustained NTG efficacy. Experiments were then conducted to validate the accuracy of time-variant dose delivery using a computer-controlled infusion pump and the ability of the theoretically derived dosing regimen to avoid hemodynamic tolerance in CHF animals.

METHODS

Animal Model of NTG Tolerance

We have studied NTG-induced hemodynamic tolerance using a rat model of congestive heart failure (CHF). This animal model has been shown to mimic humans both with respect to the hemodynamic changes and clinical symptoms of heart failure, as well as the time courses of action and tolerance development. We measured changes in left ventricular end-diastolic pressure (LVEDP) as an indicator of NTG effects. This parameter is an index of venous pressures (cardiac preload), which is significantly elevated in CHF rats and patients (7,8). Since NTG is considered a predominant venodilator, changes in LVEDP represent a meaningful index of therapeutic efficacy (9). All animal procedures were approved by the Institutional Animal Care Committee, and adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

NTG Tolerance Data

Initial simulation experiments were conducted using data of hemodynamic effect *versus* time from experiments previously conducted with CHF rats (3). Briefly, a continuous infusion of NTG (10 μg/min) was administered to male CHF rats, and LVEDP was measured prior to and during the continuous infusion regimen. This dose initially provided favorable reductions of LVEDP of about 50%, but continuous infusion at this dose led to an attenuated effect and complete tolerance within 10 hours (10). This tolerance development is not associated with any changes in steady-state plasma concentrations of NTG (11).

Pharmacokinetic/Pharmacodynamic Model of NTG Tolerance

A schematic representation of the pharmacokinetic/pharmacodynamic model used to describe NTG tolerance is shown

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in Figure 1. The model includes a classical one-compartment pharmacokinetic model which describes NTG plasma concentrations during and after intravenous infusion. Linked to this pharmacokinetic model is a pharmacodynamic model that relates NTG concentrations to the observed pharmacologic effect. NTG is assumed to produce vasodilation that is directly related to NTG plasma concentration. This model also incorporates a counter-regulatory vasoconstrictive force responsible for the development of NTG tolerance and assumes that the generation of this force is driven by the direct vasodilating effect of NTG. Thus, when NTG produces a vasodilating effect (E_d), the body produces a counter-regulatory force (E_c) to negate it, and at any time during or after NTG administration the observed pharmacologic effect is a summation of these two opposing actions. Expressed mathematically,

$$\%\text{LVEDP} = 100 - E_d + E_c \quad (1)$$

where %LVEDP is the change of LVEDP as a percentage of the baseline value. E_d, the vasodilatory effect, is presumed to be linearly related to NTG plasma concentrations (C_{NTG}) by the constant m ($E_d = m \cdot C_{NTG}$). Here we assumed vasoconstriction to develop via two sequential first-order processes (governed by the rate constants k_1 and k_2), since we have previously shown that this kinetic relationship is superior to a time-delay

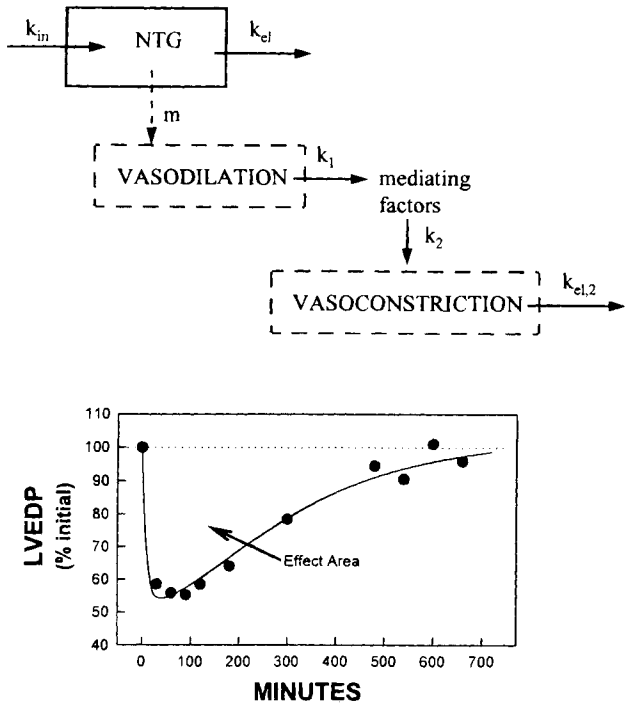

Fig. 1. Upper Panel: Schematic representation of the pharmacokinetic-pharmacodynamic model used to relate nitroglycerin input functions to nitroglycerin plasma concentrations and hemodynamic effects. Lower Panel: The effects of a continuous infusion of NTG at 10 μg/min on left-ventricular end-diastolic pressure is shown. Solid circles represent mean measurements of %LVEDP in CHF rats (n = 15), while the line is the ADAPT II fit of the data to the model represented in Figure 1. Coefficients of variation were consistently less than 20% at each timepoint. Note that in spite of the continuous administration of NTG, beneficial reductions in %LVEDP are lost by 8–10 hours (480–600 minutes).

model in describing our data. The differential equations that describe the model in Figure 1 are shown below.

$$\frac{dC_{NTG}}{dt} = \frac{k_{in}}{V} - k_{el}C_{NTG} \quad (2)$$

$$\frac{dE_d}{dt} = m \cdot \frac{dC_{NTG}}{dt} \quad (3)$$

$$\frac{dM}{dt} = k_1 \cdot m \cdot C_{NTG} - k_2 M \quad (4)$$

$$\frac{dE_c}{dt} = k_2 M - k_{el,2}E_c \quad (5)$$

where k_{in} is the mathematical function controlling the input of NTG into the system (i.e., infusion regimen), V is the volume of distribution of NTG, and k_1, k_2, k_{el}, and $k_{el,2}$ are the first order rate constants of generation of mediating factors, E_c generation, C_{NTG} elimination and E_c elimination, respectively.

The time course of NTG effects on mean %LVEDP was related to concentrations of NTG through fitting the data to Equations 1–5, using ADAPT II software (12). Nitroglycerin plasma concentrations were simulated through the use of pharmacokinetic parameters obtained from previous studies conducted with CHF rats (k_{el}: 0.127 min^{-1}, V: 2.2 L) (3), whereas k_1, k_2, m and $k_{el,2}$ were estimated by fitting the available hemodynamic data. Parameters obtained from the fitting of hemodynamic data were then used as constants in subsequent simulations to devise novel infusion regimens. In all cases the data were weighted equally.

Computer Simulation Experiments

Using the parameter estimates, we simulated the effects of varying infusion regimens on NTG efficacy and tolerance development. In these studies, the input function (k_{in}) was designed to simulate the delivery of NTG via infusion rates which increased linearly, hyperbolically or exponentially. Regardless of the mode of administration, the total dose of NTG delivered over 12 hours was held at 7.2 mg, to allow for direct comparison to results observed with a 12 hour constant rate infusion at 10 μg/min. The simulations were allowed to project NTG plasma concentration and hemodynamic effects over the 720 minute infusion period. Simulated hemodynamic effects were quantified via linear-trapezoidal measurement of the area under the %LVEDP vs. time curve from 0–12 hours (AUE).

Computer-Selected Infusion Protocol

The results of the above simulations suggested that the cumulative magnitude and time-course of NTG hemodynamic effects could be improved through manipulation of the time-course of NTG delivery. In an effort to further optimize the NTG infusion regimen, we used the mathematical model to fit a desired response profile, allowing the infusion function to be the variable. For this simulation, we set our desired response profile to be a consistent 30% reduction in %LVEDP, maintained for 12 hours. Points were selected as shown in Figure

2; these data were equally weighted (i.e., weights equalled 1). The infusion function was confined to the form of an exponentially increasing infusion, $k_{in} = Ae^{\alpha t}$, and infusion parameters A and α were allowed to float while the computer minimized the sum of squared deviations between the profiles of desired vs. simulated effects. In this manner, the computer "selected" an optimal NTG infusion function, based on the existing PK/PD model, population estimates of the parameters as constants, and the desired effect profile.

Experimental implementation of the computer-selected infusion regimen requires the construction of a system capable of rapidly changing the NTG infusion rate with time. For this purpose, we have written a subroutine in BASIC code which allows for continuous, remote personal computer (PC) control of a Harvard infusion pump (Model 22, Harvard Apparatus, South Natick, MA). The PC and the infusion pump were connected through a RS232 interface. The subroutine makes use of the internal clock of the PC to calculate and adjust k_{in} on a per-second basis. The BASIC code appears in Appendix 1.

The ability of the system to deliver the desired infusion appropriately was assessed through a feasibility experiment. In this study, a 20 ml syringe containing infusion solution was connected to a fraction collector (Model 2110, Bio-Rad Laboratories, Hercules, CA) with polyethylene infusion tubing (PE-50, Becton Dickinson, Parsippany, NJ). Culture tubes were collected from the fraction collector and weighed to determine the delivery of solvent over a 12 hour infusion period. Theoretical delivery was calculated as the numerical integration of the infusion function from initiation of the infusion to time, t (in minutes): $\int_{t=0}^{t=t} A\mu L/\text{min} \cdot e^{\alpha t}\, dt$. The measured cumulative volume delivered over 12 hours was compared to these calculated amounts.

Experimental Challenge of Model Predictions

After validation of the computer-controlled pump system, we conducted infusion experiments using CHF rats. Infusion methods and measurement of hemodynamic effects were identical to those previously described (3,10), with the exception that a computer-controlled infusion rate was used.

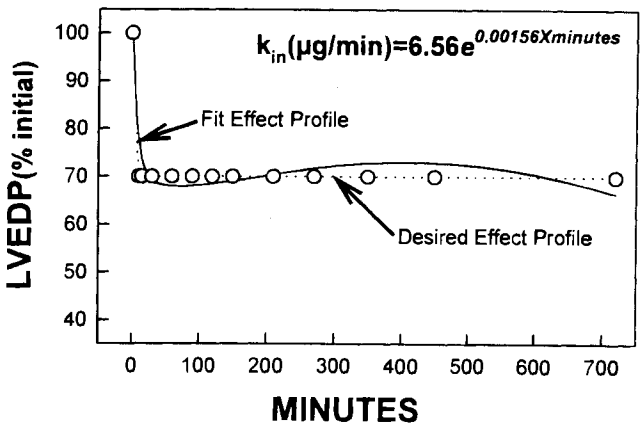

Fig. 2. Computer selection of the optimal NTG infusion regimen. ADAPT II software was directed to select an optimal infusion regimen which would produce an effect profile most closely approximating the desired effect profile (a consistent 30% reduction in LVEDP). The solid line represents the simulated effect profile of the computer selected optimal infusion. Open circles indicate the position of the desired effect data which was used in the fitting process.

RESULTS

Fitting of NTG Tolerance Data

The fitting of the hemodynamic effect data to the PK/PD model is shown in Figure 1. Pharmacodynamic parameters obtained from the ADAPT fit were: k_1, 6.26×10^{-3} min^{-1}; k_2, 6.00×10^{-3} min^{-1}; $k_{el,2}$, 5.98×10^{-3} min^{-1}; and m, 1.31 ml/ng·% (units relate percent reduction in LVEDP to NTG concentration). As shown in the lower panel of Figure 1, the computer-derived response profile closely approximates the actual mean data. As described above, parameters obtained from this data fitting were used as constants in the simulation exercises described below.

Computer Simulation Experiments

Initial simulation experiments were conducted to compare empirically derived input functions in their abilities to provide hemodynamic efficacy. Table 1 shows the predicted effect-areas (using the fitted parameter estimates as constants) during 12 hours of NTG infusion when the dose was delivered through one of the four following functions: constant, linear increasing, hyperbolic and exponential. Despite holding the total NTG dose constant for all simulations (7.2 mg over 12 hours), alteration of the mode of NTG administration dramatically modified the time-course and magnitude of NTG efficacy. Increasing the infusion of nitroglycerin exponentially was predicted to be most effective in this regard, providing a 72% increase in effect area relative to that achieved via a continuous infusion, although the total doses over 12 hours were identical (Table 1).

Computer-Selected Infusion Protocol

We then allowed the computer to "select" an appropriate NTG infusion to produce a constant 30% reduction in LVEDP. The software program determines the optimal infusion function by minimizing the sum of squared residuals between the 'optimal effect-profile' and the computer-simulated effect-profile. As shown in Fig. 2, the computer reached convergence with the infusion regimen of $k_{in} = 6.56 \times e^{0.00156 \times \text{min}}$ μg/min. Fig. 3 shows that such a time-variant infusion rate can be delivered reproducibly by a computer-controlled infusion pump with appropriate instructions (Appendix 1). The Pearson correlation coefficient of the regression line relating actual to theoretical delivery was 0.999. Although the slope of the line (0.91) was statistically different to unity ($p < 0.05$), the 95% confidence interval of the volumes delivered (7.84 ± 0.56 ml) encompassed the targeted volume (8.70 ml).

Predictability of the PK/PD Model

CHF rats were infused using the time-variant input regimen described above to test the predictability of our modeling approach. The results of these infusions are shown in Figure 4 in which the observed hemodynamic data (n = 4, mean ± SE) are plotted. The experimental data, when compared to predictions based on the theoretical model (solid line in Fig.

Table 1. A Theoretical Examination of the Effects of Changing Dose Input Function on the LVEDP Responses of NTG

Infusion	Function k_{in} =	Infusion rate vs. time	Hemodynamic effect area (%LVEDP·time)	Change from constant infusion
Constant	10 μg/min		14.31	–
Linear Increase	$\frac{t}{720} \cdot 20$ μg/min		19.69	+38%
Hyperbolic Increase	$15.11(1 - e^{0.00385\text{min}})$		17.52	+22%
Exponential Increase	$2.27(e^{0.00385\text{min}} - 1)$		24.46	+71%

4), were shown to agree with the predicted response profile, demonstrating that hemodynamic tolerance was overcome throughout the dosing interval.

DISCUSSION

Even after over a century of clinical use, NTG remains an important therapeutic agent and is a mainstay of angina and heart failure therapy. Despite this widespread popularity, nitrate tolerance is a well recognized clinical problem that has not yet been resolved. The current clinical approach to minimize tolerance employs an intermittent NTG regimen, which imposes a 8–12 hour nitrate-free interval. While this administration approach maintains initial NTG effects beginning each day of therapy (8,13,14), it does not address nitrate tolerance which develops within the daily dosage interval. Over a decade ago, we first surmised that non-linear dosing modes during the NTG-on period may improve the clinical efficacy of intermittent therapy (15), but the utility of this approach has not been evaluated. Recent advances in computer-controlled delivery systems have allowed the implementation of complex dosing strategies (16–19), and have therefore allowed us to test the pharmacodynamics of NTG resulting from such non-linear dosing regimens.

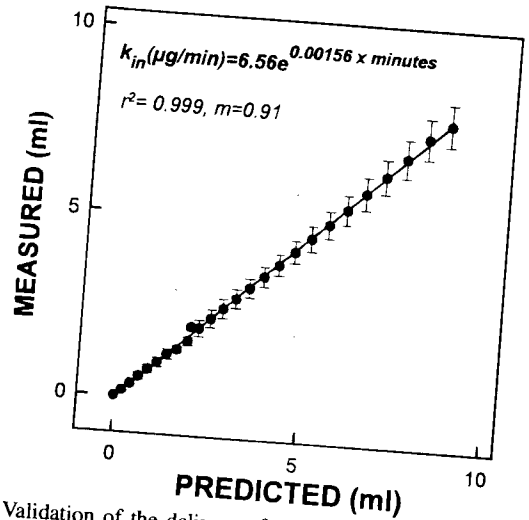

Fig. 3. Validation of the delivery of the computer-selected infusion. Correlation of measured volume delivered to the theoretical delivery volume, with linear regression line ($r^2 = 0.999$, slope = 0.91).

We have used the rat CHF animal model extensively for the study of nitrate tolerance and for devising pharmacologic strategies to avoid or overcome tolerance development (10,11). This animal model of disease is in many ways similar to the human condition, and its response to the pharmacologic effects of NTG is similar to that seen in patients. The usefulness of this animal model to predict NTG pharmacodynamics was further verified recently by the confirmation, in CHF patients (20), of a beneficial drug-drug interaction between NTG and hydralazine that we have observed in CHF rats (10). Thus, this animal model appears to be predictive of at least some aspects of clinical therapy of heart failure, and may serve as a useful tool to optimize nitrate dosing.

The pharmacokinetic/pharmacodynamic model we have employed here assumes that NTG tolerance is primarily due to an indirect vasoconstriction process driven by its direct vasodilating effect. This basic assumption is supported by animal and clinical studies showing systemic activation of endogenous vasoconstrictors during tolerance, as well as plasma volume expansion (21,22). These findings are generally consistent with the structure of our proposed model. A significant body of the nitrate literature indicates that nitrate tolerance may be mediated through vascular rather than, or in addition to, systemic changes. These changes may involve reduced nitric oxide production (23), disulfide formation at the enzyme level (24), or increased vascular production of endothelin (25), or superoxide anion (26) . The pharmacodynamic model described in Fig. 1 can, in principle, accommodate most of these vascular mechanisms since the "mediating factors" and "vasoconstriction" components of the model can well reside in the vasculature.

A salient feature of the pharmacodynamic model we employed is that the tolerance process was viewed as a physiologic response to the initial pharmacologic action during infusion (3). In previous investigations, we found that this model,

which incorporates two sequential rate constants linking NTG vasodilation to developing vasoconstriction, was statistically superior to simpler models employing either a single rate constant or a rate constant and a lag time (3). Thus the model we used here was the simplest form that described our available data adequately. In addition, our PK/PD model was formulated based on available mechanistic understanding of the processes affecting nitrate tolerance, and therefore it appeared reasonable, at least to us, when compared to other models which either evoked the use of an unknown effect-site and/or the presence of unconfirmed antagonistic metabolites (3). In our previous studies, we used solved equations and PCNONLIN to computer fit individual hemodynamic data sets. Here we used the corresponding differential equations and ADAPT II, and fit the mean hemodynamic tolerance profile. The parameter estimates were similar in magnitude using these two approaches when mean data was fit, although the variations around the parameter estimates reported by ADAPT II were large for k_1, k_2, and k_{el2} (over 100%). This result suggests a lack of confidence in the ADAPT—estimated parameter values, but convergence was reliably achieved, and was not dependent upon initial estimates. This computer-estimated variation was related to the necessary high correlation between these sequentially positioned rate constants in this model, and has been commonly observed when using such models. The present model configuration was the simplest form that we could use for an adequate description of our data. Indeed, the utility of our model was further confirmed by this study since it reliably predicted the outcome of an optimized time-variant infusion protocol.

Our results therefore indicated that novel NTG input regimens may be rationally designed through the use of an existing PK/PD model of nitrate tolerance. We targeted a desired effect profile (rather than a certain blood or plasma concentration), and used computer-controlled infusion technology to achieve the pharmacodynamic effect. The computer-optimized infusion regimen was readily achievable and it allowed input modifications on a per-second basis. Any time-dependent infusion regimen can be utilized so long as the appropriate solved equation can be provided. This approach may have general utility for probing other tolerance phenomena and optimizing dosing strategies for other drugs.

The concept of dosage escalation to overcome nitroglycerin tolerance is well accepted clinically. For example, despite the presence of NTG tolerance during continuous transdermal therapy, acute sublingual NTG is still useful in managing angina symptoms (1,2). Our simulations similarly predict that rising input functions would improve the efficacy of a given dose of NTG administered over 12 hours. Further, our PK/PD model predicts that an exponentially increasing input would be superior to linear or hyperbolic increases, and would provide a continuously maintained action during infusion. Mehra *et al.* have recently shown that escalation of isosorbide dinitrate can also overcome early attenuation of nitrate efficacy in patients with heart failure (27), suggesting that time-dependent nitrate input may serve as a useful prospective approach to sustain efficacy, at least in the short term. However, obvious limitations to this approach exist, including the finite limits in dose escalation, the risk of complicating side effects such as headaches, and/or the induction of rebound responses after abrupt drug withdrawal. These factors need to be addressed before this strategy can be employed in a clinical setting.

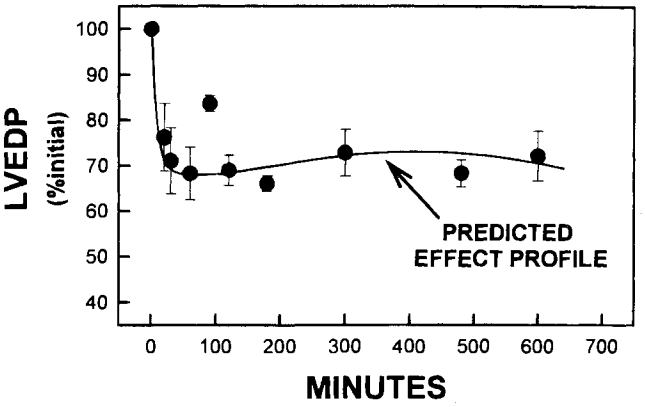

Fig. 4. Hemodynamic effects of exponential NTG infusion in CHF rats. CHF rats (n = 4, mean ± SE) were infused with NTG using the exponential infusion input, k_{in} (μg/min) = $6.56e^{0.00156 \times minutes}$ for 10 hr. The observed hemodynamic data are displayed with the PK/PD model prediction (solid line). The model predictions are consistent with the observed data.

In conclusion, we have used PK/PD modeling techniques to design a suitable dosage regimen to overcome experimental nitrate tolerance, and found that the modeling predictions were in good agreement with experimental observations. These findings support the validity of our proposed PK/PD model of NTG tolerance, and represent an example of pharmacodynamics-derived optimization of therapy. Such an approach may be useful for the study of other agents with time-dependent pharmacodynamics, and for rational improvements of their therapeutic efficacy.

ACKNOWLEDGMENTS

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APPENDIX 1

```
10 REM EXPNTG
11 KEY OFF: CLS
20 OPEN "COM1:1200, N, 8, 2" FOR RANDOM AS #1
22 PRINT #1, "STP": GOSUB 150
26 PRINT #1, "CLT": GOSUB 150
34 INPUT "ENTER RATE RANGE (MLM, MLH, ULM OR ULH):", R$
38 IF R$ = "MLM" OR R$ = "MLH" OR R$ = "ULM" OR R$ = "ULH" THEN 50
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42 IF R$ = "mlm" OR R$ = "mlh" OR R$ = "ulm" OR R$ = "ulh" THEN 50
46 PRINT "INVALID RANGE": GOTO 34
50 INPUT "ENTER INITIAL RATE:", IRATE
70 PRINT #1, USING R$ + "#####.###"; IRATE
74 GOSUB 150: IF RESPONSE$ = "" THEN 80
78 PRINT "OUT OF RANGE": GOTO 50
80 PRINT #1, "RNG": GOSUB 150: RANGE$ = RESPONSE$
82 INPUT "ENTER RAMP DURATION (SECONDS) {note: 10h = 36000s}:", TFINAL
83 PRINT: PRINT "PRESS STOP/START ON PUMP TO INTERRUPT"
84 ON TIMER (1) GOSUB 100
85 T = 0: PRINT #1, "RUN": GOSUB 150
86 TIMER ON
90 IF P$ = ">" THEN 94
92 TIMER OFF: PRINT "INTERRUPTED": PRINT: GOTO 22
94 IF T < TFINAL THEN 90
95 TIMER OFF
97 PRINT "DONE": PRINT
100 REM ********************************************
104 REM timer subroutine ___ executed once a second
106 REM ********************************************
110 T = T + 1
111 IF T > TFINAL THEN GOTO 230
120 RATE = IRATE * EXP (.0000259 * T)
122 PRINT USING " t(m) = ######.## t(hr) = ##.### ####.####" + RANGE$; T/60; T/3600; RATE
130 PRINT #1, USING R$ + "####.####"; RATE
140 GOSUB 150
141 PRINT #1, "KEY": GOSUB 150
142 RETURN
148 REM ************************************************
150 REM get response subroutine
152 REM ************************************************
160 S$ = " ": P$ = ' "' RESPONSE$ = ' "'
170 WHILE P$ <> ":" AND P$ <> ">" AND P$ <> "<" AND P$ <> "*"
180     IF LOC(1) > 0 THEN S$ = S$ + INPUT$(LOC(1), #1)
190     P$ = RIGHT$(S$, 1)
200 WEND
210 IF LEN(S$) > 3 THEN RESPONSE$ = MID$(S$, 3, INSTR(3, S$, CHR$(13)) - 3)
220 RETURN
230 CLOSE
```

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