Modeling drug- and system-related changes in body temperature:
Application to clomethiazole-induced hypothermia, long-lasting tolerance
development, and circadian rhythm in rats.

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Abbreviations: CMZ: clomethiazole; PK/PD: pharmacokinetic-pharmacodynamic; GABA: γ-aminobutyric acid; Pharmacokinetic parameters: $K_a$: absorption rate constant; $F$: bioavailability; $CL_D$: intercompartmental clearance; $V_c$: volume of central compartment; $V_t$: volume of peripheral compartment; $V_{max}$: maximum metabolic rate; $K_m$: Michaelis-Menten constant. Pharmacodynamic parameters: $\alpha$ and $\beta$: first-order rate constants for circadian rhythm; $g(t)$ and $d$: external light conditions; $T_{ref}$: reference temperature; $amp$: amplification factor; $T_{baseline}$: baseline temperature; $T_{vehicle}$: temperature during vehicle treatment; $HD$: influence of handling; $P$: magnitude of the temperature elevation; $k_{HD}$: rate of appearance and disappearance of the temporary temperature elevation; $t_{HD}$: time of handling; $k_{in}$: turnover rate production of heat; $k_{out}$: fractional turnover rate loss of heat; $T$: temperature.
temperature; \( X \): thermostat; \( A \) and \( B \): parameters for a dimensionless system; \( S_{\text{max},1} \): the maximum stimulus; \( SC_{50,1} \): concentration producing half the maximum stimulus on the temperature regulation; \( n \): factor influencing the steepness of stimulus; \( Q_0 \): unknown mediator; \( k_{\text{inQ}} \): turnover rate of production of \( Q_0 \); \( k_{\text{outQ}} \): fractional turnover rate of loss of \( Q_0 \); \( \tau \): transit time of \( Q_0 \) to \( Q_1 \) and from \( Q_1 \) to \( Q_2 \), etc.; \( S_{\text{max},2} \): the drug-induced rate of loss from compartment \( Q_0 \); \( SC_{50,2} \): concentration giving half the maximum stimulus on the tolerance development.

**Section:** Neuropharmacology
ABSTRACT

The aim of the present investigation was to develop a pharmacokinetic-pharmacodynamic (PK/PD) model for the characterization of clomethiazole (CMZ)-induced hypothermia and the rapid development of long-lasting tolerance in rats while taking into account circadian rhythm in baseline and the influence of handling. CMZ-induced hypothermia and tolerance was measured using body temperature telemetry in male Sprague Dawley rats, which were given subcutaneous (sc) bolus injections of 0, 15, 150, 300, and 600 µmol·kg⁻¹ and 24-h sc continuous infusions of 0, 20, and 40 µmol·kg⁻¹·h⁻¹ using osmotic pumps. The duration of tolerance was studied by repeated injections of 300 µmol·kg⁻¹ at 3-32 day intervals. Plasma exposure to CMZ was obtained in satellite groups of catheterized rats. Fitted population concentration-time profiles served as input for the pharmacodynamic analysis. The asymmetric circadian rhythm in baseline body temperature was successfully described by a novel negative feedback model incorporating external light-dark conditions. An empirical function characterized the transient increase in temperature upon handling of the animal. A feedback model for temperature regulation and tolerance development allowed estimation of CMZ potency at 30 ± 1 µM. The delay in onset of tolerance was estimated via a series of 4 transit compartments at 7.6 ± 2 h. The long-lasting tolerance was assumed to be caused by inactivation of a mediator with an estimated turnover time of 46 ± 3 days. This multicomponent turnover model was able to quantify the CMZ-induced hypothermia, circadian rhythm in baseline, and the rapid onset of a long-lasting tolerance to CMZ in rats.
INTRODUCTION

Clomethiazole (CMZ) has sedative, hypnotic, anticonvulsive, and neuroprotective properties and has been used clinically in elderly patients as a useful sedative/hypnotic for 40 years (Green, 1998). In a recent investigation, we showed that CMZ is able to induce hypothermia in rats and that even a single injection of CMZ rapidly induces complete tolerance lasting for more than 10 days, with a 50% return of effect after a month (Visser et al., 2005). This observation of tolerance partly explained why conflicting reports exist regarding the ability of CMZ to induce hypothermia and the relationship between hypothermia and neuroprotective properties in vivo (Cross et al., 1991; Green, 1998; Chaulk et al., 2003). For CMZ, it was observed that the tolerance is induced directly by the drug, rather than via a decrease in body temperature, since hypothermic tolerance to CMZ did not affect hypothermic responses to other drugs and could not be attenuated (Visser et al., 2005).

Moreover, the tolerance appeared to last for more than 15 days, which suggests that CMZ down-regulated a necessary but so far unknown mediator, which by itself has a slow turnover. In the present investigation the objective was to quantify the CMZ-induced hypothermia and the rapid onset of long-lasting tolerance by developing a pharmacokinetic-pharmacodynamic (PK/PD) model.

The regulation of body temperature is a complex homeostatic control mechanism, the purpose of which is to maintain body temperature at a constant level (Simon et al., 1986). It is affected by, among other things, the neurotransmitter systems of serotonin, GABA, glutamate, and dopamine and modulated by inflammatory processes (Salmi and Ahlenius, 1998; Zarrindast and Oveissi, 1988; Corbett et al., 1990; Perachon et al., 2000; Blatteis, 2000; Briese, 1998). The effect of a drug on body temperature is the result of multiple time-dependent processes with their own specific rate constants, such as compound distribution and elimination, temperature baseline behavior, the effects on body temperature regulation, and the development of tolerance. The pharmacokinetics can practically be determined and quantified separately. Information on drug exposure can then be used as the driving force of the pharmacodynamics. For the modeling of body temperature modulation, a turnover model or a model for homeostatic regulation of body temperature has been applied accounting for the time delay between maximum exposure and effect (Ackerman et al., 1964; Gabrielsson and Weiner, 1997; Zuideveld et al., 2001). For the CMZ-induced effects on body temperature and tolerance development,
a systematic approach based on previously described models was adopted to build a pharmacodynamic model composed of various subsystems with their specific rate constants.

It is also commonly known that body temperature is subject to circadian rhythm at around a constant level with a 1°C lower temperature during the day compared to the night in rats (Lobo et al., 1999; Benstaali et al., 2001). So far, models based on trigonometric functions such as the cosine function or Fourier series have been applied for the description of circadian rhythm (Hempel et al., 1998; Chakraborty et al., 1999). However, here we apply a novel feedback model for circadian rhythm, which is described in more detail elsewhere (Sällström et al., 2005). This is a generic model, which is able to describe the (asymmetric) biorhythm of biomarkers.

Tolerance development can be considered as a homeostatic process of the body to reduce the effect of altered conditions, such as receptor down-regulation, adaptation of signal-transduction processes, etc. during drug administration. The best studied is the glucose-insulin system (Ackerman et al., 1964), but also nicotine and nitroglycerine tolerance are studied well (Porchet et al., 1988; Fattinger et al., 1997; Bauer et al., 1997). A number of tolerance models have been proposed and applied depending on the proposed mechanism of action, such as the formation of antagonistic metabolites, tolerance compartment, counter-regulation, feedback, pool and precursor models (Ekblad and Licko, 1984; Holford and Sheiner, 1982; Mandema and Wada, 1995; Wakelkamp et al., 1996; Gardmark et al., 1999; Movin-Osswald and Hammarlund-Udenaes, 1995; Gabrielsson and Weiner, 1997).

In the present investigation, the CMZ-induced hypothermia and the rapid onset of long-lasting tolerance was quantified by developing a PK/PD model consisting of different submodels for circadian rhythm in baseline, handling events, drug effects, and tolerance development.
METHODS

Temperature and exposure measurements

The in vivo experiments have previously been described (Visser et al., 2005). In short: male Sprague-Dawley rats (n = 117, weight (mean ± SD): 301 ± 23 g, B&K Universal AB, Sollentuna, Sweden) were used in pharmacodynamic experiments 7 days after implantation of a telemetric transmitter into the peritoneal cavity (TA10TA-F20, Data Sciences, St Paul, Minn., USA). Satellite groups of male animals were used in separate pharmacokinetic experiments 3 days after implantation of a catheter in the carotid artery and the telemetric transmitter in the peritoneal cavity. The animals were kept in rooms where the artificial light followed a 12-h cycle (lights on at 6 AM). Throughout the experiments the rats had access to food and tap water ad libitum. Ethical permission was obtained from the Stockholm Animal Ethics Committee, Sweden. Body temperature was recorded 4 days prior to dosing until 4–6 days after the end of dosing. The telemetry signals were measured every 2 min for a 10-s period and signaled to a receiver. Using the software package Dataquest ART 2.2 (Data Sciences), the temperature data were processed, analyzed, and visualized. The animals were disturbed as little as possible throughout the experiment by allowing entry to the experimental room only during dosing and care. In the assessment of CMZ exposure, blood samples were taken at predefined intervals, up to 2 mL of blood being withdrawn. The plasma samples were analyzed using HPLC-UV. Precision and accuracy were previously reported (Visser et al., 2005).

Drugs, Dosages, Dosing

Clomethiazole (Clomethiazole edisilate, MW 523.46 g/mol, denoted test compound) was obtained from Compound Management (AstraZeneca R&D Södertälje, Sweden). The concentration of clomethiazole (CMZ) in all solutions was calculated on the basis of the clomethiazole base (MW: 161.7 g/mol). All injection solutions were made in saline (9 mg/mL, Braun Medical AB, Bromma, Sweden) on the day of the experiment. The rats were randomly assigned to dosing groups and received a weight-adjusted subcutaneous (sc) injection dose or saline as vehicle treatment. The dosing design for the characterization of the hypothermia and tolerance development is given in Table 1. A validation data set was obtained in an additional experiment, in which CMZ was administered three
times at an interval of 15 days. Alzet® osmotic pumps (model 2001D, Scanbur BK AB, Sollentuna, Sweden) with an average pump rate of 7.4 µL/h were used for sc drug infusion at a continuous rate. The osmotic pumps were filled with a solution of CMZ in saline. The pumps were preincubated in saline for a minimum of 2.5 h at 37°C before being inserted sc into the neck of the rat under light isoflurane anesthesia.

Data analysis

A PK/PD model was developed to describe body temperature in vehicle- and CMZ-treated rats (see Figure 1). It consists of a pharmacokinetic model describing the exposure profile after drug administration, a circadian rhythm (biorhythm) model describing the asymmetric day/night temperature profile with a correction for stress-induced temperature elevation seen when animals were handled for drug administration or surgery, and a turnover model comprising CMZ-induced hypothermia and tolerance development.

Exposure to CMZ

The time course of the CMZ plasma concentration after iv infusion has previously been described by a two-compartment disposition model with capacity-limited elimination (Gabrielsson and Weiner, 1997). These data were included to allow better estimation of the maximum metabolic rate and the Michaelis-Menten constant following sc dosing. The bolus injection was administered to a sc compartment at time zero, and the decrease in the amount of CMZ at the sc compartment was described by:

\[ \frac{dA_{sc}}{dt} = -K_a \cdot A_{sc} \]  

where \( K_a \) is the absorption rate constant. The amount of CMZ in the sc compartment during the 24-h sc continuous infusion via an osmotic pump was described by:

\[ \frac{dA_{sc}}{dt} = \frac{D_{sc}}{t_{inf}} - K_a \cdot A_{sc} \]  

(1b)
where $D_{sc}$ equals the total amount of CMZ infused under the duration of implantation ($t_{inf}$) of the osmotic pump. After removal of a pump, the amount in the sc compartment equals zero. The CMZ concentrations in the central ($p$) and peripheral ($t$) compartments were described by:

$$\begin{align*}
\frac{dC_p}{dt} &= \left( F \cdot K_a \cdot A_{sc} - CL_D \cdot C_p + CL_D \cdot C_t - CL \right) / V_c, \\
\frac{dC_t}{dt} &= \left( CL_D \cdot C_p - CL_D \cdot C_t \right) / V_t,
\end{align*}$$

(2)

where $F$ is the sc bioavailability, $CL_D$ is the intercompartmental distribution term, and $V_c$ and $V_t$ are the volumes of the central and peripheral compartment, respectively. The capacity-limited clearance ($CL$) is defined as:

$$CL = \frac{V_{max} \cdot C_p}{K_m + C_p},$$

(3)

where the parameters $V_{max}$ and $K_m$ define the maximum metabolic rate and the Michaelis-Menten constant, respectively.

**Baseline behavior**

A turnover model was developed for the description of asymmetric circadian rhythm in baseline temperature in rats (Sällström et al., 2005). The biorhythm model consists of two interconnected compartments $B_1$ and $B_2$. The input and output rates of the compartments are governed by:

$$\begin{align*}
\frac{dB_1}{dt} &= \alpha \cdot (B_1 - B_2) - B_1^3 + g(t), \\
\frac{dB_2}{dt} &= \beta \cdot (B_1 - B_2),
\end{align*}$$

(4)

where $\alpha$ and $\beta$ are first-order rate constants and the timekeeper $g(t)$ describes the external light conditions following a squared wave function:

$$g(t) = \begin{cases} 
0 & 6AM - 6PM \quad (\text{light - on}) \\
r & 6PM - 6AM \quad (\text{light - off})
\end{cases}$$

(5)
where $d$ represents the intensity of a night stimulus because the animals are more active at night. The model can account for a low but slowly increasing temperature during the day and a high and relatively stable temperature during the night. The baseline body temperature was defined as:

$$T_{\text{baseline}} = T_{\text{ref}} \cdot (1 + \text{amp} \cdot B_1),$$  \hspace{1cm} (6)

where $T_{\text{ref}}$ is the reference temperature level and $\text{amp}$ adjusts the amplitude of the circadian rhythm in $B_1$ around the reference temperature. Hence, $T_{\text{baseline}}$ represents the model for circadian rhythm, describing the day/night temperature profile of normal rats in an artificial 12/12-hour light/dark cycle environment.

**Vehicle treatment**

It was observed that handling of the animals caused a temporary temperature increase that was independent of dose level. Therefore, for the individual estimation of the temperature profile upon vehicle treatment (sc injection or continuous pump), the model was corrected by an empirical function $HD$ mimicking this handling effect:

$$HD = k_{HD} \cdot (t - t_{HD}) \cdot P \cdot e^{-k_{HD}(t-t_{HD})} \text{ when } t > t_{HD}$$  \hspace{1cm} (7)

where $P$ determines the magnitude of the temperature elevation, whereas $k_{HD}$ determines the rate of the appearance and disappearance of the temporary temperature elevation and $t_{HD}$ is set to the time of handling. Hence, the temperature profile during vehicle treatment is defined as:

$$T_{\text{vehicle}} = T_{\text{ref}} \cdot HD \cdot (1 + \text{amp} \cdot B_1).$$  \hspace{1cm} (8)

**Drug-induced hypothermia**

The model of temperature regulation applied in this investigation has been described previously and utilizes concepts of turnover principles and feedback regulation (Ackerman et al., 1964; Gabrielsson and Weiner, 1997; Zuideveld et al., 2001; Zuideveld et al., 2004). It is based on a turnover model for body temperature ($T$) combined with a thermostat-like regulation of body temperature ($X$):
The parameters $k_{in}$ and $k_{out}$ are the turnover rate and the fractional turnover rate governing the production and loss of heat, respectively. The parameter $T_{drug}$ is dependent on a function of the drug plasma concentration and is defined by:

$$T_{drug} = T_{ref} \cdot (1 - f(C))$$

where $T_{ref}$ is the reference temperature level in the absence of the drug and baseline variation (see equation (6)). The relationship between the plasma concentration and the stimulus ($S_1$) on the temperature system is defined by:

$$f(C) = S_1 = \frac{S_{max,1} \cdot C_p}{SC_{50,1} + C_p}$$

where $C_p$ is the plasma concentration of CMZ, $SC_{50,1}$ is the concentration producing half the maximum stimulus and $S_{max,1}$ is the maximum stimulus. Because CMZ induced a temperature decrease down to 31°C at the highest dose (600 µmol/kg), it was assumed that CMZ can exert the maximum possible reversible effect on temperature, so $S_{max,1}$ was fixed at 1.

The model for temperature regulation with four system parameters to be estimated can lead to parameter unidentifiability (see for details Zuideveld et al., 2001). In a rescaling strategy, one parameter can be eliminated by making the system dimensionless. The dimensionless system ($x, y$) is obtained by:

$$x = \frac{X}{X_0} \quad \text{and} \quad y = \frac{T}{T_0},$$

where $T_0$ is equal to $T_{ref}$ and $X_0$ is the reference signal of $X$:

$$X_0 = \left(\frac{k_{out} \cdot T_{ref}}{k_{in}}\right)^{\frac{1}{\gamma}}.$$  

The dimensionless system is given by:
\[
\begin{aligned}
\frac{dy}{dt} &= B\left(1 - y \cdot x^{-\gamma}\right) \\
\frac{dx}{dt} &= A \cdot \left(1 - S_1 - y\right),
\end{aligned}
\] (14)

where the new parameters \(A\) and \(B\) are defined by:

\[
A = \frac{a \cdot T_0}{X_0} = a \cdot \left(\frac{k_{in}}{k_{out}}\right)^{\frac{1}{\gamma}} \cdot T_0^{1-\left(\frac{1}{\gamma}\right)}
\] (15)

and

\[
B = \frac{k_{in}}{T_{ref}}.
\] (16)

The temperature model in equation (14) can describe drug-induced hypothermia. It must be noted that equation (14) is only valid in the absence of tolerance development. In the next section, equation (14) will be further elaborated with a model for tolerance development.

**Tolerance model**

It was proposed that CMZ induces long-lasting tolerance by the irreversible removal of an unknown mediator \(Q_0\) (e.g., irreversible degradation of a receptor or second messenger), which has a slow turnover (Visser et al., 2005). Preliminary analysis of the data showed also that the tolerance development did not occur instantaneously but needed time to develop. In order to estimate this delay, it was assumed that \(Q_0\) was cascaded through a number of transit compartments before reducing the effect of CMZ on the temperature (Sun and Jusko, 1998). The proposed full model for tolerance is given by:
The parameters $k_{inQ}$ and $k_{outQ}$ are the turnover rate and the fractional turnover rate governing the production and loss of $Q_0$, respectively. $\tau$ reflects the transit time of $Q_0$ to $Q_1$ and from $Q_1$ to $Q_2$, etc. $S_2$ is a sigmoidal function of the plasma concentration:

$$S_2 = \frac{S_{max,2} \cdot C_p}{SC_{50,2} + C_p}$$

(18)

in which $S_{max,2}$ is the maximum rate of loss from compartment $Q_0$ and $SC_{50,2}$ is the concentration giving half the maximum stimulus. $Q_0$ is normalized to unity (=1) which means that $k_{outQ} = k_{outQ}$. The turnover time of $Q_0$ and the half-life of the irreversible emptying of $Q_0$ are defined by, respectively:

$$t_{1/2} k_{outQ} = \frac{\ln 2}{k_{outQ}}$$

(19)

and

$$t_{1/2} S_{max,2} = \frac{\ln 2}{S_{max,2}}$$

(20)

The complete model for temperature regulation and acute tolerance development after administration of CMZ is defined by:

$$\begin{cases}
\frac{dy}{dt} = B \left(1 - y \cdot x^{-\gamma} \right) \\
\frac{dx}{dt} = A \cdot \left(1 - S_{tol} \right) - y 
\end{cases}$$

(21)

in which $S_{tol}$ is given by:

$$S_{tol} = S_1 \cdot Q_n$$

(22)
In this way, tolerance development occurs with a delay and the total stimulus \( S_{\text{tot}} \) is equal to 0 when CMZ exposure is 0, and therefore tolerance will not affect the body temperature itself but only by means of CMZ.

**Modeling procedure**

A sequential modeling approach was adapted. All fitting procedures were performed using NONMEM V. First, the individual concentration-time profiles were fitted simultaneously to equation (2). Population pharmacokinetic parameter estimates were used to simulate concentrations to the temperature measurements. For the determination of the circadian rhythm in temperature, 6 days of baseline recordings (each 2 min) in vehicle groups (1A and 6A, n=6 per group) were averaged to a 24 h profile and fitted to equation (6). 3 hours of data after the time of handling were removed to avoid interference.

In the subsequent analysis, all temperature data were reduced to one observation per 20 min in order to reduce computational time. Individual temperature profiles in all vehicle groups were analyzed by means of equation (8), allowing a different magnitude of temperature elevation after sc injection and implantation of the pump. The population parameter estimates for the circadian rhythm and handling were used to simulate the vehicle baseline temperature \( T_{\text{vehicle}} \). This profile was used to normalize the temperature during drug treatment to vehicle treatment. The temperature data were rescaled following:

\[
T_{\text{rescaled}} = \frac{T_{\text{obs}} - T_{\text{min}}}{T_{\text{vehicle}} - T_{\text{min}}}.
\]  

The rescaling of the temperature data in order to make the data dimensionless was necessary to enable the use of the reduced parameter temperature model (see Equation (21)). For graphical purposes, the final temperature predictions \( (\text{pred}) \) were recalculated to \( T_{\text{body, pred}} \) via:

\[
T_{\text{body, pred}} = \text{pred} \cdot T_{\text{vehicle}} - \text{pred} \cdot T_{\text{min}} + T_{\text{min}}.
\]  

The interindividual variability of all estimated parameters was modeled by an exponential equation:

\[
P_i = \theta_i \cdot \exp(\eta_i),
\]
where $\theta$ is the population estimate for parameter $P$, $P_i$ is the individual estimate, and $\theta_i$ the random deviation of $P_i$ from $P$. The values of $\eta_i$ are assumed to be independently normally distributed with mean zero and variance $\omega^2$. The covariance structure of the variability parameters was assumed to be diagonal. The residual error in the plasma drug concentration and pharmacodynamics was characterized by a constant coefficient of variation (CCV) error model:

$$y_{mij} = y_{p_{ij}} \cdot \left(1 + e_{ij}\right),$$

where $y_{p_{ij}}$ represents the $j^{th}$ observation for the $i^{th}$ individual predicted by the model, $y_{mij}$ represents the predicted concentration or temperature, and $e_{ij}$ accounts for the residual deviation of the model-predicted value from the observation. The values for $e$ were assumed to be independently normally distributed with mean zero and variance $\sigma^2$. The first-order estimation method (FOCE interaction) was used to estimate the population $\theta$, $\omega^2$, and $\sigma^2$ for the pharmacokinetic analysis. A centering first-order conditional estimation method was used for the pharmacodynamic analysis. Individual parameter estimates were obtained in a Bayesian posthoc step.
RESULTS

Pharmacokinetics

Concentration-time profiles of CMZ displayed typical nonlinear kinetics with increasing doses of the test compound. A two-compartment model with capacity-limited elimination described the exposure profiles best. The population and individual fits are shown in Figure 2. Population parameter estimates are summarized in Table 2. Iv infusion data (Gabrielsson and Weiner, 1997) were used to estimate the Michaelis-Menten constants. The bioavailability was around 100%. The absorption rate from the sc site ($K_a$) decreased with increases in the CMZ dose (table 2). The CMZ concentration in the injection solutions for 15, 150, 300, and 600 µmol/kg sc bolus doses and 20 and 40 µmol/kg/h sc infusion doses were 10, 100, 200, 400, 680, and 1380 µmol/mL, respectively. The pH decreased in this concentration range from 7 to 1 and was not adjusted for reasons of low solubility at higher pH.

Baseline temperature

Six days of temperature observations in groups 1A and 6A were averaged for all individuals (n=6x6 days = 36 days per treatment group). Three hours of observations were removed after each handling event to avoid influence of handling. Baseline profiles were fitted using equation (6). The asymmetric temperature baseline is shown in Figure 3A. During the day a gradual increase in temperature was observed, followed by an abrupt increase in temperature when the lights were turned off. During the night, the body temperature was reasonably stable around 38°C and decreased in 1 to 2 hours to 37°C when the lights were turned on. Population parameter estimates can be found in Table 3.

Vehicle treatment

Two days of temperature recordings in the vehicle treatment groups (1A, 1B, 6A, and 6B) were analyzed simultaneously using equation (8), allowing a different magnitude of temperature elevation after sc injection (n=15) and implantation of the pump (n=9). Injection or implantation of the pump occurred between 9 and 12 AM on the first day. The population parameter estimates are given in Table 3. Two typical profiles are shown for sc vehicle injection and implantation of the pump in
Figure 3B. The temporary elevation of temperature after administration varies between animals and is three times higher after sc injection than after implantation of the pump.

**Drug-induced hypothermia and tolerance**

The population prediction for the vehicle response was used to rescale the temperature data after drug treatment (see equation 23). The exposure-related decrease in body temperature after sc bolus injections of 15, 150, 300, and 600 µmol·kg⁻¹ CMZ or following a 24-h sc continuous infusion of 0, 20, or 40 µmol·h⁻¹·kg⁻¹ CMZ could simultaneously be described by the temperature-tolerance model (equation (21)). The observed and fitted time-effect profiles are shown in Figure 4 and all population pharmacodynamic parameter estimates are given in Table 4.

The differences in profiles after a sc bolus injection or sc continuous infusion were crucial for determination of the number of transit compartments. For the sc bolus injections, a clockwise hysteresis was observed and the return phase shifted to the right with increasing doses, indicating the tolerance development. In contrast, for the sc continuous infusion, a counterclockwise hysteresis was observed (Figure 5). This difference arose due to the pharmacokinetics being the rate-limiting step for the sc continuous infusions, whereas for the sc bolus injections, the time constants in temperature regulation were rate-limiting. A delay for tolerance development was observed and on the basis of the combined analysis of sc bolus and sc continuous infusion, the optimal number of transit compartments could be determined at 4. The delay in tolerance development, defined as 4 transit times, was 7.6 ± 2 h. After a single sc bolus injection of 600 µmol/kg, the theoretical hypothermic effect is reduced by 28% due to tolerance development, whereas after a sc continuous infusion of 40 µmol/kg/h, the maximum possible temperature effect was reduced by 66%. This is graphically illustrated in Figure 6.

The single dose data did not yield information on the slow turnover of $Q_0 (k_{\text{outQ}})$ and were therefore fitted with $k_{\text{outQ}}$ fixed at 0.022 day⁻¹. This corresponded to a turnover time of 32 days. This value was graphically derived from the average minimum effect (mean ± SD) for all individuals measured at 100 min after injection on days 1, 3, 6, 10, 16, 24 and 32. This relationship is shown in gray in Figure 7. Subsequently, parameter $k_{\text{outQ}}$ was estimated in the analysis of the repeated dosing.
data with all other pharmacodynamic parameters fixed at the single dose estimates. The turnover rate constant $k_{outQ}$ was estimated at $0.015 \pm 0.001 \ (10\%) \ \text{day}^{-1}$, yielding a turnover time of $46 \pm 3$ days. The results are shown in Figure 7. The predicted time courses of $Q_0$ to $Q_4$ are shown in Figure 8.

The predictive performance of the model was tested by simulation of the profile of the validation data set (Figure 9). The model successfully predicted the effect on days 1 and 15 but underestimated the effect on day 32.
DISCUSSION

In the present investigation, a pharmacodynamic model using various components was put forward for the quantification of the CMZ-induced hypothermia, vehicle treatment, baseline behavior, and tolerance development.

Exposure to CMZ was described by means of a pharmacokinetic model with nonlinear elimination. The absorption rate constant was dose-dependent, which is most likely explained by the decrease in pH of injection fluids with higher doses, resulting in slower absorption. Adjustment of the pH to physiological values was avoided for reasons of low solubility at physiological pH. The variability observed in the bioavailability may be due to the different occasions of the pharmacokinetic experiments. Nevertheless, the primary goal of the pharmacokinetic model was to predict the population exposure profiles as adequately as possible to function as driver of the pharmacodynamics.

In order to characterize the complex dynamic system with circadian rhythm in baseline, hypothermia, and tolerance development, a systematic approach was adopted to building a pharmacodynamic model. The baseline and vehicle temperature profiles were first analyzed and fixed in the analysis of CMZ-induced temperature decreases. The asymmetric behavior, with a relatively constant temperature during the night followed by a sudden fall of 1°C at the start of the day and a gradually increasing temperature during the day, was successfully captured (Achermann, et al., 1999; Lobo et al., 1999; Benstaali et al., 2001, Sällström, 2005). The advantage of quantifying baseline behavior lies in improving quantification of the pure drug effect for low doses or low efficacy agonists that cannot produce a maximum decrease in temperature. The maximal possible physiological reversible decrease in body temperature is approx. 5°C. A larger temperature decrease might result in a very long recovery time or even death. Thus, a circadian rhythm of 1°C represents 20% of the maximum physiological temperature decrease.

The baseline model, combined with an empirical function of temporary elevation of temperature due to handling events, served as model for vehicle treatment. Transient elevation of temperature during insertion of the osmotic pump was lower than after injections, which is probably due to opposing effects of the short period of isoflurane anesthesia necessary for pump implantation and removal (Prudian et al., 1997). However, the significant influence of handling underlines the need
to control experimental settings. It may therefore be likely that the size of the drug effect depends on the time of drug administration and experimental conditions such as handling (Lemmer, 1999).

For the temperature regulation, both a turn-over and a feedback model were tested. In the model optimization step, it appeared that a basic turnover model fitted the data reasonably well (Daynkea et al., 1993), although the feedback model performed better due to increased flexibility (Zuideveld et al., 2001). The feedback model might also describe the physiological regulation of the temperature in a more mechanistic way by using a feedback loop. This model, however, might also be preferred in studying a low efficacy agonist as oscillatory behavior of the model (and observations) are more pronounced at low stimulation (Zuideveld et al., 2004). On the other hand, for application in routine analysis, the complexity and calculation time for the feedback model might be a disadvantage (Sällstrom et al., 2005).

Parameter estimability of the feedback model was improved by rescaling the data to the maximum observed temperature decrease. Combined with a correction for vehicle treatment and baseline (see equation (23)), this enabled the characterization of the pure drug effect. The drug-induced hypothermia and counteracting tolerance could not be independently measured in a single response profile, but only when multiple doses and infusion rates were analyzed simultaneously. In this investigation, the data obtained by sc continuous infusion were crucial for the characterization of the tolerance, whereas the sc bolus doses were more informative regarding the temperature regulation (as illustrated in Figure 6).

The tolerance was modeled by means of a separate turnover model that counteracted the CMZ-induced hypothermic effect and to describe the long-lasting nature of the tolerance. During exposure to CMZ, the compartment $Q_0$ was irreversibly emptied with a half-life of 8 min (see equation (20)). It was estimated via transit compartments that the tolerance development was delayed by $7.6 \pm 2$ h. Therefore, the tolerance is less apparent (only 22%) after a single sc bolus injection (a fair amount of CMZ has already been eliminated) but more pronounced (66%) during sc continuous administration. Similar observations include nitroglycerine and nifedipine as examples where the separation of the beneficial effect and tolerance development to the same effect is highly dependent on the rate of administration (Kleinbloesem et al., 1987; Bauer et al., 1997; Wang et al., 2004). Also a
similar tolerance pattern (although less long lasting) was observed for the prolactin release after administration of remoxipride (Movin-Osswald and Hammerlund-Udenaes, 1995).

For tolerance modeling, standard feedback or pool models were not appropriate because of the predicted rebound effects upon withdrawal of the drug. For CMZ, only a slight rebound of 0.3°C could be observed during daytime after the highest doses (300 and 600 µmol/kg) after averaging data and comparing to baseline. The question is whether this is physiologically significant. It might be the result of a slight disturbance of the circadian rhythm similar to that observed after anesthesia (Prudian et al., 1997). Many approaches have been proposed to modeling delays between exposure and response, such as a link model for distributional delay (Sheiner et al., 1979), a model for slow on/off binding to a receptor (Shimada et al., 1996), various turnover models for time-dependent transductions (see Mager and Jusko, 2001 for review), and empirical transit compartments (Sun and Jusko, 1998). Here we have applied transit compartments. The delay in onset of tolerance could indicate that a metabolite of CMZ (Green et al., 2000) is responsible for the observed tolerance instead of CMZ. However, this possibility will not alter the proposed turnover model for tolerance but rather account for a part of the delay as formulated by means of the transit compartments.

The turnover time of $Q_0$ was estimated to be 46 ± 3 days. This was longer than graphically derived from the data in Figure 6 as the maximum decrease in temperature does not linearly reflect the return of the effect. This long turnover time suggests that in order to elucidate the mechanism of tolerance, experiments should be designed to find the mediator responsible with a similar turnover rate. It has previously been shown before that GABA_A receptors, 5-HT_1A, and NMDA receptors at least are not involved in tolerance development (Visser et al., 2005). We are not aware of any reports on such an extensive adaptation in a small animal like the rat.

The prediction of the effect after three dose occasions was not completely satisfactory because it underestimated the effect at 32 days, whereas days 1 and 15 were well predicted. Our model assumes that after a second dose of CMZ on day 15, $Q_0$ is irreversibly emptied again and that the effects of the third dose on day 32 will be almost similar to those of the second dose. However, the remarkable observation was that a third dose gave a larger effect than on day 15 with a larger variability. One reason could be that in this group of rats the actual turnover of $Q_0$ was faster and so
the predicted effects would be larger. Another factor, which might influence the observations, is that the actual plasma levels during the final dose were higher than simulated. Doses were adjusted to body weight, which was considerable over 32 days but clearance may have remained relatively constant. However, this discrepancy remains to be investigated in further experiments.

The model design was solely dependent on information derived from temperature data. An approach in which mechanistic information is derived from various parts using multiple biomarkers (e.g., the information on the molecular mechanism of the tolerance and circadian rhythm) might improve future generations of complex dynamic models in terms of mechanistic significance. Furthermore, a future step in model development for temperature regulation could be the integration of the model for circadian rhythm into the set-point model in such a way that $T_{ref}$ is subject to a biorhythm. Another focus could be the independent estimation of $k_{out}$, with keeping the flexibility of the set-point model. Due to rescaling of the temperature model, it was not possible to independently derive an estimate (see equations (9) and (14)). However, based on an analysis using an indirect response model $k_{out}$ was estimated between 0.006 - 0.02 min$^{-1}$ yielding a 30-80 min half life $k_{out}$.

Although the prediction of other dose regimens in routine research would benefit from simpler models with a high predictive value, the development of simpler models should still recognize specific patterns and the overall rate-limiting step in drug response.

For CMZ in particular, it allowed us to estimate the in vivo potency and to predict and understand the absence of hypothermia upon repeated dosing. The modeling of the CMZ-induced hypothermia and tolerance provided an example of modeling of various complex dynamic systems such as baseline temperature, handling effects, tolerance development, delays, and temperature regulation.
REFERENCES


Zuideveld KP, Maas HJ, Treijtel N, Hulshof J, van der Graaf PH, Peletier LA, and Danhof M (2001)
A set-point model with oscillatory behavior predicts the time course of 8-OH-DPAT-induced

Zuideveld KP, van der Graaf PH, Newgreen D, Thurlow R, Petty N, Jordan P, Peletier LA, and
receptor agonists: estimation of *in vivo* affinity and intrinsic efficacy on body temperature in rats.
FOOTNOTES

Preliminary results were presented at the annual meeting of the Population Approach Group Europe, June 2004, Uppsala, Sweden: Sandra A.G. Visser, Björn Sällström, Tomas Forsberg, Lambertus A. Peletier and Johan Gabrielsson: Modeling of drug- and system-related changes in body temperature: application to drug-induced hypothermia, long-lasting tolerance development and circadian rhythm in body temperature.
LEGENDS FOR FIGURES

**Fig. 1.** The proposed pharmacokinetic-pharmacodynamic model consisting of three components. A: CMZ pharmacokinetics with a capacity-limited clearance. B: Circadian rhythm in baseline and handling-induced elevation of temperature (Sällström et al., 2005). C: Temperature regulation according to the set-point model (Zuideveld et al., 2001) including the tolerance model with a slow turnover and a delay in onset via transit compartments. The data were analyzed in a sequential way. First the pharmacokinetics were analyzed and the population exposure profiles were simulated at each time point of the temperature observations (A). Secondly, the fitted population vehicle response including circadian rhythm was used for rescaling the body temperature after CMZ treatment (B). Last, the body temperature and tolerance development after drug administration was modeled (C).

**Fig. 2.** Individual observed (markers) and fitted individual (thin lines) and population (thick lines) CMZ plasma concentrations after sc injection (panel A) of 15 (○), 150 (□), 300 (△), and 600 (◇) µmol/kg and during (panel B) 24-h osmotic pump delivery of 20 (▽) and 40 (○) µmol/kg/h. Osmotic pumps were implanted between 0 and 24 h. Note the different time scale. The steady-state plasma concentrations of CMZ were 8 and 16 µM for the 20 and 40 µmol·h⁻¹·kg⁻¹ infusion rates, respectively.

**Fig. 3.** Left: Averaged (mean ± SE) and fitted profiles for baseline profile. The average profiles were derived by averaging 6 consecutive days of 6 animals (n = 36 days) in each vehicle group (1A and 6A). 3 hours of data were removed after handling events. Right: Two typical temperature profiles after sc injection of vehicle and implantation of osmotic pump loaded with vehicle. Markers represent observations and lines the fitted baseline following equation (7). The white/black bar represents the light/dark cycle and time = 0 represents 06:00 AM. Drug administration for these individuals occurred between 10:10 AM and 10:30 AM, respectively.
Fig. 4. Individual observed (markers) and fitted individual (solid lines) and population (dotted lines) temperature profiles for typical individuals after single sc injection and implantation of osmotic pump. The predicted CMZ concentration is shown as a gray line scaled to the right y-axis. Horizontal gray bar represents implantation of osmotic pump. T = 0 represents 06:00 AM. Drug administration occurred between 09:00 and 12:00 AM.

Fig. 5. Individual observed (markers) and fitted individual (solid lines) and population (dotted lines) concentration-effect relationships for typical individuals (same as in Figure 4) after single sc injection and implantation of osmotic pump. The loop indicates a delayed effect and the arrows indicate the time order of the observations. Note that the injections revealed a clockwise loop, whereas the continuous administration revealed a counterclockwise loop.

Fig. 6. Left: Time course of the CMZ stimulus on temperature with ($S_{tol}$, solid line) and without tolerance development ($S_I$, dotted line). The area under the $S_{tol}$ curve is 66% for the injection and 28% for the pump compared to the $S_I$ curve. Right: Concentration-stimulus relationship for CMZ on temperature with ($S_{tol}$, solid line) and without tolerance development ($S_I$, dotted line). The individuals shown in panel B and C are the individuals shown in Figures 4 and 8 that received a sc injection of 600 µmol/kg or pump of 40 µmol/kg/h.

Fig. 7. Observed (markers) and fitted (solid lines) individual temperature profiles after repeated sc injection of 300 µmol/kg on day 1 with a second injection on days 3 (△), 6 (□), 10 (∗), 16 (∧), 24 (○), or 32 (△). Drug administration occurred around 10:00 AM. All markers represent different individuals. The gray line with large gray circles represents the average minimum effect (mean ± SD) for all individuals measured 100 min after injection. The predicted CMZ concentration is shown as a gray line scaled to the right y-axis.
Fig. 8. Time course of amount in transit compartments $Q_0$–$Q_4$. The inserted graph shows the first day of the time course in detail.

Fig. 9. Prediction of the time course of temperature for the validation data set. Predictions are superimposed on the averaged observations after three occasions of dosing on days 1, 15, and 32. Standard error of measurements are shown in gray.
**TABLES**

**Table 1:** Dosing groups for characterization and validation of the hypothermia and the onset and duration of tolerance to clomethiazole (CMZ). Solutions of 1.5 mL/kg with various concentrations of CMZ were injected sc. The concentrations of CMZ in the osmotic pump were 680 µmol/mL and 1350 µmol/mL, respectively. Groups denoted X-A received only treatment on day 1, while groups X-B received treatment on both day 1 and day 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>Treatment t=0</th>
<th>Route</th>
<th>Day</th>
<th>Treatment</th>
<th>Route</th>
<th>n</th>
</tr>
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<tbody>
<tr>
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<tr>
<td><strong>CMZ-mediated hypothermia and onset of tolerance</strong></td>
<td></td>
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<td></td>
<td></td>
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<td>1A/1B</td>
<td>1</td>
<td>VEH</td>
<td>sc</td>
<td>3</td>
<td>300 µmol/kg</td>
<td>sc</td>
<td>6/9</td>
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<td>2</td>
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<td>15 µmol/kg</td>
<td>sc</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>150 µmol/kg</td>
<td>sc</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
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<td>1</td>
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<td>300 µmol/kg</td>
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<td>sc</td>
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<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
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<td>6/3</td>
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<td>7A/7B</td>
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<td>sc</td>
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<td><strong>Onset and duration of tolerance to CMZ</strong></td>
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</tr>
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<td><strong>Validation data set</strong></td>
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<td>15</td>
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<td>sc</td>
<td>15</td>
<td>300 µmol/kg</td>
<td>Sc</td>
<td>5</td>
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</tbody>
</table>


Table 2: Population pharmacokinetic parameter estimates for capacity-limited elimination. The absorption rate constant $K_a$ was found to be dependent on the dose. CMZ was absorbed more slowly at higher doses (concentration in dose solutions). Intraindividual variability was 20%. SE: standard error of estimate.

<table>
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<th>Parameter</th>
<th>Unit</th>
<th>Estimate ± SE</th>
<th>Interindividual variability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{max}$</td>
<td>µmol·min$^{-1}$·kg$^{-1}$</td>
<td>1.5 ± 0.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>$K_m$</td>
<td>µmol·L$^{-1}$</td>
<td>34 ± 5</td>
<td>21</td>
</tr>
<tr>
<td>$V_c$</td>
<td>L·kg$^{-1}$</td>
<td>1.6 ± 0.2</td>
<td>10</td>
</tr>
<tr>
<td>$V_t$</td>
<td>L·kg$^{-1}$</td>
<td>2.4 ± 0.2</td>
<td>23</td>
</tr>
<tr>
<td>$CL_D$</td>
<td>L·min$^{-1}$·kg$^{-1}$</td>
<td>0.08 ± 0.02</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$K_a$ min$^{-1}$ (42%)</th>
<th>$F$ (%) (9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate ± SE</td>
<td>Estimate ± SE</td>
</tr>
<tr>
<td>15 µmol/kg</td>
<td>2.0 ± 4</td>
</tr>
<tr>
<td>150 µmol/kg</td>
<td>0.14 ± 0.024</td>
</tr>
<tr>
<td>300 µmol/kg</td>
<td>0.050 ± 0.007</td>
</tr>
<tr>
<td>600 µmol/kg</td>
<td>0.028 ± 0.003</td>
</tr>
<tr>
<td>20 µmol/h/kg</td>
<td>0.010 ± 0.001</td>
</tr>
<tr>
<td>40 µmol/h/kg</td>
<td>0.015 ± 0.002</td>
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</table>
**Table 3:** Population parameter estimates for the description of the circadian rhythm in body temperature baseline and the temperature profile after vehicle treatment. Intraindividual variability was <1%. SE: standard error.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Interindividual variability (%)</th>
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<td>α</td>
<td>h⁻¹</td>
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<td>±</td>
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<tr>
<td>β</td>
<td>h⁻¹</td>
<td>0.66</td>
<td>±</td>
<td>0.06</td>
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<tr>
<td>T&lt;sub&gt;reference&lt;/sub&gt;</td>
<td>°C</td>
<td>37.2</td>
<td>±</td>
<td>0.02</td>
<td>&lt;1</td>
</tr>
<tr>
<td>amp</td>
<td>-</td>
<td>0.04</td>
<td>±</td>
<td>0.01</td>
<td>11</td>
</tr>
<tr>
<td>d</td>
<td>-</td>
<td>0.06</td>
<td>±</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>k&lt;sub&gt;HD&lt;/sub&gt;</td>
<td>h⁻¹</td>
<td>1.8</td>
<td>±</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>P (pump)</td>
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<td>0.004</td>
<td>±</td>
<td>0.004</td>
<td>146</td>
</tr>
<tr>
<td>P (injection)</td>
<td>-</td>
<td>0.03</td>
<td>±</td>
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**Table 4:** Population parameter estimates for the estimation drug- and system-related parameters for temperature regulation and tolerance. Intraindividual variability was 6%. SE: standard error.

<table>
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<td>$S_{\text{max},1}$</td>
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<td>$SC_{50,1}$</td>
<td>µM</td>
<td>30</td>
<td>± 1</td>
<td>48</td>
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<tr>
<td>$S_{\text{max},2}$</td>
<td>h</td>
<td>5.2</td>
<td>± 1.7</td>
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</tr>
<tr>
<td>$SC_{50,2}$</td>
<td>µM</td>
<td>69</td>
<td>± 57</td>
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<tr>
<td>$k_{in}$</td>
<td>°C·min⁻¹</td>
<td>2.7</td>
<td>± 0.9</td>
<td>-</td>
</tr>
<tr>
<td>$A$</td>
<td>min⁻¹</td>
<td>0.012</td>
<td>± 0.003</td>
<td>58</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>-</td>
<td>1.1</td>
<td>± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>$k_{\text{outQ}}$</td>
<td>day⁻¹</td>
<td>0.015</td>
<td>± 0.001</td>
<td>10</td>
</tr>
<tr>
<td>$\tau$</td>
<td>h</td>
<td>1.9</td>
<td>± 0.6</td>
<td>31</td>
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</table>

**Derived estimates**

<table>
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<tr>
<th>$t_{1/2}$ $k_{\text{outQ}}$</th>
<th>day</th>
<th>46</th>
<th>± 7</th>
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<tbody>
<tr>
<td><em>Delay onset tol (4·\tau)</em></td>
<td>h</td>
<td>7.6</td>
<td>± 2</td>
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<tr>
<td>$t_{1/2}$ $S_{\text{max},2}$</td>
<td>min</td>
<td>8</td>
<td>± 2</td>
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</tbody>
</table>
FIGURES

Fig. 1.

**A: CMZ pharmacokinetics**

\[
\text{Plasma} \quad C_p \\
\text{Tissue} \quad C_t
\]

\[\text{dose} \xrightarrow{\text{CL}_p} \text{Diurnal variation} \quad B_1 \quad \beta \]

\[\text{Intrinsic oscillator} \quad B_2 \]

\[g(t) \xrightarrow{\ast} \]

\[\text{Kin:} \quad \frac{\text{CL}}{\text{CL}} = \frac{V_{\text{cm}} \cdot C_p}{K_a + C_p}
\]

**B: Diurnal variation and handling**

\[\text{Diurnal variation} \quad B_1 \quad \beta \]

**C: Temperature regulation and tolerance development**

\[\text{Temperature} \quad T \xrightarrow{k_{in}} \text{Thermostat} \quad X\]

\[\text{Thermostat} \quad X \xrightarrow{a} \text{Tref} \]

\[s_{cm} \cdot C_p \]

\[\frac{s_{cm} \cdot C_p}{C_p + C_p} \cdot Q_c
\]
Fig. 2.

A

B

Concentration (µM)

Time (h)

Concentration (µM)

Time (h)
Fig. 3.

Temperature (°C)

0 6 12 18 24

Time (h)

37 38

injection

pump

A

B

Time (h)

0 6 12 18 24 30 36 42 48

injection

pump

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Fig. 4.
Fig. 5.
Fig. 6.
Fig. 7.
Fig. 8.
Fig. 9.