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Modelling of body temperature in rats: circadian rhythm, chemically induced hypothermia and tolerance development

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Title (English)

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Abstract

A mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) model was developed to describe body temperature in untreated and CmpX-treated rats. The model included CmpX exposure levels, normal circadian rhythm, CmpX-induced hypothermia, tolerance development and temperature increases during animal handling. Model fitting and validation was performed with data from two experiments with different routes of administration.

Keywords

pharmacokinetic, pharmacodynamic, PK/PD, circadian rhythm, hypothermia, tolerance

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Modelling of body temperature in rats: circadian rhythm, chemically induced hypothermia and tolerance development

Björn Sällström

Sammanfattning

Innan ett nytt läkemedel kan testas på människor måste man genomföra noggranna prekliniska studier. Förutom att testa hur effektiv den nya substansen är mot en viss åkomma måste man också kontrollera att den inte ger biverkningar såsom ökat blodtryck, störningar av hjärtrytmen eller förändrad kroppstemperatur. Resultaten från dessa studier används som underlag för att besluta om en substans ska testas och utvecklas vidare i kliniska studier.

Syftet med det här projektet har varit att utveckla en matematisk modell för att beskriva hur en substans (CmpX) påverkar kroppstemperaturen hos råttor. Modellen beskriver hur substansen absorberas och bryts ner i kroppen, hur den orsakar en temperatursänkning (hypotermi) och hur kroppen utvecklar tolerans mot substansen så att kroppstemperaturen återgår till normal nivå. En del av modellen beskriver också hur temperaturen förändras med den normala dygnsrytmen hos obehandlade råttor.

Fördelen med att beskriva effekten av ett läkemedel med matematiska modeller är dels att man kan få en bättre förståelse för substansens verkningsmekanismer, dels att man bättre kan förutsäga resultaten av framtida experiment. Simuleringar har visat att den nya modellen har god förmåga att förutsäga vad som händer om man till exempel ger substansen oftare, i en högre dos eller på ett nytt sätt.

Examensarbete 20p i Molekylär bioteknikprogrammet
Uppsala Universitet Februari 2004

Summary

Preclinical *in vivo* experiments are used in drug discovery to investigate the efficacy and toxicity of a substance. Pharmacokinetic/pharmacodynamic (PK/PD) modelling can analyze drug responses in order to predict and optimise the experimental design of future studies. Body temperature can be used as a biomarker for drug safety assessment and can be measured continuously for several consecutive days in freely moving rats.

In the present investigation, a mechanism-based PK/PD model was developed to describe chemical exposure and body temperature in un-treated and CmpX-treated rats. The model accounted for CmpX exposure levels, circadian temperature rhythm, chemically induced temperature decreases (hypothermia), tolerance development and increased body temperature in connection with animal handling. The model described temperature effects during five days continuous administration, including the shape of the asymmetric circadian baseline. The estimated potency of CmpX was 137 nM and the half-life of the induced hypothermia was about 60 min. It was predicted that tolerance to CmpX would persist long after the substance had been eliminated from the body. Simulations with the obtained parameter estimates showed a high ability to predict the temperature response in an experiment with a repeated oral dosing design.

In conclusion, a detailed mechanism-based PK/PD model for body temperature was developed and challenged. The model had good descriptive and predictive properties.

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1 Introduction

Modern drug development is a demanding and time-consuming process, requiring large financial investments. In order to stay competitive, pharmaceutical companies are under constant pressure to reduce time of development and to prevent late termination of candidate drugs. Improved methods are needed to explain and predict both desired and toxic effects of a new compound already at an early stage in the development phase. These methods should acknowledge the fact that drugs act within complex biological systems. This study proposes mathematical modelling of preclinical *in vivo* experiments as a powerful tool for describing and predicting physiological effects before, during and after chemical treatment.

1.1 Preclinical kinetic-dynamic modelling

Pharmacokinetics (PK) describes the processes that a drug undergoes in the body, including absorption, distribution and elimination of the compound. Its aim is, for example, to estimate the concentration(s) of a drug and its metabolites at various body sites during and after drug delivery. Pharmacodynamics (PD) describes the relationship between drug concentration and pharmacological effect(s), which may be directly or indirectly related to the ultimate clinical outcome, *e.g.* decreased mortality or increased well-being. Pharmacokinetics may be defined as "what the body does to the drug" and pharmacodynamics as "what the drug does to the body". The integration of kinetic and dynamic relationships is called pharmacokinetic/pharmacodynamic (PK/PD).

Preclinical PK/PD modelling and simulation have proven useful for rational drug development, *e.g.* by characterising drug- and system-specific properties and predicting optimal dosing regimens. PK/PD modelling can provide estimates of a drug's intrinsic activity (maximum effect) and the

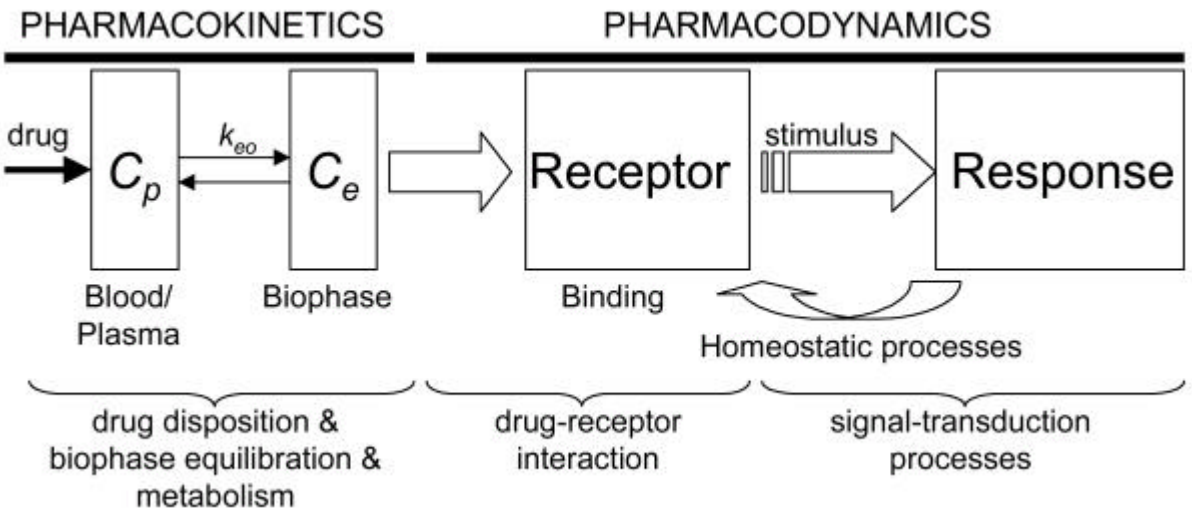

Figure 1. *Schematic illustration of the principal parts in kinetic-dynamic reasoning. Pharmacokinetics characterises the distribution, metabolism and elimination of the drug, e.g. to describe the time-dependent drug concentration(s). Pharmacodynamics describes the cascade from drug-receptor interaction to functional response. Drug-receptor interactions give drug-specific parameters such as potency and efficacy. The signal-transduction cascade is described by system-specific parameters. The illustration was used with permission from Sandra Visser at AstraZeneca R&D Södertälje.*

potency (required exposure for half the maximum effect) based on concentration rather than on the given amount of drug. This is important since the free plasma concentrations required to produce a particular effect are often fairly similar in experimental animals and in humans, although kinetic and metabolic differences between species may require very different dose levels (Levy, 1993).

Mechanism-based PK/PD models seek to capture characteristics of the biochemical events underlying a physiological response. For example, the model can feature a drug-specific part, describing the interaction between a drug and its target receptor, and a system-specific part, describing the downstream transduction processes leading to the induced physiological response (Figure 1). Preclinical *in vivo* studies can help to describe normal and drug-induced effects in the complex biological systems of a complete animal. Many of these systems could be retained between species, providing knowledge of use for the design and the interpretation of future clinical studies. For example, PK/PD models that can account for complex homeostatic control mechanisms (*e.g.* the control of body temperature) may be used to predict potential risks and possibilities, such as rebound effects of drug-withdrawal or desired tolerance of side-effects (Kleinbloesem, 1987).

1.2 Biomarkers

If we are to describe and model the influence that a drug has, we need to define what we mean by a drug effect. A biomarker may be defined as "a characteristic that is objectively measured and evaluated as an indication of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention" (Frank and Hargreaves, 2003). In brief, a biomarker is any measurable effect of a biological process and can range from protein levels in a cell culture to a functional response in patients.

Biomarkers can be divided into *clinical endpoints* and *surrogate endpoints*. A clinical endpoint reflects the ultimate desired effect of a drug, *e.g.* decreased mortality or increased well-being. In preclinical development, clinical endpoints are quite often impractical or impossible to use and must be replaced by surrogate endpoints. Blood pressure and cholesterol levels, for example, are widely accepted as surrogate endpoints for mortality resulting from heart attack and stroke (Frank and Hargreaves, 2003). Although very few surrogate endpoints have yet reached the legal status for drug approval, they may still play an important role during drug development in measuring drug effects and in revealing mechanisms of drug action.

1.3 Circadian rhythms

Stable biological cycles with an approximately 24 h period are found in almost all organisms, reflecting evolutionary adaptation to the surrounding day/night environment. In mammals, the master clock for the circadian rhythm is thought to be located in the suprachiasmatic nuclei of the anterior hypothalamus, and it has been reported that about 2–10% of all mammalian genes are expressed

according to stable circadian oscillations (Reppert and Weaver, 2002). Several disorders and conditions of the central nervous system (CNS), *e.g.* major depression, sleep disorders and jet lag, are linked to genetic or environmental disturbances of the circadian system.

For PK/PD modelling, a correct description of the normal circadian baseline can be important to avoid biased or misleading interpretations of drug effects and tolerance development. Several methods have been used to describe circadian rhythms, most of which are based on trigonometric functions such as a single cosine wave or a combination of sinus and cosine functions, *e.g.* Fourier series (Lemmer, 1997; Chakraborty *et al.*, 1999). However, such methods are purely descriptive and cannot aid our understanding of the underlying mechanisms. So far, there exists no mechanistic PK/PD model that can describe the asymmetric circadian profiles seen for example in heart rate, blood pressure and body temperature in rats and other mammals. For lower organisms such as *Drosophila* and *Neurospora*, detailed mechanistic circadian models based on molecular interactions have been proposed (Leloup and Goldbeter, 2000; Smolen *et al.*, 2001), although for pharmacological purposes, such models are as yet too complex. Instead, a simple generic model developed from a mechanistic approach that can describe asymmetric circadian baselines would be beneficial for PK/PD modelling and could increase our understanding of the circadian clock and how drugs can interfere with it.

1.4 Body temperature regulation

Changes in heart rate and blood pressure can indicate the toxicity of a substance. Increased (hyperthermia) or decreased (hypothermia) body temperature can also indicate unwanted side-effects. Heart rate, blood pressure and body temperature can be routinely used as biomarkers for drug safety assessment in preclinical development. Telemetric systems with implanted transmitters allow continuous measurements in freely moving animals over long periods of time. This is advantageous since no animal handling is needed during the experiment and measurements can be made simultaneously with drug administration (Refinetti and Menaker, 1992).

A subject tries to maintain a stable temperature at a certain reference level, called the set-point temperature. Body temperature can change due to two different mechanisms; either the temperature is forced away from the reference level, or the reference level itself is changed to a new value (Refinetti and Menaker, 1992). Studies in rats (Briese, 1985) and humans (Shoemaker and Refinetti, 1996) suggest that the fluctuations in body temperature caused by the normal circadian rhythm reflects internally forced deviations from the setpoint, rather than changes of the setpoint itself. In contrast, some drugs are thought to change the setpoint. For example, a PK/PD model has previously been applied to describe the hypothermic effects induced by 5-HT_{1A} receptor agonists (Zuideveld *et al.*, 2001).

2 Aims

CmpX is a CNS active substance. The object of the present study was to develop a PK/PD model to describe the body temperature of rats before, during and after CmpX treatment.

The first aim was to identify plasma exposure to CmpX after different routes of administration and dose levels, using a pharmacokinetic model. The second aim was to develop a mechanism-based model for the circadian rhythm of body temperature. The third aim was to develop a pharmacodynamic model for the CmpX-induced lowering of body temperature coupled with tolerance development.

3 Materials and methods

3.1 *In vivo* pharmacological experiments and analytical procedure

All *in vivo* body temperature data and blood samples were collected at the Department of General Pharmacology, AstraZeneca R&D Södertälje, Sweden.

3.1.1 Animals

Male Sprague-Dawley rats (Supplier B&K Universal AB, Sollentuna, Sweden) that had been acclimatised for at least five days were used for the *in vivo* studies. During the experiments, the rats were housed individually in transparent cages with wood shavings as bedding and with free access to food (Lactamin, Stockholm, Sweden) and tap water under the following environmental conditions: temp: 18–22 °C; humidity: 40–60%; ventilation: 15–20 air changes per hour; artificial illumination: 12 h light/12 h dark (lights on at 6 a.m.). Permission was obtained from the Animal Ethics Committee, Stockholm South, Sweden.

3.1.2 Body temperature measurement

Body temperature was continuously monitored for 5–7 days in freely moving rats using a telemetric system (PhysioTel Telemetry system, DSI). A transmitter (Physiotel implant TA10TA-F20 system, Data Sciences, St Paul, Minn., USA) was surgically implanted into the peritoneal cavity of each rat at least three days prior to the start of measurement. The cage was placed on a corresponding receiver plate (PhysioTel™ Receiver models, RLA2000, RLA 1020 and RPC-1, Data Sciences, St Paul, Minn., USA) connected to a computer through a Dataquest Exchange Matrix. Body temperature, heart rate and blood pressure were collected at regular intervals (every 2 – 60 min) and exported to Microsoft Excel (Figure 2).

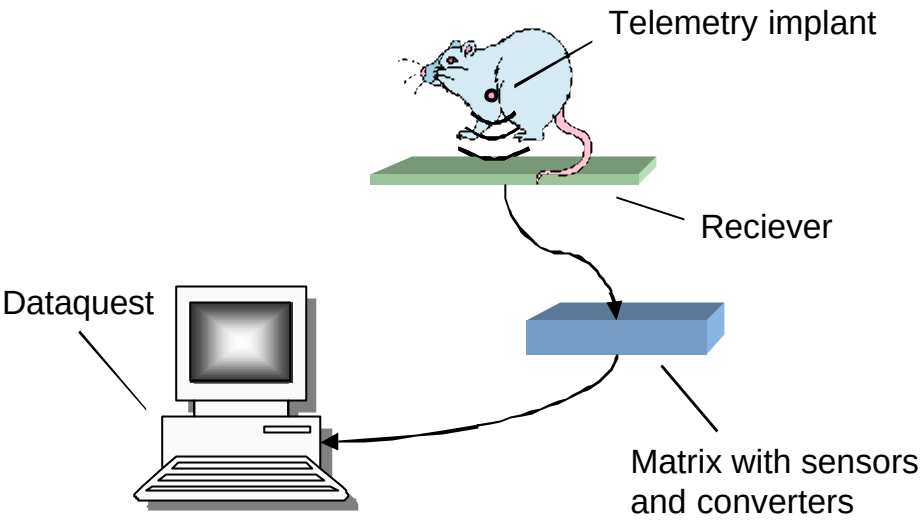

Figure 2. *Continuous measurement of body temperature with an implanted telemetry transmitter, sending information on body temperature to a receiver plate placed under the animal cage. The data was collected to a computer every 2-60 min via a data processing matrix. Animals were not handled during data collection, providing undisturbed recordings.*

3.1.3 Chemicals and dosing

In the temperature measurement experiments, CmpX (AstraZeneca) was given through continuous *s.c.* administration or oral administration (Table 1). Solutions were prepared on the day of use. Physiological saline (9 mg/mL, Braun Medical AB, Bromma, Sweden) was used as vehicle.

Alzet® osmotic minipumps (model 2001d, 8 µL/h over 5 days) were used for continuous *s.c.* CmpX delivery. Three different infusion solutions were used corresponding to 25, 100 and 200 nmol/kg/h CmpX infusion rates. The pumps were preincubated in saline for 3 h at 37 °C prior to being inserted *s.c.* in the back of the rats. At the time of dosing, a small incision was made and the pump was immediately inserted (with the flow modulator pointing away from the incision) in order to give a

Table 1. *Dose, route, regimen, duration and number of rats in the body temperature measurement experiments. Plasma samples were collected in most individuals for estimation of PK parameters.*

Dose	Units	Route	Regimen	Duration	No. of rats
25	nmol·kg^{-1}·h^{-1}	*s.c.*	continuous input	5 days	3
100	nmol·kg^{-1}·h^{-1}	*s.c.*	continuous input	5 days	6
200	nmol·kg^{-1}·h^{-1}	*s.c.*	continuous input	5 days	3
0	-	*p.o.*	*bid*[*]	7 days	3
250	nmol·kg^{-1}	*p.o.*	*bid*[*]	7 days	6
750	nmol·kg^{-1}	*p.o.*	*bid*[*]	7 days	5

[*] *bid denotes twice daily*

constant *s.c.* input of CmpX over five days. After this time the pumps were removed. The animals were briefly anaesthetised with 5% enflurane during the insertion and the removal operations.

For oral administration, two different solutions corresponding to 250 and 750 nmol/kg of CmpX were used. Vehicle treated animals were used as control. Oral doses were given twice daily at 9 a.m. and 3 p.m. for seven days.

3.1.4 Blood sampling and plasma concentration analysis

Plasma samples were taken during the continuous *s.c.* administration experiments. Plasma samples were also taken after an extra oral dose given the day after the end of the 7 days measurement in the oral administration experiments. More information on pharmacokinetics was obtained with additional experiments. Samples were taken after a single 400 nmol/kg oral dose and after a single 100 nmol/kg intravenous bolus dose. The plasma samples were analysed for CmpX at Bioanalytical Chemistry, AstraZeneca R&D Södertälje, Sweden.

3.2 Integrated body temperature model

A mathematical model was developed to describe body temperature regulation in untreated and CmpX-treated rats. The model consists of a pharmacokinetic part describing the exposure profile after administration of CmpX, a circadian rhythm part describing the asymmetric day/night temperature profile in untreated rats, and a concentration driven pharmacodynamic part including chemically induced hypothermia and tolerance effects.

3.2.1 Pharmacokinetics

The relationship between time and CmpX plasma concentration was characterised by means of a standard one-compartment, pharmacokinetic model. The model was designed to simultaneously describe different dose levels of CmpX administered through continuous *s.c.* infusion, oral administration or intravenous injection (Figure 3).

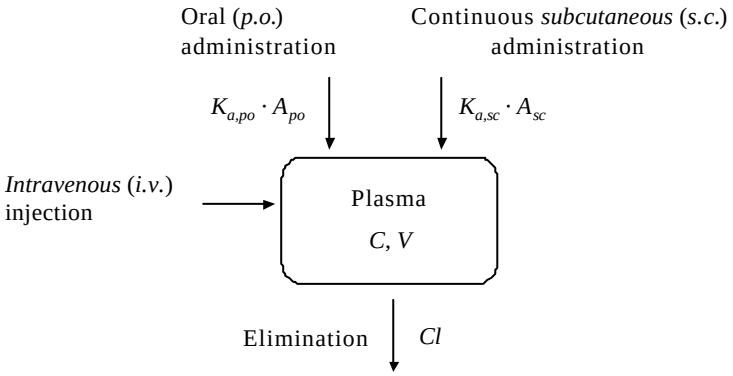

Figure 3. *A one-compartment pharmacokinetic model, describing absorption and elimination of CmpX during and after continuous s.c., p.o. and i.v. administration.*

In the case of continuous *s.c.* administration, the amount (A_{sc}) of CmpX at the absorption site (*subcutaneous* space) was described by

$$\frac{dA_{sc}}{dt} = \frac{F_{sc} \cdot D_{sc}}{t_{\inf}} - K_{a,sc} \cdot A_{sc} \qquad (1)$$

where F_{sc} denotes the *s.c.* bioavailability, D_{sc} denotes the dose, $K_{a,sc}$ denotes the first order absorption rate constant and t_{inf} is the duration of delivery. For oral administration, the amount (A_{po}) of CmpX at the absorption site (oral/intestine space) was described by

$$\frac{dA_{po}}{dt} = F_{po} \cdot D_{po} - K_{a,po} \cdot A_{po} \qquad (2)$$

where F_{po} is the oral bioavailability, D_{po} corresponds to the oral dose and $K_{a,po}$ denotes the first order absorption rate constant.

The concentration (C) of CmpX in the plasma was described by

$$\frac{dC}{dt} = \frac{Inf - Cl \cdot C}{V} \qquad (3)$$

where *Inf* denotes the input rate to the plasma after continuous *s.c.*, oral or *i.v.* dosing. *Cl* denotes clearance and *V* the distribution volume.

3.2.2 Modelling of the circadian rhythm baseline

The circadian rhythm model describes the normal temperature baseline in untreated rats, with a low but slowly increasing temperature during the day and a high temperature during the night. The model consists of two interconnected compartments, one baseline compartment (B_1) and one modulator compartment (B_2) (Figure 4). The input and output rates of the compartments are governed by

$$\begin{cases} \frac{dB_1}{dt} = a \cdot (B_1 - B_2) - B_1^3 + g(t) \\ \frac{dB_2}{dt} = b \cdot (B_1 - B_2) \end{cases} \qquad (4)$$

where a and b are first order rate constants and $g(t)$ describes the external light conditions (see below). The input rates into both compartments are governed by B_1, whereas the loss is governed by B_2. Thus, a negative feedback loop is formed by the modulator compartment, an increase in B_1 stimulating an increase in B_2, which in turn stimulates a decrease in B_1. In addition, the model features complex self-regulation of B_1 described by the term ($-B_1^3$). If $g(t) = 0$, the right-hand sides of the equations (4) are independent of time, thus forming an autonomous system of differential equations. If $a > b$, this system will oscillate around zero.

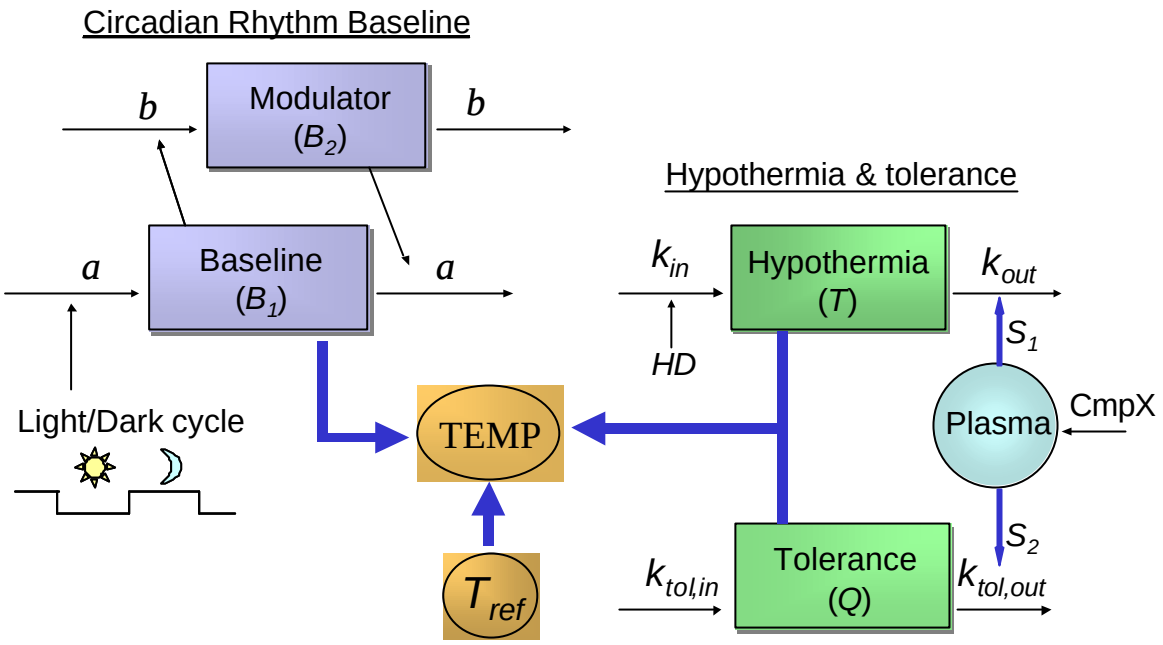

Figure 4. *The PK/PD model takes into account CmpX exposure levels, circadian temperature rhythm, CmpX-induced temperature decrease (hypothermia), temperature increase on animal handling and tolerance development to CmpX. The timing and the period of the circadian rhythm baseline are determined by the external light/dark cycle. S_1 and S_2 represent the stimuli induced by exposure to CmpX. TEMP is the actual body temperature and T_{ref} is the reference level. HD represents the animal handling correction.*

The influence of light as an external Zeitgeber was modeled in the function *g(t)*. The sudden onset and offset of light in the animal room was described by a simple squared wave function

$$light = \begin{cases} 1 & 6AM - 6PM \quad (Day) \\ 0 & 6PM - 6AM \quad (Night) \end{cases} \qquad (5)$$

Since rats are more active at night than during the day, *g(t)* was modelled as a night stimulus with the intensity parameter denoted *dark*

$$g(t) = dark \cdot (1 - light) \qquad (6a)$$

or, by combining equation (5) and (6a),

$$g(t) = \begin{cases} 0 & 6AM - 6PM \quad (Day) \\ dark & 6PM - 6AM \quad (Night) \end{cases} \qquad (6b)$$

By introducing the definition of *g(t)* into the system (Equation 4), B_1 will represent the circadian profile. To reflect body temperature, B_1 simply needs to be amplified to the level and amplitude of the observed profile (approximately 37.5 ± 0.5 °C). The baseline temperature is therefore defined as

$$T_{baseline} = T_{ref} \cdot (1 + amp \cdot B_1) \qquad (7)$$

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

3.2.3 Modelling of hypothermia

A sigmoid S_{max} model was used to describe the CmpX-induced stimulus (S_1)

$$S_1 = \frac{S_{1,\max} \cdot C^n}{SC_{50}^n + C^n} \quad (8)$$

where C is the plasma concentration of CmpX (Equation 3). $S_{1,max}$ is the relative maximum stimulus and SC_{50} is the concentration giving half the maximum stimulus. The n parameter represents the sigmoidicity factor influencing the steepness of response around SC_{50}.

For the hypothermic effect, a single turnover model was chosen (Figure 4), with S_1 stimulating the outflow according to

$$\frac{dT^*}{dt} = k_{in} - k_{out} \cdot (1 + S_1) \cdot T^* \quad (9)$$

where k_{in} and k_{out} are zero- and first-order rate constants, respectively, and T^* represents the hypothermic effect compartment before correction for animal handling (see below). The steady-state level ($dT^*/dt = 0$) at constant drug stimulus is given by

$$T_{ss}^* = \frac{k_{in}}{k_{out} \cdot (1 + S_1)} \quad (10)$$

For the special case where no CmpX is present ($S_1 = 0$, $T^* = T^*_0$), it is assumed that T^* equals the same reference level T_{ref} as was used in the circadian rhythm model described above (Equation 7), and the following relationship is obtained;

$$T_0^* = \frac{k_{in}}{k_{out}} = T_{ref} \quad (11)$$

which can be rearranged to give

$$k_{in} = k_{out} \cdot T_{ref} \quad (12)$$

3.2.4 Animal handling correction

It was observed that handling of the animals during dose delivery caused a temporary temperature increase that was independent of dose level and occurred also after vehicle treatment. The primary effect model was therefore corrected by a function HD describing this handling effect

$$HD = P \cdot e^{-k_{HD} \cdot (t - t_{HD})} \quad (13)$$

where t_{HD} is set to the time-point when the animal was handled for dose administration. At the time of dosing, $t = t_{HD}$ and $HD = P$. Therefore, P determines the magnitude of the temperature elevation,

whereas k_{HD} determines how fast the effect disappears. Long after handling, *i.e.* when $t >> t_{HD}$, *HD* approaches 1. This is also the case at the start of each experiment before dose administration. Thus,

$$HD_{ss} = HD_0 = 0 \tag{14}$$

The handling function was incorporated as a stimulation of the input rate k_{in}, forming the corrected model for hypothermia

$$\frac{dT}{dt} = k_{in} \cdot (1 + HD) - k_{out} \cdot (1 + S_1) \cdot T \tag{15}$$

Since $HD_{ss} = HD_0 = 0$ (Equation 14), the initial and steady-state levels defined in equations (10) – (12) still hold true for the corrected model:

$$T_{ss} = \frac{k_{in}}{k_{out} \cdot (1 + S_1)} \tag{16}$$

$$T_0 = \frac{k_{in}}{k_{out}} = T_{ref} \tag{17}$$

$$k_{in} = k_{out} \cdot T_{ref} \tag{18}$$

3.2.5 Modelling of tolerance development

Tolerance (*Q*) was modelled as desensitisation to the induced hypothermia. The tolerance development depends on the CmpX plasma concentration, but not on the actual temperature decrease (Figure 4). The induced stimulus was defined as

$$S_2 = \frac{S_{2,\max} \cdot C}{SC_{50} + C} \tag{19}$$

having similar potency SC_{50} but a separate maximum level $S_{2,max}$ compared to the stimulus driving the hypothermia (Equation 8). S_2 stimulates the outflow from a turnover model

$$\frac{dQ}{dt} = k_{tol,in} - k_{tol,out} \cdot (1 + S_2) \cdot Q \tag{20}$$

where $k_{tol,in}$ and $k_{tol,out}$ are zero- and first-order rate constants, respectively. It was assumed *a priori* that no tolerance is reflected by $Q = 1$, giving the initial and steady-state conditions

$$Q_0 = \frac{k_{tol,in}}{k_{tol,out}} = 1 \tag{21}$$

$$Q_{ss} = \frac{1}{1 + S_2} \tag{22}$$

If the maximum level is high ($S_{2,max} >> 1$), Q_{ss} will approach zero when the CmpX plasma concentration is large ($C >> SC_{50}$).

3.2.6 Full temperature model

The full temperature model with all its integrated parts is given by

$$TEMP = T_{baseline} + \left(T - T_{ref}\right) \cdot Q \qquad (23)$$

where $TEMP$ is the predicted body temperature, and $T_{baseline}$, T and Q are given by equations (7), (15) and (20). Before dosing, the concentration of CmpX is zero and $T - T_{ref} = 0$ (Equation 17). On prolonged administration, the decrease in Q towards zero will mask the hypothermia and $TEMP$ will return towards the baseline. Initial and steady-state temperatures are therefore only determined by the baseline circadian rhythm,

$$TEMP_0 = T_{baseline} \qquad (24)$$

$$TEMP_{ss} \approx TEMP_0 \qquad (25)$$

The full temperature model takes into account normal circadian temperature rhythm, a CmpX-induced temperature decrease, a temperature increase during animal handling, and complete tolerance development to CmpX (Figure 4).

3.3 Parameter estimates, simulations and data management

The pharmacokinetic and temperature model parameters were fitted to observed data in the population mixed-effect statistical program NONMEM (NONMEM project group, University of California, San Francisco, USA) Based on the obtained individual (where available) or population kinetic parameters, NONMEM was used to simulate the CmpX plasma concentration for each animal at every time-point where a temperature had been recorded. The simulated concentrations were used as inputs for S_1 and S_2 (Equations 8 and 19) when fitting the parameters in the pharmacodynamic temperature model to observed temperature data.

Simulations of model behavior including sensitivity analyses were performed in the simulation program Berkeley Madonna (University of California, Berkeley, USA).

Sudden, non-physiological values (*e.g.* zeroes) in the measured data were assumed to be due to transmission errors and were removed. In animals treated with continuous drug administration, blood samples for drug plasma concentration analyses were taken during the experiments. The artificially large temperature increases seen at these times did not coincide with the induced responses. Therefore, data-points from 0 up to approximately 3 h after each blood-sampling event were removed in these animals.

For the circadian baseline analysis, temperature data was collected for 9 days in 6 rats during continuous *s.c.* vehicle administration. A prototype 24-hour temperature profile was constructed by averaging the observed temperatures from all the days. The circadian rhythm model was fitted to the 24-hour profile using WinNonlin Professional (Pharsight Corporation, USA).

Initial estimates of the parameters a, b, $dark$ and SC_{50},were obtained by trial simulations. Initial estimates of the parameters T_{ref}, amp, k_{out}, P, $S_{2,max}$, and $k_{tol,out}$ were obtained by graphic methods.

4 Results

4.1 Exposure to CmpX

A one-compartment pharmacokinetic model was able to describe the CmpX plasma concentration data. Observed and fitted time-concentration profiles for five days' continuous *s.c.* and single oral administration are shown in Figure 5. The other dose groups were not used as input for the response models and are not shown. The population parameter estimates are presented in Table 2. The half-life of CmpX in plasma was about 60 min.

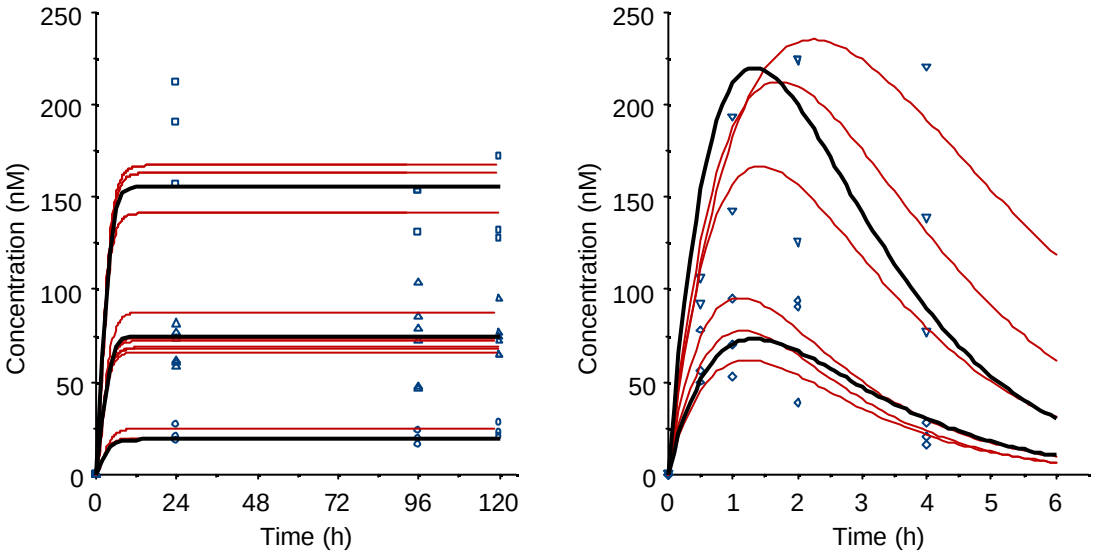

Figure 5. *Linear plasma concentration-time plots for CmpX after continuous s.c. infusions for 5 days (left) at 25 (circles), 100 (triangles) and 200 (squares) nmol·kg^{-1}·h^{-1} and single oral dose (right) at 250 (diamonds) and 750 (triangles) nmol·kg^{-1}. Individual observed (markers) and fitted individual (thin lines) and population (thick lines).*

Table 2. *Parameters, population estimates, precision, inter-individual variability and routes for the one-compartment pharmacokinetic model fitted to CmpX plasma concentration data.*

Parameter	Units	Estimate	SE %	Inter-individual variability (CV%)	Route
Cl	L·kg^{-1}·min^{-1}	0.013	11	20	*s.c., p.o., i.v.*
V	L·kg^{-1}	1.2	3	- [b]	*s.c., p.o., i.v.*
F_{sc}	-	0.7	12	- [b]	*s.c.*
$K_{a,sc}$	min^{-1}	0.012	67	- [b]	*s.c.*
$F_{1,po}$	-	0.58	11	- [b]	*p.o.*
$F_{2,po}$	-	1[a]	-	- [b]	*p.o.*
$K_{a,po}$	min^{-1}	0.014	12	39	*p.o.*

Cl = clearance, V = volume, F_{sc} = s.c. bioavailability, $K_{a,sc}$ = s.c. absorption rate constant, $F_{1,po}$ = p.o. bioavailability (400 nmol/kg dose experiment), $F_{2,po}$ = p.o. bioavailability (250 and 750 nmol/kg doses experiment), $K_{a,po}$ = p.o. absorption rate constant. SE = standard error, CV = coefficient of variation. [a)] Fixed at 1. [b)] Fixed at zero.

The bioavailability could be determined for several dosing groups in comparison to the i.v. profile. The bioavailability in the experiment with 250 and 750 nmol/kg oral doses ($F_{2,po}$) was estimated to be above 1 in previous runs, which has no physical meaning, and it was therefore fixed at 1. The difference in bioavailability between the oral administration experiments ($F_{1,\mathrm{po}}$ and $F_{2,po}$) reflected the fact that the observed concentration levels were not dose-proportional between experiments.

4.2 Modelling of the circadian rhythm baseline

The circadian rhythm model (Equation 7) was fitted to data representing a prototype day (see Materials and Methods). The model adequately described the asymmetric day/night temperature profile, with a low (~37.2 °C) but slowly increasing temperature during the day, an abrupt increase when the light is turned off, a relatively constant temperature (~37.8 °C) during the night and a rapid fall when light is turned on (Figure 6). Rapid fluctuations during both day and night appeared to occur randomly in individuals and were not modelled as a part of the circadian clock.

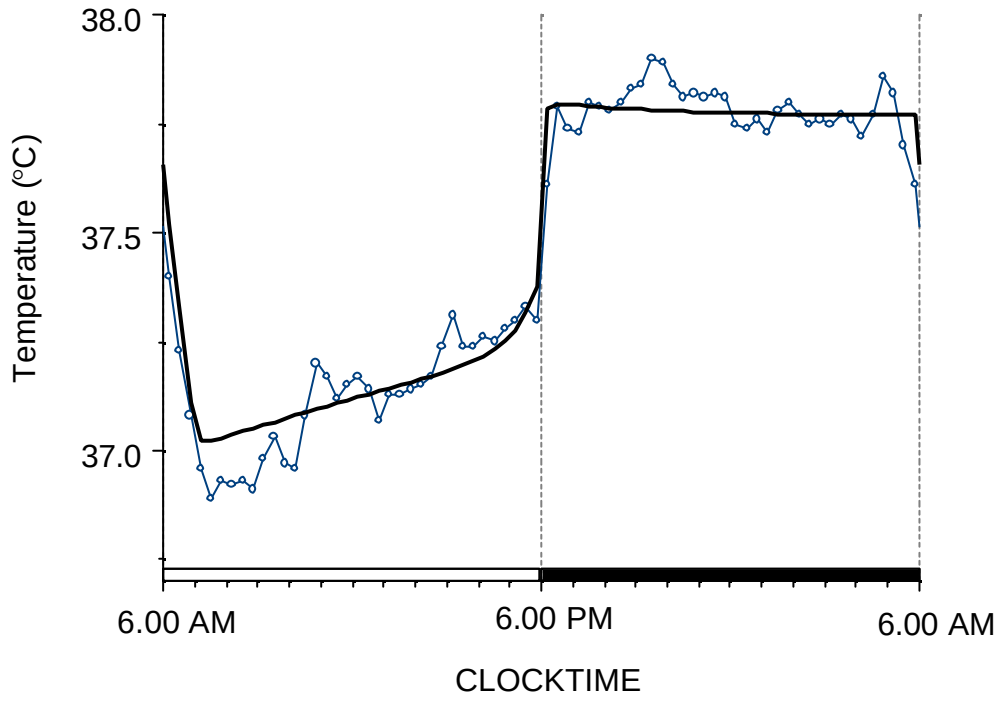

Figure 6. C*ircadian rhythm of body temperature in untreated rats with a 12/12-hour light/dark cycle. The observed 24 h temperature profile (circles) was constructed by averaging the observed values at corresponding time-points from a large number of days. The fitted circadian rhythm model (line) described the shape, timing and amplitude of the baseline. The white/black bar represents the light/dark cycle. For parameter values, see Table 3.*

The estimated parameter values obtained by fitting the circadian rhythm model to the 24-h body temperature profile are shown in Table 3.

Table 3. *Parameters, estimates and precision for the temperature circadian rhythm model fitted to the body temperature profile of untreated rats.*

Parameter	Units	Estimate	SE %
a	min^{-1}	0.026	5
b	min^{-1}	0.0037	3
dark	-	0.053	2
T_{ref}	°C	37.3	0.02
amp	-	0.033	2

Simulation of the model behaviour using the estimated parameters (Table 3) revealed stable oscillations also in the absence of an external Zeitgeber, *i.e.* when *g(t)* was set to zero. The profile of the system was symmetric and had lower amplitude, shorter period and less pronounced temperature shifts compared to what was seen when the external Zeitgeber was present (Figure 7).

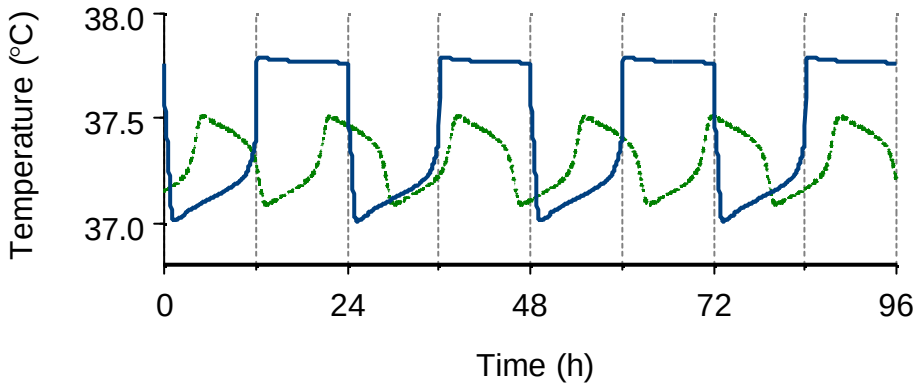

Figure 7. *Simulation of the circadian rhythm model in the absence of an external light/dark cycle (dotted line) showed stable oscillations with shorter period and lower amplitude compared to when the light/dark cycle was present (solid line).*

4.3 Modelling of hypothermia and tolerance development

4.3.1 Fitting: 5 days' continuous *s.c.* administration

The full temperature model was fitted to body temperature observations from 12 rats that had undergone 5 days' continuous *s.c.* administration corresponding to either 25, 100 or 200 nmol/kg/h. During the fit, the circadian rhythm parameters were fixed at the values obtained earlier (Table 3), except for the reference temperature T_{ref}.

The full temperature model managed to describe CmpX-induced hypothermia and full tolerance development, as well as temperature elevations caused by animal handling (Figure 8 and 9).

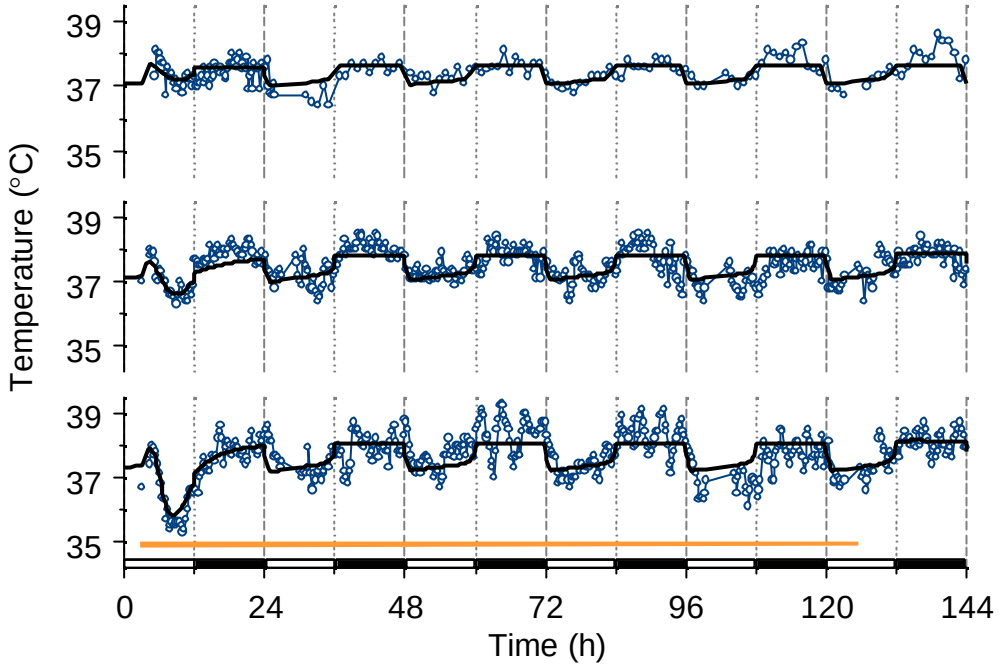

Figure 8. *Observations and fits for 3 representative individuals at doses 25 (upper panel), 100 (middle panel) and 200 (lower panel) nmol/kg/h for 5 days. The yellow bar represents implementation of the osmotic minipump. Note the absence of rebound effects at pump removal. The white/black bar represents the light/dark cycle.*

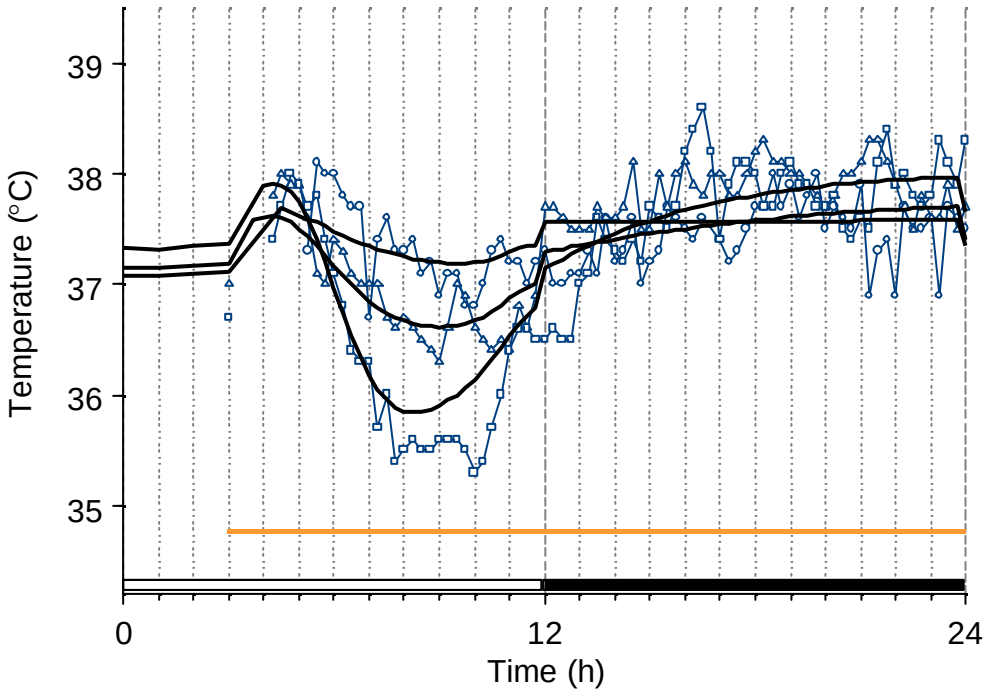

Figure 9. *Comparison between dose levels for 3 representative individuals at doses 25 (circles), 100 (triangles) and 200 (squares) nmol/kg/h showed dose-dependent hypothermia and complete tolerance development within 24 hours. The yellow bar represents implementation of the osmotic minipump. The white/black bar represents the light/dark cycle.*

The population parameter estimates for the full temperature model fitted to 5 days' continuous *s.c.* administration are shown in Table 4.

Table 4. *Parameters, estimates and precision for the full temperature model fitted to temperature data for 5 days continuous s.c. administration. Except for T_{ref}, the circadian rhythm parameters were fixed at profile values (Table 3).*

Parameter	Units	Estimate	SE %
$S_{1,max}$	-	0.2*	-
SC_{50}	nM	137	11
n	-	2*	-
k_{out}	min^{-1}	0.026	31
$S_{2,max}$	-	74	81
$k_{tol,out}$	min^{-1}	$9.5 \cdot 10^{-5}$	74
P	-	0.017	31
k_{HD}	min^{-1}	0.01*	-
T_{ref}	ºC	37.8	0.1

**) Fixed*

Since tolerance development prevented direct observation of the maximum CmpX-induced temperature decrease, $S_{1,max}$ was fixed to give a maximum possible effect corresponding to a body temperature of around 30 °C. The potency SC_{50} of CmpX was estimated to 137 nM and the response half-life was about 30 min. Trial runs showed improved fits if the steepness of the primary drug stimulus S_1 was somewhat increased, and a fixed n was chosen based on simulations. Visual inspection of vehicle-treated animals showed that the temperature elevation after animal handling had a half-life of ~70 min, and a corresponding fixed value of k_{HD} was used. The estimated $S_{2,max}$ corresponds to a maximum tolerance development of over 98% (Equation 22). The half-life of the induced tolerance was about 5 days, predicting that tolerance effects can persist long after CmpX has been eliminated from the body.

4.3.2 Predictions: Twice daily oral dosing for 7 days

A prediction of the temperature response during twice daily oral dosing for 7 days was performed with the parameter estimates (Table 4) obtained from the analysis reported in 4.3.1. A comparison with measured observations showed that both maximum response profiles and sustained tolerance after CmpX elimination were well predicted by the model (Figure 10). The main difference appeared to be an earlier onset (~30 min) of the induced temperature decrease in the predictions compared to the observations.

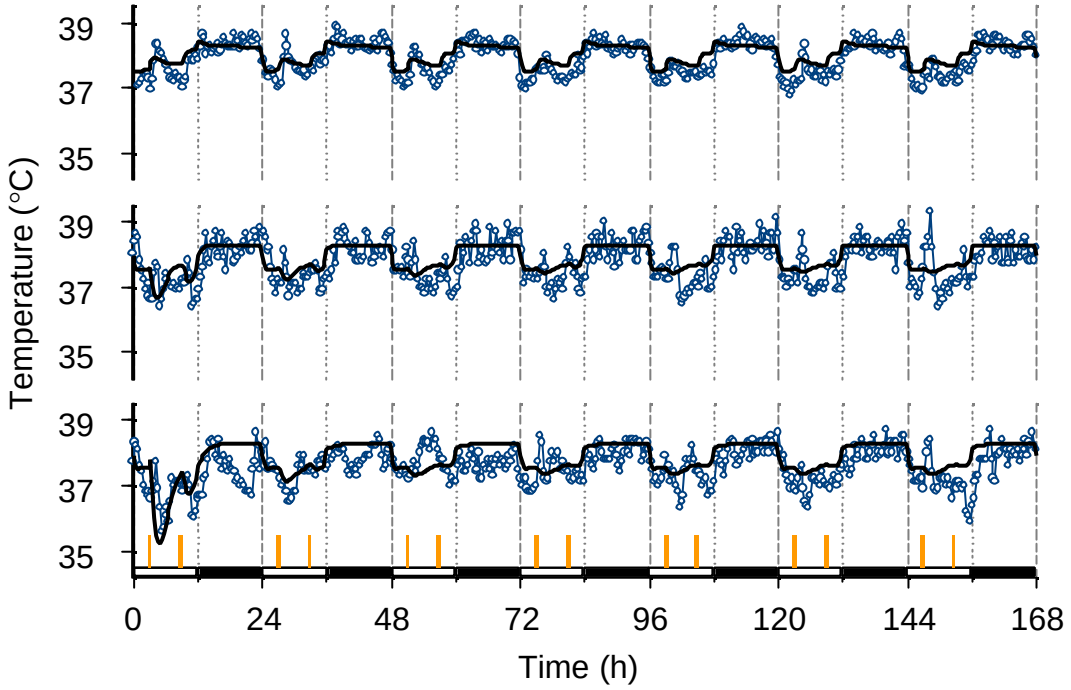

Figure 10. *Comparison between observed (circles) and predicted (lines) temperature response during twice daily oral dosing for 7 days, using the estimated parameter values from Table 4. Doses are 0 (upper panel), 250 (middle panel) and 750 (lower panel) nmol/kg. The yellow vertical lines represent dosing times. The white/black bar represents the light/dark cycle.*

5 Discussion

This study presents a complex model for temperature regulation in untreated and CmpX-treated rats, based on *in vivo* pharmacological experiments. The model predicts the concentration time-courses, the temperature baseline, CmpX-induced hypothermia, tolerance development and temporary temperature elevations caused by animal handling.

5.1 Modelling of the circadian rhythm baseline

A novel two-compartment system was developed for the temperature baseline behaviour. The system can describe the basic features of the body temperature profiles seen in untreated and vehicle-treated rats, including asymmetric day/night profiles and adaptation to external light. The system fulfils a number of basic conditions set up for circadian rhythm models (Moore-Ede *et al.*, 1982):

1) Stable oscillations in the absence of a light/dark function
2) Adaptation of the oscillation timing (phase) and period when a light/dark function is introduced
3) Preservation of timing (phase), but not period, when the light/dark is removed

Moreover, initial analysis of heart rate and blood pressure in the conducted experiments has revealed similar circadian profiles to those for body temperature, making the proposed model generic for several physiological biomarkers.

The circadian rhythm model predicts qualitatively different behaviour under constant light (LL) *versus* constant dark (DD) conditions. Indeed, LL and DD conditions have been reported to cause different temperature responses in rats (Deprés-Brummer *et al.*, 1995). These reported experiments in rats show stable oscillations under DD conditions and suppressed oscillations under LL conditions. The proposed model predicts stable oscillations under LL conditions and no oscillations under DD conditions. Reversing this behaviour will be a challenge for the next generation of the circadian rhythm model.

No mechanism-based PK/PD model has previously been reported for circadian rhythm baselines in rats, although more descriptive models have been proposed and applied (Chakraborty *et al.*, 1999). For lower organisms such as *Drosophila* and *Neurospora*, the molecular basis for the circadian clock is better understood than for mammals, and mathematical models have been developed that can quantitatively describe the underlying molecular systems, allowing detailed predictions and analyses (Leloup and Goldbeter, 2000; Smolen *et al.*, 2001). These models feature negative feedback loops, which are not too different from what is proposed in this study. Moreover, homologues of many of the genes found in the circadian clock in lower organisms have been found in mammals (Young and Kay, 2001), and preliminary molecular descriptions of the mammalian circadian system have been proposed (Shearman *et al.*, 2000; Reppert and Weaver, 2002). Modelling experiences from lower organisms and extended molecular knowledge can aid the development of improved circadian PK/PD models.

The proposed circadian baseline model describes oscillations around a fixed reference temperature T_{ref}, which is in agreement with the conclusions of Briese (1985) and Shoemaker and Refinetti (1996) that circadian temperature changes are not due to a change of the set-point level. However, Refinetti and Menaker (1992) have reviewed studies supporting these findings as well as studies that have come to the exact opposite conclusion. Further experiments may be needed to clearly resolve this question, although the successful application of the proposed circadian model supports the former view.

5.2 Hypothermia and tolerance development

Hypothermia induced by CmpX was described with a standard turnover model where the decrease is driven by the stimulation of outflow. The strength of the stimulation depends on the predicted drug concentration in the central compartment of the pharmacokinetic model. If no tolerance is present, the model predicts a maximum temperature decrease of about –7.5 °C, corresponding to a body temperature of around 30 °C.

Complete tolerance development was observed during prolonged CmpX administration. Despite this, no temperature rebound effect was seen on withdrawal of the substance. Previous models such as the feedback model and the pool model (Ekblad and Licko, 1984) predict withdrawal responses of the same magnitude as the tolerance development and could not be used. Instead, tolerance was described as desensitisation to the hypothermic effect, thereby avoiding any rebound. Moreover, tolerance was assumed to depend directly on the exposure to CmpX, rather than on the induced temperature decrease. As a consequence, it is predicted that tolerance development could occur even if the actual temperature decrease were blocked, *e.g.* by another chemical.

Relevant descriptions of tolerance mechanisms have important implications for drug development. Several PK/PD models have been proposed for different compounds, *e.g.* glucose (Ackerman *et al.*, 1964), histamine (Ekblad & Licko, 1984) and nitroglycerin (Bauer and Fung, 1994). Few of these models have been challenged, however, with features such as complete tolerance development or data from experiments ranging over several days. The present study clearly shows that several days of continuous as well as repeated dosing may be necessary in order to obtain steady-state drug effect levels and to capture possible tolerance effects after drug washout. Measurements before and after drug treatment are also necessary for good predictions. In this study, one reason for the low precision of the tolerance parameter estimations ($S_{2,max}$ and $k_{tol,out}$, Table 4) was probably the lack of individual measurements before the beginning of CmpX administration and the poor information on tolerance after drug washout.

5.3 Model predictions

To have pharmacological relevance, a PK/PD model should be able to predict the response to different dose levels and dosing regimens. After the proposed model had been fitted to the observed

temperatures from an experiment with continuous administration, the model behaviour was validated with data from an experiment with repeated oral administration. Comparison between simulated and observed responses showed that the model had good predictive capacity. The main difference appeared to be an earlier onset of the induced response in the predictions compared to the observations, and it is therefore of interest to mention that only approximate dosing times were available for the oral dosing experiments. Future studies in different species could provide information to enable scaling of the model predictions, *e.g.* from preclinical to clinical studies.

5.4 Perspectives and study design

Further development of the circadian rhythm system will be conducted, alongside its application to different biomarkers. PK/PD models can be developed for CmpX-induced responses in heart rate and blood pressure in order to quantify and evaluate similarities and differences in, for example, tolerance development. The temperature PK/PD model can also be applied to temperature responses induced by other compounds in order to validate the model and to analyse whether it has captured general properties of the temperature regulation system.

The present study shows that *in vivo* experiments with continuous drug delivery generate a lot of useful information and are very well suited to the modelling and prediction of pharmacological effects. For future studies, it is recommended that data should be collected a few days before and after dosing in order to establish the individual baselines and to identify possible rebound effects.

Further cooperation between *in vivo* pharmacology scientists and PK/PD modellers is desirable to improve study design and to gain more knowledge from preclinical experiments.

6 Conclusions

A novel circadian rhythm model has been developed that can describe the asymmetric baseline observed in the body temperature of rats.

The proposed PK/PD model captures CmpX exposure levels, the circadian baseline, CmpX-induced hypothermia and complete tolerance development without rebound effects.

Simulation of an oral administration experiment revealed good predictive properties of the model.

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8 References

Ackerman E, Rosevear JW, McGuckin WF (1964) A mathematical model of the glucose-tolerance test. *Phys.Med.Biol.* **9**:203-213

Bauer JA and Fung HL (1994) Pharmacodynamic models of nitroglycerin-induced hemodynamic tolerance in experimental heart failure. *Pharm.Res.* **11**:816-823.

Briese E (1985) Rats prefer ambient temperatures out of phase with their body temperature circadian rhythm. *Brain Res.* **345**:389-393.

Chakraborty A, Krzyzanski W, and Jusko WJ (1999) Mathematical modeling of circadian cortisol concentrations using indirect response models: comparison of several methods. *J.Pharmacokinet.Biopharm.* **27**:23-43.

Depres-Brummer P, Levi F, Metzger G, and Touitou Y (1995) Light-induced suppression of the rat circadian system. *Am.J.Physiol* **268**:R1111-R1116.

Ekblad EB and Licko V (1984) A model eliciting transient responses. *Am.J.Physiol* **246**:R114-R121.

Frank R and Hargreaves R (2003) Clinical biomarkers in drug discovery and development. *Nat.Rev.Drug Discov.* **2**:566-580.

Kleinbloesem CH, van Brummelen P, Danhof M, Faber H, Urquhart J, and Breimer DD (1987) Rate of increase in the plasma concentration of nifedipine as a major determinant of its hemodynamic effects in humans. *Clin.Pharmacol.Ther.* **41**:26-30.

Leloup JC and Goldbeter A (2000) Modeling the molecular regulatory mechanism of circadian rhythms in Drosophila. *Bioessays* **22**:84-93.

Lemmer B. (1997) Chronopharmacological aspects of PK/PD modelling. *Int.J.Clin.Pharmacol.Ther.* **35**:458-464

Levy G. (1993) The case for preclinical pharmacodynamics. In: Yacobi A., Skelly J. Shah V., et al., editors. Integration of pharmacokinetics, pharmacodynamics and toxicokinetics in rational drug development. New York: Plenum Press, 1993:7-13

Moore-Ede M., Sulzman F., Fuller C. (1982) *The Clocks That Time Us*. Harvard University Press, Cambridge, Massachusetts and London, England.

Refinetti R and Menaker M (1992) The circadian rhythm of body temperature. *Physiol Behav.* **51**:613-637.

Reppert SM and Weaver DR (2002) Coordination of circadian timing in mammals. *Nature* **418**:935-941.

Shearman LP, Sriram S, Weaver DR, Maywood ES, Chaves I, Zheng B, Kume K, Lee CC, van der Horst GT, Hastings MH, and Reppert SM (2000) Interacting molecular loops in the mammalian circadian clock. *Science* **288**:1013-1019.

Shoemaker JA and Refinetti R (1996) Day-night difference in the preferred ambient temperature of human subjects. *Physiol Behav.* **59**:1001-1003.

Smolen P, Baxter DA, and Byrne JH (2001) Modeling circadian oscillations with interlocking positive and negative feedback loops. *J.Neurosci.* **21**:6644-6656.

Young MW and Kay SA (2001) Time zones: a comparative genetics of circadian clocks. *Nat.Rev.Genet.* **2**:702-715.

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 hypothermia. *Am.J.Physiol Regul.Integr.Comp Physiol* **281**:R2059-R2071.