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Population pharmacokinetic–pharmacodynamic modelling of the anti-hyperalgesic effect of 5′ deoxy-N^6-cylopentyladenosine in the mononeuropathic rat

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Abstract

The objective of this investigation was to characterise the pharmacokinetic–pharmacodynamic correlation of 5′ -deoxy-N^6-cyclopentyl-adenosine (5′ dCPA) in the chronic constriction injury model of neuropathic pain. Following intravenous administration of 5′ dCPA (0.30 or 0.75 mg kg^{-1}), the time course of the drug concentration in plasma was determined in conjunction with the effect on (1) the mechanical paw pressure and (2) the Von Frey Hair monofilament withdrawal threshold. Population pharmacokinetic–pharmacodynamic analysis was applied to derive individual concentration–effect relationships. For mechanical paw pressure a composite model consisting of an E_{max} model for the anti-hyperalgesic effect in combination with a linear model for the anti-nociceptive effect accurately described the data. The EC_{50} for the anti-hyperalgesic effect was 178±51 ng ml^{-1} and the slope of the anti-nociceptive effect 0.055±0.008 g ml ng^{-1}. For the Von Frey Hair monofilament withdrawal threshold responders and non-responders were observed. Typically, in responders, full pain relief was observed at concentrations exceeding 100 ng ml^{-1}. The high plasma concentrations required for the anti-hyperalgesic effect relative to the receptor affinity are consistent with restricted transport of 5′ dCPA to the site of action in the spinal cord and/or the brain.

Keywords: Adenosine A_1 receptor; Chronic constriction injury model; Neuropathic pain; Pharmacokinetic–pharmacodynamic modelling; Sparse data analysis; Anti-hyperalgesic effect; Anti-nociceptive effect

1. Introduction

The physiological role of adenosine in pain perception is well established. Early animal studies in mice and rats have shown the anti-nociceptive effects of adenosine and synthetic adenosine analogues (Post, 1984; Ahlijanian and Takemori, 1985; Holmgren et al., 1986) and have provided evidence that spinal adenosine receptors are involved in this effect (Karlsten et al., 1990; Sawynok, 1991; Lee and Yaksh, 1996). Specifically, it has been demonstrated that the adenosine A_1 receptor subtype is involved in the inhibition of spinal sensory transmission, suppressing the C-fiber-evoked responses in dorsal horn neurons (Nakamura et al., 1997). Interestingly, adenosine and analogues also suppress allodynia resulting from intrathecal (i.t.) administration of strychnine (Sosnowski and Yaksh, 1989) and prostaglandin $F_{2\alpha}$ (Minami et al., 1992), indicating that these compounds could play a role in the treatment of neuropathic pain. In the meantime, the effects of adenosine A_1 receptor agonists have been convincingly demonstrated in a variety of animal models of neuropathic pain (Yamamoto and Yaksh, 1991; Lee and Yaksh, 1996; Sjölund et al., 1996, 1998; Cui et al., 1998; Lavand'homme and Eisenach, 1999). Furthermore,

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the beneficial effects of adenosine (Sollevi et al., 1995; Belfrage et al., 1999; Sjölund et al., 2001) and adenosine A_1 receptor agonists (Karlsten and Gordh, 1995; Lindblom et al., 1997) in neuropathic pain have been confirmed in clinical investigations.

The clinical application of adenosine A_1 receptor agonists in the treatment of neuropathic pain however is limited by side effects. Due to the ubiquitous distribution of adenosine receptors, selective adenosine A_1 receptor agonists display an array of side effects on for example the cardiovascular system (Mullane and Williams, 1990; Olsson, 1996). An additional complication is that due to the presence of the ribose moiety in the molecule, the blood–brain barrier transport of adenosine A_1 receptor agonists is restricted (Schaddelee et al., 2003). Presumably, transport across the blood–spinal cord barrier, which is similar in anatomy and function to the blood–brain barrier (Noble et al., 1996), is restricted as well.

Recent research efforts on improving the selectivity of action of adenosine A_1 receptor activation have focussed on the design of partial agonists (van der Wenden et al., 1995; Roelen et al., 1996) and allosteric modulators (G). It has been shown that due to differences in receptor density and the efficiency of receptor–effector coupling between tissues, low efficacy adenosine A_1 receptor agonists exhibit indeed a much improved selectivity of action in vivo (van Schaick et al., 1998; van der Graaf et al., 1999). Specifically, various low efficacy 8-alkylamino and deoxyribose analogues of N^6-cyclopentyladenosine (CPA) still display a full anti-lipolytic effect, but are devoid of cardiovascular side-effects. An important question is whether low efficacy agonists still display a full agonistic effect on the central nervous system, and more specifically also in neuropathic pain. In this respect, it is important that adenosine A_1 receptor expression in various parts of the brain and spinal cord is indeed high (Fredholm et al., 2001).

In this report, we describe the development of an integrated pharmacokinetic–pharmacodynamic model for quantification of the effects of selective adenosine A_1 receptor agonists in an animal model of neuropathic pain. This pharmacokinetic–pharmacodynamic model may ultimately be applied to characterise in a strictly quantitative manner the anti-hyperalgesic effects of adenosine A_1 receptor partial agonists in neuropathic pain. An important factor in this respect is that in these animal models, only a limited number of observations are possible. For this reason, sparse data sampling in combination with non-linear mixed effects modelling was applied to derive individual concentration effect relationships. Another aspect is the distinction between on one hand the anti-hyperalgesic effect and on the other hand the anti-nociceptive effect. It has been demonstrated that selective adenosine A_1 receptor agonists display both types of effects (Sawynok, 1998; Sjölund et al., 1998; Bastia et al., 2002). For therapeutic application, however, only the anti-hyperalgesic effect is of interest. The proposed population pharmacokinetic–pharmacodynamic model therefore aims specifically at the distinction between these two effects.

2. Material and methods

2.1. Chemicals

5-deoxy-N^6-cyclopentyladenosine (5′ dCPA) was a gift from Parke Davis (Ann Arbor, MI). GR79236 was kindly provided by GlaxoSmithKline (Stevenage, United kingdom). Methanol (HPLC application), acetonitrile (HPLC gradient application) and water (HPLC application) were obtained from Fisher Chemicals (Loughborough, United Kingdom). All other chemicals were of analytical grade (Fisher Chemicals).

2.2. Animals

The protocol of the study was approved by the institutional ethics committee. Male random hooded rats (Rodent Breeding unit-Bioscience Support GlaxoSmithKline, United Kingdom), weighing between 200 and 225 g, were used in this study. The animals were housed in groups in plastic cages with a normal 12 h light–dark cycle, fed on laboratory chow (Special Diet Services, Maldon, Essex, United Kingdom) and tap water ad libitum.

2.3. Pharmacokinetic study

The full pharmacokinetic profile was investigated in a group of 10 rats. 5′ dCPA was administered in a 0.75 or 0.30 mg kg^{-1} intravenous bolus injection through the tail vein. Each dosage group consisted of five rats. Blood samples (200 to 400 μl) were drawn from a semi-permanent cannula in the tail vein at predefined time-points: 5, 15, 30, 45, 75, 105, 165 min after drug administration and directly centrifuged to plasma (5 min, 13.000 rpm).

2.4. Chronic constriction injury model

The chronic constriction injury model of neuropathic pain, as previously described by Bennett and Xie (1988), was used in the investigations. Briefly, rats were anaesthetised with isoflurane. The common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through biceps femoris. Proximal to the sciatic's trifurcation, the nerve was freed from adhering tissue and four ligatures (4.0 chromic gut, Braun Surgical, Melsungen, Germany) were tied loosely around it with 1 mm spacing. Ten days after surgery, the chronic constriction injury rats displayed neuropathy. The surgical procedure for the sham-operated rats was identical except that the sciatic nerve was not ligated.

2.5. Experimental protocol

To determine the effect of 5′ dCPA, mechanical hypersensitivity was measured on basis of mechanical paw pressure using an algesymeter (Ugo Basile, Comerio, Italy). In addition, mechanical allodynia was measured using von Frey hair monofilaments (Ugo Basile). The following von Frey hair monofilaments were used: 5.07, 5.18, 5.46, 5.88 and 6.1 corresponding to the weights of 7.37, 12.5, 20.9, 46.54, 84.96 g, respectively. One week after surgery, the behavioural measurements started. The pharmacokinetic–pharmacodynamic experiments were performed 3 weeks after surgery, when the mechanical hypersensitivity and allodynia had reached a stable minimum. 5′ dCPA was administered in doses of 0.75 or 0.30 mg kg^{-1} as intravenous bolus injection through the tail vein. Each dose group contained 14 chronic constriction injury and 10 sham rats. In each rat, three serial bleeds through the tail vein were obtained and five behavioural nociceptive effect measurements were conducted according to a randomised sampling design. Behavioural nociceptive effect measurements were performed at the following pre-defined time-points: at 5, 15, 45, 60, 105, 180 min after administration of the 0.75 mg kg^{-1} dose and at 5, 15, 45, 60, 90, 120 min after administration of the 0.30 mg kg^{-1} dose. At 24 h pre- and post-dosage, behavioural nociceptive effect measurements were conducted for establishment of the baseline values. Blood samples (200 to 400 μl) were drawn at the following pre-defined time-points: at 0, 10, 20, 50, 65, 110, 185 min after administration of the 0.75 mg kg^{-1} dose and at 0, 10, 20, 50, 65, 95, 125 min after administration of the 0.30 mg kg^{-1} dose and directly centrifuged to plasma (5 min, 13.000 rpm).

2.6. Drug analysis

The concentration of 5′ dCPA in plasma samples was determined by high performance liquid chromatography with tandem mass spectrometry (LC-MS-MS). GR79236 (*N*-[(1S, trans)-2-hydroxycyclopentyl]adenosine) (20 μl, 1 μg ml^{-1}) was used as internal standard. The samples were extracted with acetonitrile (3 to 4 volumes). After centrifugation (5 min, 13000 rpm), the supernatant was transferred into clean tubes and dried under nitrogen at 40 °C. The residues were dissolved in 100 μl of a mixture of water and methanol (95:5 v/v) and a volume of 30 μl was injected into the LC-MS-MS system. HPLC was performed using a Hewlett-Packard 1100 instrument (Hewlett-Packard, Waldbronn, Germany). Chromatography was performed on a C18 column (50×2.1 mm I.D.; 5 μM particle size) (Capital HPLC, Broxburn, United Kingdom) at a flow rate of 1.0 ml/min. In the chromatography, gradient elution was applied. The mobile phase consisted of a mixture of 2 solvents: (A) 100% water+0.1% formic acid and (B) 100% acetonitrile+0.1% formic acid. The profile was 0–1 min: 95% A; 1–2 min linear gradient to 90% B; 2–3 min: 90% B; 3–3.1 min: linear gradient to 95% A; 3.1–5 min: 95% A. Mass spectrometry was performed on a PE-Sciex API2000 instrument (Perkin-Elmer Sciex Instruments, Foster City, CA, USA) equipped with a turbo ion spray source for electrospray ionisation in the positive mode. Detection by tandem mass spectrometry was based on precursor ion transitions to the strongest intensity. Instrumental conditions were optimised to yield best sensitivity. The limit of quantification for a 50-μl plasma sample was 0.05 ng ml^{-1} and the within-day and between-day variation were 7% and 10%, respectively.

2.7. Data analysis

2.7.1. Pharmacokinetics

A population approach was applied to quantify the pharmacokinetics and pharmacodynamics of 5′ dCPA. In this approach, the data from all individual rats both from the pharmacokinetic study and from pharmacokinetic–pharmacodynamic experiments could be fitted simultaneously while explicitly taking into account both the interindividual variability in the model parameters as well as intraindividual residual error (Schoemaker and Cohen, 1996). All fitting procedures were performed in the nonlinear-mixed-effect-modelling software NONMEM (GloboMax, Hanover, MD, US). The model was fitted using subroutine ADVAN 7 in NONMEM, which is a general linear model that makes use of numerical approximations to the matrix exponential.

To allow a stepwise building of the final integrated pharmacokinetic–pharmacodynamic model, the pharmacokinetic model was a one-compartment model with linear elimination, as selected on the basis of the Akaike information criterion (Akaike, 1974). The plasma concentration vs. time data were modelled according to the following mono-exponential equation:

$$C_{(t)} = C_0 e^{-\frac{CL}{V_{ss}}t} \quad (1)$$

where $C_{(t)}$ is the plasma concentration at time t, C_0 is the initial plasma concentration, CL is the total plasma clearance and V_{ss} is the volume of distribution at steady state. Interindividual variability on the parameter CL was modelled according to an exponential equation. Thus, it was assumed that the value of this parameter is log-normally distributed:

$$\theta_i = \theta \exp(\eta_i) \quad (2)$$

where θ is the population mean parameter value, θ_i is the individual parameter value and $\exp(\eta_i)$ is a random term from a normal distribution with mean zero and variance ω^2. The η_i values quantifies the deviation of the individual parameters to the population mean. The variance ω^2 associated with parameter θ provides a measure of inter-individual variation in θ, which relates to the biological variation and experimental errors.

The residual error was characterised by a proportional error model:

$$Cm_{ij} = Cp_{ij}\left(1 + \varepsilon_{ij}\right) \quad (3)$$

where Cp_{ij} is the jth plasma concentration for the ith individual predicted by the model, Cm_{ij} is the measured plasma concentration, ε_{ij} is a random noise term from a normal distribution with mean zero and variance σ^2. The size of σ^2 is a measure of the intra-individual residual error in the model; the difference between the observed and the predicted values. In the pharmacokinetic–pharmacodynamic study, plasma samples were collected 5 min after the effect measurement. For each individual rat, actual plasma concentrations at the time-points of the effect measurements were estimated on basis of the population pharmacokinetic model. The elimination half-life was calculated following standard procedures (Gibaldi and Perrier, 1989).

2.7.2. Pharmacodynamics—mechanical hypersensitivity

No hysteresis was observed between plasma concentration and the effect on mechanical hypersensitivity and the two were directly related to each other. The effect on mechanical hypersensitivity can be divided into two effects, the anti-hyperalgesic effect and the anti-nociceptive effect. The anti-hyperalgesic effect was defined as the increase in paw pressure threshold from the baseline value of a chronic constriction injury rat to the level of the baseline value in the sham rats. Therefore, per definition, the anti-hyperalgesic effect is observed only in the chronic constriction injury rats. The anti-hyperalgesic effect was described with the following E_{max} model:

$$E_{an}(t) = E_{0,CCI} + \left(\frac{E_{max} Cp(t)}{EC_{50} + Cp(t)}\right) \quad (4)$$

where $E_{an}(t)$ is the anti-hyperalgesic effect at time t, $Cp(t)$ the plasma concentration at time t, $E_{0,CCI}$ the baseline threshold value of the chronic constriction injury rats, E_{max}, the maximum anti-hyperalgesic effect, EC_{50}, the concentration where 50% of the anti-hyperalgesic effect is reached. The E_{max} was identical to the population mean baseline value of the sham rats. In the analysis, the anti-nociceptive effect was defined as the observed increase in paw pressure withdrawal threshold above the baseline value of the sham rats. The anti-nociceptive effect was described with the following linear model:

$$E_{no(t)=E_{0,sham}+aC(t)} \quad (5)$$

where $E_{no}(t)$ is the anti-nociceptive effect at time t, $C(t)$, the plasma concentration at time t, $E_{0,sham}$, the baseline value of the sham rats, and a is the slope of the linear concentration–effect relationship. The concentration effect profiles in the chronic constriction injury rats were described with a combined model of the E_{max} model and the linear model:

$$E_{CCI}(t) = E_{an}(t) + E_{no}(t) \quad (6)$$

where $E_{CCI}(t)$ is the effect at time t. The concentration–effect profiles in the sham-operated rats were described by the linear model (Eq. (5)), as per definition, only an anti-nociceptive effect could be observed. The statistical model used for the concentration effect profiles had the following general form:

$$E_{ij} = f\left([Cp]_{ij}, \theta_i\right) + \varepsilon_{ij} \quad (7)$$

where E_{ij} and $[Cp]_{ij}$ correspond to effect and concentration of the agonist for the jth data point in the ith concentration–effect curve, f is the function, θ_i is the individual parameter value (e.g. E_{max}, EC_{50} and a) of the concentration–effect curve i and ε_{ij} is a random noise term from a normal distribution with mean zero and variance σ^2. The interindividual variability in the pharmacodynamic parameters was modelled according to the exponential equation used in the pharmacokinetic model (Eq. (2)). All pharmacokinetic and pharmacodynamic data, both obtained in the chronic constriction injury- and sham-operated rats were fitted to the integrated pharmacokinetic–pharmacodynamic model simultaneously. The first-order Bayesian estimation method implemented in the NONMEM software was used to calculate population and individual parameter estimates. All fitting procedures were performed on an IBM-compatible personal computer (Pentium®, 133 MHz) under Windows NT using the Microsoft FORTRAN Powerstation 4.0 compiler with NONMEM version IV, level 2 (double precision) and Visual NONMEM version 2.2.2 (RDPP, Montpellier, France).

2.7.3. Pharmacodynamics—mechanical allodynia

In order to allow classification of compounds into silent, partial or full agonists, the data obtained with the von Frey hair monofilaments were transformed into three categories, no, partial and full pain relief. Descriptions of each of the three classes are listed in Table 1. A confounder in the analysis was the presence of responders and non-responders in the population. Therefore, a full statistical analysis of the concentration–effect relationship was not feasible with the available data.

2.7.4. Statistical analysis

An improvement in the goodness-of-fit with a ΔMOF of 6.6 ($p<0.05$) was considered significant to including a

Table 1
Description of the pain relief categories

Score	Degree of pain relief	Description
0	No pain relief	Pain threshold did not change compared to baseline value
1	Partial pain relief	Pain thresholds increased compared to baseline value; did not reach the same level as control paw
2	Full pain relief	Pain thresholds increased to pain threshold of control paw

covariate on one of the pharmacokinetic or pharmacodynamic parameters. The pharmacokinetic and pharmacodynamic post hoc Bayesian estimates of the different doses were statistically compared using an unpaired Student's *t*-test (Graphpad Instat® version 3.00). A value of $p<0.05$ was considered as a statistically significant difference. All data are reported as mean±standard error.

3. Results

3.1. Pharmacokinetics

The plasma concentration time profiles of 5′ dCPA after intravenous administration of 0.3 and 0.75 mg kg^{-1} of 5′ dCPA are shown in Fig. 1. The pharmacokinetic data could be described adequately by a one-compartment PK model with linear elimination. In the population analysis, dose was considered as a covariate on the pharmacokinetic parameters V_{ss} and CL, but this did not improve the minimum value of the objective function. This shows that the pharmacokinetics is independent of the administered dose. Also no pharmacokinetic differences were found between normal, sham-operated and chronic constriction injury rats. In the population analysis, the interindividual variability in clearance was 15%. No interindividual variability in V_{ss} was identifiable (<<0.01%). From the population analysis, the individual values of the pharmacokinetic parameters were estimated by Bayesian post hoc analysis. The values of the pharmacokinetic parameters including the population parameter estimates are summarised in Table 2. No significant differences in the parameters were observed between the low and high dose, confirming that the pharmacokinetics were indeed dose-independent.

3.2. Pharmacodynamics—mechanical hypersensitivity

At the start of the experiment, a wide and stable window existed in baseline values of the paw withdrawal threshold between the chronic constriction injury-operated rats vs. the sham-operated rats. The baseline values were 77.1±1.4 and 122±1.6 g for chronic constriction injury and sham-operated rats, respectively. The time course of mechanical hypersensitivity after intravenous administration of 5′ dCPA for both dosages is shown in Fig. 2. Administration of 5′ dCPA produced a rapid increase in the paw pressure withdrawal threshold, in both the chronic constriction injury- and sham-operated rats. 5′ dCPA had a dual effect, an anti-hyperalgesic effect (the reversal from chronic constriction injury baseline to the sham baseline level) and an anti-nociceptive effect (increase in paw withdrawal threshold above sham baseline level). No effect-compartment was necessary to link the pharmacokinetics to the pharmacodynamics. The relationship between plasma concentration and mechanical hypersensitivity could accurately be described on basis of the composite pharmacodynamic model featuring an E_{max} model for the anti-hyperalgesic effect in combination with a linear model for the anti-nociceptive effect. The observed plasma concentration–effect relationship is shown in Fig. 3. In the analysis, the E_{max} of the anti-hyperalgesic effect was identical to the baseline value of the sham-operated rats. A full anti-hyperalgesic effect was observed at both the low and the high dose of 5′ dCPA. The anti-nociceptive effect on the other hand was much more profound at the high dose compared to the low dose. Dose was considered as a covariate in the pharmacodynamic analysis specifically with

Table 2
Pharmacokinetic parameter estimates of 5′ dCPA following intravenous administration, on basis of post hoc Bayesian estimates (mean±S.E.) and on basis of population analysis

	n	$T_{1/2}$ (min)	CL (ml min^{-1})	V_{ss} (ml)
Post hoc estimates				
0.3 mg kg^{-1}	29	19.9±2.5	9.0±1.2	260±15
0.75 mg kg^{-1}	29	22.8±2.5	7.9±0.9	260±15
Population estimates				
Population mean	58	22.7±1.8	7.9±0.4 (15%)	260±15 (≪0.01%)

The inter-subject variability in the parameter estimates is shown between brackets.

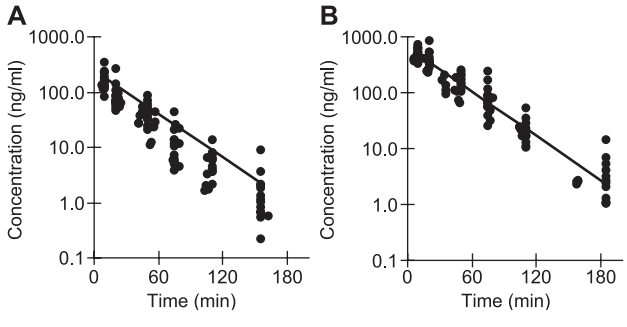

Fig. 1. Plasma concentration vs. time after intravenous bolus injection of 0.3 mg kg^{-1} (A) and 0.75 mg kg^{-1} (B) of 5′ dCPA. The solid line in the graph represents the population mean predicted plasma concentration on basis of fitting a one-compartment model to all the data.

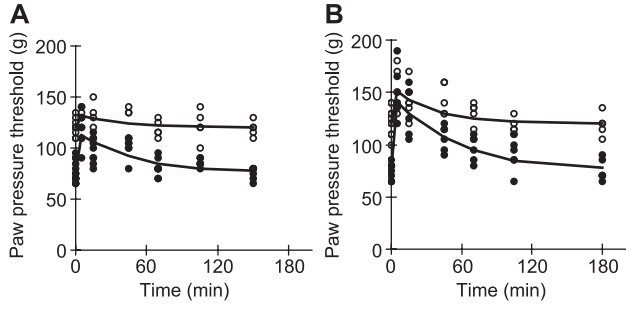

Fig. 2. Paw pressure withdrawal threshold (mechanical hypersensitivity) vs. time after intravenous bolus injection of 0.3 mg kg^{-1} (A) and 0.75 mg kg^{-1} (B) of 5′ dCPA. The solid line in the graph represents the population mean predicted effect obtained fitting the combined E_{max} and linear model to the data. (●) Chronic constriction injury, (○) sham.

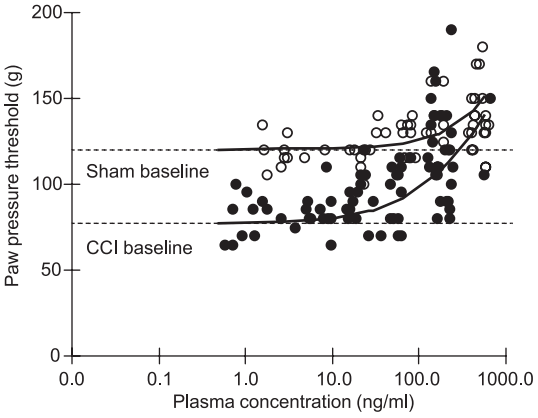

Fig. 3. The plasma concentration effect relationship for the paw pressure withdrawal threshold. The solid lines in the graph represent the population predicted concentration effect relationships. (●) Chronic constriction injury, (○) sham.

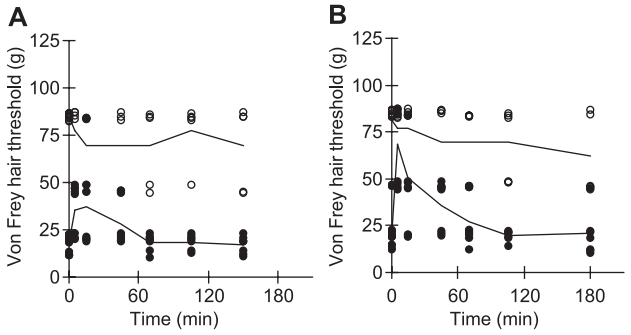

Fig. 4. Von Frey Hair monofilament withdrawal threshold (mechanical allodynia) time-profiles after intravenous bolus injection of 0.3 mg kg^{-1} (A) and 0.75 mg kg^{-1} (B) of 5′ dCPA. (●) Chronic constriction injury, (○) sham.

regard to the parameters EC_{50}, E_{max} and slope, however, this did not improve the minimum value of the objective function. Thus, the observed pharmacodynamic parameter estimates are independent of the administered dose confirming the validity of the model. The individual values of the pharmacodynamic parameters were estimated from the population analysis on the basis of a Bayesian post hoc analysis. The values of the post hoc pharmacodynamic parameter estimates including the population parameter estimates are summarised in Table 3. No statistically significant difference in the parameters was observed between the low and the high dose.

3.3. Pharmacodynamics—mechanical allodynia

At the start of the experiment, a wide window existed in baseline values of the monofilament withdrawal threshold with values of 19.4±6.6 and 83.6±8.6 g in the chronic constriction injury- and sham-operated rats, respectively. After intravenous administration of 5′ dCPA, an increase in pain threshold was observed in the chronic constriction injury-operated rats but not in the sham-operated rats. The time course of von Frey hair monofilament withdrawal threshold following the low and high dose, are shown in Fig. 4. In order to explore the concentration vs. effect relationship, the data were transformed into three classes: no pain relief, partial pain relief and full pain relief (Table 1). Fig. 5 shows the relationship between the grade of pain relief and the plasma concentration. A wide interindividual variability in response is observed, which complicates the characterisation of the concentration–effect relationship on the basis of this data set. Specifically at high drug concentrations in a number of animals, a full anti-hyperalgesic response was observed, whereas in other animals, no response was observed at the same concentration. This indicates that there are 'non-responders' in the population. Typically, in the responders, a full anti-hyperalgesic response was observed at the high plasma concentrations exceeding the value of 100 ng ml^{-1}.

4. Discussion

The anti-hyperalgesic effect of adenosine A_1 receptor agonists in neuropathic pain has been demonstrated in

Table 3
Pharmacodynamic parameter estimates following intravenous administration of 5′ dCPA, on basis of post hoc Bayesian estimates (mean±S.E.) and on the basis of the population analysis

	Treatment	*n*	Baseline paw withdrawal threshold (g)	EC_{50} (ng ml^{-1})	Slope (g ml ng^{-1})
Post hoc estimates					
0.3 mg kg^{-1}	chronic constriction injury	14	76.7±3.9	212±102	0.057±0.016
	sham	10	120±2.7		0.060±0.025
0.75 mg kg^{-1}	chronic constriction injury	12	75.9±3.1	186±85	0.066±0.029
	sham	10	120±2.7		0.060±0.026
Population estimates					
Population mean	chronic constriction injury	26	77.1±1.2 (8%)	178±51 (76%)	0.055±0.008[1] (21%)
	sham	20	120±2.7 (<<0.01%)		

The inter-subject variability in the parameter estimates is shown between brackets.
[1] One population value was estimated for both chronic constriction injury and sham-operated rats (*n*=46).

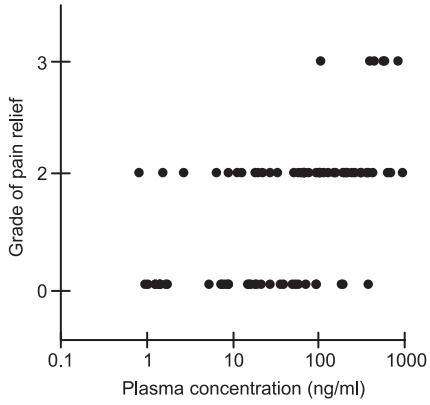

Fig. 5. The grade of pain relief in Von Frey Hair monofilament withdrawal threshold vs. plasma concentration after intravenous bolus injection of 0.3 and 0.75 mg kg^{-1} of 5′ dCPA.

several investigations in experimental animals (Yamamoto and Yaksh, 1991; Sjölund et al., 1996; Nakamura et al., 1997; Cui et al., 1998) and in humans (Karlsten and Gordh, 1995; Lindblom et al., 1997). The clinical application of adenosine A_1 receptor agonists however is limited by side effects. This raises the question how the selectivity of action of adenosine A_1 receptor agonists could be improved. Important issues in this respect are (1) the ubiquitous distribution of adenosine A_1 receptors in the body and (2) the presence of the blood–brain barrier/blood–spinal cord barrier, which limits the distribution into the central nervous system. Research efforts to improve the selectivity of action have focussed on the development of partial agonists (van der Wenden et al., 1995; Roelen et al., 1996) and allosteric modulators (Bruns and Fergus, 1990; van der Klein et al., 1999). So far, the effects of these compounds in nociceptive tests have not been evaluated. Specifically, effect of these drugs in neuropathic pain models has so far not been established. An important question is whether (a) adenosine A_1 receptor low efficacy agonists still exhibit full anti-hyperalgesic properties in neuropathic pain and (b) whether such effects occur at reasonable concentrations and dose levels. An integrated pharmacokinetic–pharmacodynamic approach is ideally suited to answer these questions. The objective of this investigation was to develop an integrated population pharmacokinetic–pharmacodynamic model for the anti-hyperalgesic effect of synthetic adenosine A_1 receptor agonists in an animal model of neuropathic pain which can ultimately be used to characterise the effects of partial agonists and allosteric modulators.

Over the years, several experimental models for neuropathic pain have been developed which all display the behavioural signs of both evoked and ongoing neuropathic pain as reflected in an increased sensitivity to tactile stimuli (Bennett and Xie, 1988; Seltzer et al., 1990; Kim and Chung, 1992; DeLeo et al., 1994; Na et al., 1994). In several of these models, anti-hyperalgesic effects of adenosine analogues have been demonstrated (Yamamoto and Yaksh, 1991; Lee and Yaksh, 1996; Sjölund et al., 1996, 1998; Cui et al., 1998; Lavand'homme and Eisenach, 1999). However, so far, relationships between drug concentration and effect have not been reported for these models. In the present investigation, the effect of the prototypical A_1 agonist 5′ dCPA was characterised in the chronic constriction injury rat model as described by Bennett and Xie (1988). This model has the advantage that a stable level of neuropathy develops in 2–3 weeks. The effects of 5′ dCPA were quantified on basis of (i) mechanical paw pressure withdrawal threshold and (ii) the von Frey hair monofilament withdrawal threshold.

In this investigation, 5′ dCPA was chosen as a model drug since it acts as a full agonist at the adenosine A_1 receptor in various in vitro and in vivo test systems (van der Wenden et al., 1995; Mathôt et al., 1995) and because of its favourable pharmacokinetic properties with a relatively long terminal half-life. Furthermore, 5′ dCPA appears to have favourable blood–brain barrier transport characteristics as was recently demonstrated in an experimental in vitro model of bovine brain capillary endothelial cells and rat astrocytes (Schaddelee et al., 2003) A population approach was applied to characterise the pharmacokinetics. This approach offers the advantage that the plasma concentration vs. time profile in the individual rats can be characterised in detail on the basis of a limited number of concentration measurements. Such an approach is especially attractive in situations where only a limited number of observations is possible, as is often the case in behavioural pharmacology investigations (Della Pasqua et al., 1998). The pharmacokinetics of 5′ dCPA was independent of the administered dose and no differences between the chronic constriction injury- and sham-operated rats were observed. The values of the pharmacokinetic parameters are consistent with those in previous investigations, using the more traditional sampling designs and data analysis techniques (Mathôt et al., 1995).

No hysteresis was observed between the plasma concentration and the effect of 5′ dCPA on the paw pressure withdrawal threshold and the two were therefore directly correlated to each other. An important feature of the paw pressure withdrawal threshold is that it provides a continuous measure of the response (Dingemanse et al., 1988). A disadvantage is however that the effect is non-specific in the sense that it reflects both the anti-hyperalgesic and the anti-nociceptive effect. A composite pharmacodynamic model was used to distinguish between the two effects. This model bears similarity with a previously proposed model for the anti-convulsant effect of midazolam (Hoogerkamp et al., 1996). The model converged and the pharmacodynamic parameter estimates were obtained for each of the effects. The E_{max} part of the model successfully described the anti-hyperalgesic effect on the assumption that the maximum effect is identical to the baseline in sham-operated controls. A linear model was applied to describe the anti-nociceptive effect. An important observation is that the value of the

slope of the linear concentration–effect relationship is identical in chronic constriction injury- and sham-operated controls, confirming that indeed a true measure of the anti-nociceptive response is obtained. Furthermore, the pharmacodynamic parameter estimates were independent of the administered dose. This confirms the validity of the proposed pharmacodynamic model for the effect on mechanical hypersensitivity.

With regard to the analysis of the pharmacodynamic data obtained with the von Frey hair monofilaments, it is important that these are categorical rather than continuous. In the present analysis, the data were divided into three categories (i.e., no, partial and full pain relief) as shown in Table 3. This division into three categories was chosen to allow a distinction between full and partial response, while maintaining maximum statistical power to establish concentration–effect relationships. In Fig. 5, it is shown that the probability of observing partial and full pain relief increases with increasing drug concentration. However, a considerable interindividual variability in the data was observed which complicated a formal pharmacodynamic analysis on basis of logistic regression. Although in general, full pain relief was observed only at high plasma concentrations (i.e., values exceeding the concentration of 100 ng ml^{-1}), in several animals, no pain relief was observed at equally high concentrations. In particular, the presence of these 'non-responders' precludes a formal pharmacokinetic–pharmacodynamic analysis of the present data set.

The results of the present investigation show that the high-efficacy agonist 5′ dCPA displays clear anti-hyperalgesic and anti-nociceptive responses in the chronic constriction injury rat. These effects occur at concentrations that largely exceed those required for significant anti-lipolytic effect (EC_{50}=0.21 ng ml^{-1}, van der Graaf et al., 1999) and haemodynamic effect (EC_{50}=16 ng ml^{-1}, van der Graaf et al., 1997). However, the adenosine A_1 receptor expression in the brain and spinal cord as in fat tissue is higher than in the heart (Fredholm et al., 2001). Therefore, on basis of receptor density, a lower EC_{50} value was expected for the anti-hyperalgesic response, in a similar range of that previously found for the anti-lipolytic response. The relatively high EC_{50} value indicates that the transport to the site of action, either at the spinal or the supra-spinal level, is restricted and explains the relatively high doses required to achieve the full anti-hyperalgesic response. It has been demonstrated that the blood–brain barrier transport of adenosine A_1 receptor agonists is highly restricted due to the hydrophilic nature of these compounds (Schaddelee et al., 2003). Thus, the observation of high EC_{50} values corroborates in vitro data on the restricted distribution of these compounds across the blood–brain barrier. As mentioned earlier, a much-improved selectivity of action in favour of the anti-lipolytic effect of low efficacy agonists has been demonstrated (van der Graaf et al., 1999). An important question is whether a similar approach could be applied in the treatment of neuropathic pain. Further research is required to investigate whether low efficacy agonists still display a full anti-hyperalgesic effect in the mononeuropathic rat.

In conclusion, the results of this investigation show that mechanical paw pressure in the chronic constriction injury model provides a useful endpoint for characterisation of the pharmacokinetic–pharmacodynamic correlation of adenosine A_1 receptor agonists in the chronic constriction injury model, allowing a distinction between the anti-hyperalgesic and the anti-nociceptive effect. The high value of the EC_{50} of 5′ dCPA relative to the affinity at central adenosine A_1 receptors the transport to the site of action is restricted. This model will ultimately be useful to characterise the anti-hyperalgesic effect of adenosine A_1 receptor partial agonists and allosteric modulators, which may have a much-improved selectivity of action.

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