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Pharmacokinetic–Pharmacodynamic Modelling of the Analgesic and Antihyperalgesic Effects of Morphine after Intravenous Infusion in Human Volunteers

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Abstract: Using a modelling approach, this study aimed to (i) examine whether the pharmacodynamics of the analgesic and antihyperalgesic effects of morphine differ; (ii) investigate the influence of demographic, pain sensitivity and genetic (OPRM1) variables on between-subject variability of morphine pharmacokinetics and pharmacodynamics in human experimental pain models. The study was a randomized, double-blind, 5-arm, cross-over, placebo-controlled study. The psychophysical cutaneous pain tests, electrical pain tolerance (EPTo) and secondary hyperalgesia areas (2HA) were studied in 28 healthy individuals (15 males). The subjects were chosen based on a previous trial where 100 subjects rated (VAS) their pain during a heat injury (47°C, 7 min., 12.5 cm^2). The 33% lowest- and highest pain-sensitive subjects were offered participation in the present study. A two-compartment linear model with allometric scaling for weight provided the best description of the plasma concentration–time profile of morphine. Changes in the EPTo and 2HA responses with time during the placebo treatment were best described by a linear model and a quadratic model, respectively. The model discrimination process showed clear evidence for adding between-occasion variability (BOV) on baseline and the placebo slope for EPTo and 2HA, respectively. The sensitivity covariate was significant on baseline EPTo values and genetics as a covariate on the placebo slope for 2HA. The analgesic and antihyperalgesic effects of morphine were pharmacologically distinct as the models had different effect site equilibration half-lives and different covariate effects. Morphine had negligible effect on 2HA, but significant effect on EPTo.

Pain sensitivity varies substantially among individuals and is determined by complex interactions between nociception, ethnic [1,2], genetic [3], physiological [4,5], psychological [6–8] and social factors [9]. These interactions together with the complex pharmacokinetic/pharmacodynamic (PK/PD) relationships for the majority of analgesic drugs explain the less than straightforward and predictable outcome of pharmacological pain treatment.

Strong opioids are generally considered efficacious key players in the management of high-intensity acute and chronic pain, and opioids are known to possess important antihyperalgesic efficacy. However, opioid therapy is also associated with development of tolerance and a paradoxically increased sensitivity to pain, that is opioid-induced hyperalgesia [10,11]. This is obviously of great clinical concern as opioid therapy *per se* may aggravate pain not only following surgical procedures but also in chronic pain patients jeopardizing adequate pain control [10].

Due to the complex actions of opioids, treatment for pain in the clinical setting often bears resemblance with a trial and error process. However, as population PK/PD modelling characterizes the relationship between drug concentration and effect for the typical individual in the population; in addition, considering specific factors involved in individual patients' responses from the population mean, PK/PD modelling may provide a basis for an evidence-guided therapy for the individual patient [12]. In clinical pain, a number of confounding factors are present. Human experimental pain models may be preferable adjuncts in defining the underlying mechanisms, as the duration, frequency and intensity of nociceptive input can be minutely controlled in an experimental setting, resulting in substantially fewer variables confounding pain measures [13].

The analgesic effects of opioids mainly result from activation of opioid receptors located in the central nervous system (CNS) at both spinal and supraspinal levels. Peripherally mediated suppression of sensitized nociceptors by opioids has been reported, but the absence of antihyperalgesic effects in clinical studies suggest that the role of opioids inhibiting central sensitization is of minor importance [14]. Acute receptor desensitization via uncoupling of the receptor from G-proteins, up-regulation of the cAMP pathway, activation of the N-methyl-D-aspartate (NMDA)-receptor system, as well as activation of descending facilitation, have been proposed as potential mechanisms underlying opioid-induced hyperalgesia

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[11]. Furthermore, experimental studies and clinical observations suggest a possible role for μ-receptor agonists to induce hyperalgesia [10,11]. Therefore, different mechanisms have been proposed for opioid-induced analgesia and antihyperalgesia, which may result in different pharmacodynamic profiles of the analgesic and antihyperalgesic effects [15].

Polymorphisms in the gene expressing the μ-opioid receptor (OPRM1) have been associated with differences in pain perception and have been recognized as an important element in human pain sensitivity. The relationships between A118G single-nucleotide polymorphism (SNP), altered pain thresholds and analgesic responses have been well characterized. Although a number of studies have reported decreased pain thresholds and increased pain responses associated with the G118 allele [16,17], contradictory results have recently been reported [18]. Therefore, the hypothesis that A118G, single-nucleotide polymorphism, in the OPRM1 could explain the interindividual differences in the analgesic response was explored.

The aim of this study was to model the analgesic and antihyperalgesic responses after intravenous administration of morphine, a full μ-receptor agonist, in healthy volunteers and to examine whether different pharmacodynamic profiles of the analgesic and antihyperalgesic effects of morphine exist. A secondary aim was to investigate the influences of demographic, pain sensitivity and genetic variables on between-subject variability in pharmacokinetics and pharmacodynamics of morphine in human experimental pain models.

Subjects, Design and Methods

The study protocol was approved by the Regional Ethics Committee (H-2-2010-115), Danish Medicines Agency, Danish Data Protection Agency and registered at ClinicalTrials.gov (NCT01296334). The study was approved as a part of the study 'morphine- and buprenorphine-induced analgesia and antihyperalgesia in a human inflammatory pain model: a double-blind, randomized, placebo-controlled, 5-arm, cross-over study' [19]. The study was conducted according to the guidelines for Good Clinical Practice (GCP) and audited by the GCP Unit of Copenhagen University Hospital.

Subjects.

Healthy volunteers were recruited from participants in a prior study by Ravn *et al.*[20]. Inclusion criteria were 20–40 years of age. Exclusion criteria were insufficient proficiency in Danish, participation in other clinical trials 4 weeks prior to this study, skin lesions on the lower leg, intake of any medication 48 hr prior to the investigation, intake of analgesics 7 days prior to the investigation (except paracetamol p.n.), allergy to morphine, buprenorphine, hydrocortisone, ondansetrone or DHB, current or former drug abuse, smoking, body mass index (BMI) > 28 kg/m^2 and in females, pregnancy or planning of pregnancy or no use of contraception. Following verbal and written information, all volunteers provided written informed consent before inclusion. All volunteers had a routine medical examination by a physician prior to inclusion.

Design.

The study was a randomized, placebo-controlled, double-blind, 5-arm, cross-over study.

Several psychophysiological pain tests were performed as previously described [19]. The electrical pain tolerance (EPTo) and secondary hyperalgesia (2HA) were further examined in the present PK/PD analyses. Based on the data from a previous trial [20], 100 subjects were categorized as low-, intermediate- or high pain-sensitive subjects based on their pain ratings (VAS) during a heat injury (47°C, 7 min., area 12.5 cm^2). The low- and high pain sensitive subjects (the subjects, divided into males and females, with the 33% lowest and highest ratings) were offered participation in the present study (fig. 2).

Electrical pain tolerance and 2HA were measured during baseline (before drug infusion) and at three post-burn (PB) measurements at time (min.) 205, 265 and 325. The infusion rate was decreased after 15 min. and infusion was discontinued after 210 min., after the first post-burn (PB1) measurement (fig. 1).

Methods.

Electrical pain tolerance. Transcutaneous electrical stimuli were applied using a computerized, constant current stimulator (PainMatcher, Cefar Medical AB, Lund, Sweden) [21]. The stimulator delivered square-wave impulses with a frequency of 10 Hz and an amplitude of 15 mA. The stimulation intensity was automatically modulated by increased pulse width, in 4 μsec. increments, from 4 to a maximum of 396 μsec. The subject pinched the two opposed rubber electrodes between the non-dominant thumb and index finger. By holding a steady grip on the electrodes, an incremental increase in the electrical energy was delivered. When releasing the pinch grip, an arbitrary value between 1 and 100, reflecting the energy delivered, was registered. The subjects were told to release when their pain tolerance was reached. The test had a maximum obtainable value of 100.

Secondary hyperalgesia. The area of secondary hyperalgesia in normal skin surrounding the area of the heat injury was determined with a calibrated nylon filament {nominal value 18 [0.89 ± 0.05 N (mean ± S.D.)], Stoelting, IL, USA} [22]. The border was determined by stimulating in eight radial lines each separated by an angle of 45° converging towards the centre of the heat injury. The subjects reported the occurrence of a definite uncomfortable change in sensation to a burning

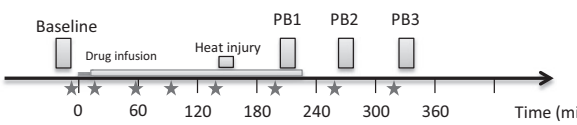

Fig. 1. Study flowchart. Illustration of time for baseline measurements, the heat injury and post-burn 1, 2 and 3, drug infusion (15 min. loading dose follow by 195 min. maintenance dose) and times for blood sampling (indicated by stars). Abbreviations: PB, post-burn (at 1, 2 and 3 hr after the heat injury)

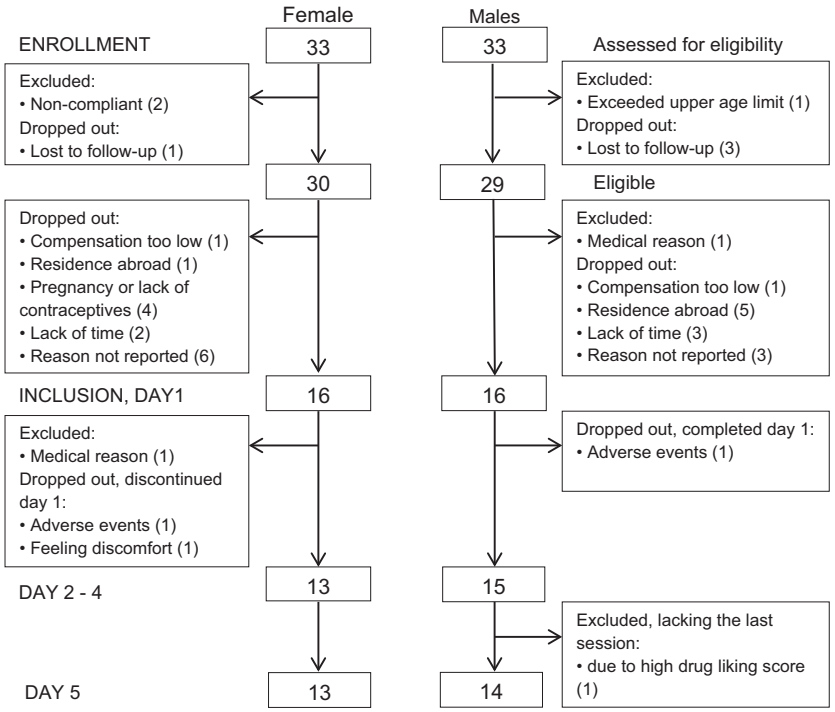

Fig. 2. Subject flow diagram. Illustration of patient enrolment, inclusion, the two study days and enrolment- and eligibility analysis.

or stinging sensation. The secondary hyperalgesia areas were calculated using a computer-based vector algorithm (Canvas 12.0, ACD Systems International, Victoria, Canada). The area of the thermode (12.5 cm^2) was the minimum obtainable value of the test.

Study drug.

Morphine (20 mg/ml; Morphine SAD) was mixed with 480 ml of 0.9% saline. Morphine (10 mg and 20 mg) and placebo (0.9% saline) were administered as i.v. infusions over a 210-min. period. The dose was administered according to the following infusion regimen: between 0 and 15 min., one-fourth of the dose was infused; the remaining three quarters of the dose was infused between 15 and 210 min. Thus, the infusion rates were 166.7 μg/min. and 333.3 μg/min. during the first 15 min., and 38.5 μg/min. and 77.0 μg/min. during the subsequent 195 min. (10 and 20 mg, respectively). The target-controlled infusion regimens were chosen to obtain steady-state conditions (until 1 hr after the heat injury, PB1). Simulations based on a morphine model [23] were performed with Berkeley Madonna (v. 8.3.18, UC, Berkeley, CA, USA) to develop these dose regimens. The dose regimens were chosen as an attempt to minimize potential concentration-dependent adverse events, especially nausea and vomiting, and at the same time, to reach a significant difference between the high and low doses.

As a preventive measure, antiemetics (25 mg hydrocortisone succinate and 2 mg ondansetron) were administered initially to all subjects and a drip of 1000 ml 5.5% isotonic glucose was administered intravenously to prevent fasting symptoms.

The randomization, blinding and packaging of drugs were performed by Herning Hospital Pharmacy, who used the second generator at *randomization.com*. The second generator creates random permutations of treatments for situations where subjects receive all of the treatments in random order. The packaging was identical for all study drugs, and the drug solutions were prepared 1–12 hr before use by pharmaceutically trained staff not involved in other parts of the study.

Vital signs.

An electrocardiogram was taken before morphine infusion was initiated, and exact values of blood pressure, pulse rate, respiratory rate and arterial oxygen saturation were registered before each blood sampling and monitored during the entire sessions.

Blood sampling.

Eight blood samples (10 ml each) were collected before drug administration and 15, 60, 95, 140, 200, 260 and 320 min. after initiation of drug administration. After centrifugation at 1400 × *g* for 10 min., plasma was separated and stored at −20°C until drug analysis.

Quantification of morphine.

The analytes and the internal standard (morphine-D_3) were extracted from plasma using solid-phase extraction (SPE). Chromatographic separation from other constituents of the sample was achieved by ultra performance liquid chromatography (Acquity, Waters, Waters Corp., Milford, CT, USA), followed by tandem mass spectrometry (MS/MS) detection (API 5000,

Applied Biosystems/MDS Sciex, Concord, Ontario, Canada). The analytes were separated by an Atlantis dC18 (5 μm, 150 × 2.1 mm ID) column at ambient temperature, using 8% aqueous ammonium formate (10 mM) in methanol as the eluent at a flow rate of 0.5 ml/min. turbo-ion spray in positive ion mode with selected reaction monitoring mode (MRM). The retention time for morphine was 2.8 min. Morphine and morphine-D_3 were detected at parent/daughter molecular mass of 286.2/152.1 and 289.2/152.1 m/z, respectively, using a cone voltage of 5000 V and a collision energy of 4.0 eV. Peak areas correlated linearly ($r^2 > 0.998 \pm 0.0005$; Mean ± S.D., n = 3) with morphine concentrations in the range 20–1000 ng/ml. Accuracy and precision (mean ± S.D., n = 6) of back-calculated morphine concentrations from plasma calibration standards were 20.1 ± 0.15, 39.6 ± 0.46, 99.9 ± 3.5 ng/ml, 204 ± 3.4 ng/ml and 1020 ± 11.7 ng/ml for the 20, 40, 100, 200 and 1000 ng/ml calibration standards, respectively. To avoid censoring of data, the data were re-analysed with the standard curved extrapolated through zero, and morphine plasma concentrations between 3 and 20 ng/ml were included.

Genetic analyses.
Blood samples were taken to perform an allelic discrimination assay in regard to the single-nucleotide polymorphism (SNP) 118 in the OPRM1 gene, using polymerase chain reaction (PCR) technique. The polymorphism was determined in 27 of the 28 subjects, and the subjects were characterized as being either homozygotes (AA) or heterozygotes (AG). The test was repeated with no divergence. The analyses were performed at the University Hospital of Copenhagen.

PK/PD analysis.
The PK/PD analyses were performed using nonlinear mixed effect (population) modelling with the 'NONMEM' software (Version 7 level 2.0, ICON Development Solutions, Ellicott City, MD, USA) with the Wings For NONMEM interface available from Nick Holford (version 720; http://wfn.sourceforge.net/). The R data analysis language (version 2.14.1) was used for most graphical output and data analysis [24].

Population parameter variability (PPV) was described using either an exponential distribution, which approximates a log-normal distribution:

$$\theta_i = \theta \cdot e^{\eta i} \quad (1)$$

or a normal distribution

$$\theta_i = \theta + \eta i, \quad (2)$$

where θ_i is the value of the parameter for the *i*th subject, θ is the typical value of the parameter in the population, and η_i is a random vector with a mean of zero and a normal distribution with a variance-covariance matrix of between-subject variability (BSV) ω. If data were available on more than one occasion, then between-occasion variability (BOV) was also tested as a random effect. For example, assuming log-normally distributed parameter:

$$\theta_{i,k} = \theta \cdot e^{\eta i + \eta i,k}, \quad (3)$$

where θ_i, k is the value of the parameter for the *i*th subject on the *k*th occasion.

Residual unexplained variability (RUV) model was described using a combination of additive and/or proportional models or an exponential model.

Selection criteria for the final models were based on standard diagnostic plots and parameter precision estimates. For the PK model development, a drop of 3.84 in objective function value (OBJ) for one nested parameter was considered a significant improvement in model fit ($p < 0.05$). The Akaike information criterion was calculated from the OBJ obtained from NONMEM (AIC = OBJ + 2 × npar, where npar is the total number of model parameters). ΔAIC more than -2 was used as indicator of improved model fit [25] for placebo and PD models. Evaluation of final models employed visual predictive checks (VPC) based on 500 simulations of the index dataset using the final model and its parameter estimates with nominal sampling times from the original data.

Pharmacokinetic modelling. The 1-, 2- and 3-compartment models were fitted to the morphine plasma concentration–time data. The covariate analyses included age, sex and BMI as covariates on clearance (CL), volumes (V_1) and intercompartmental clearances (Q). Weight was tested as a covariate on each parameter individually or as allometrically scaled on all parameters.

Pharmacodynamic modelling. Using the final pharmacokinetic model, Bayesian MAP (maximum *a posteriori* probability) estimates of the PK parameters for each individual subject were used to provide the concentrations of morphine used in the PD analyses (i.e. a sequential PK–PD approach). Important aspects of modelling drug effects on pain metrics include a dataset with a realistic representation of the baseline (time zero, drug-free) distribution of the pain metric and a realistic representation of the time course of the metric in the placebo group. The latter is necessary as pain metrics may demonstrate time-dependent changes in the placebo group due to accommodation or sensitization to the stimulus. Avoiding accounting for these changes may confound estimates of any concentration–effect relationship.

Baseline distribution. Inspection of the baseline pain metric data showed that pain metrics were censored. Some EPTo readings were at the maximum obtainable value of 100, while some readings of 2HA were at the minimum possible value of 12.5 cm^2. Furthermore, some subjects on occasions had readings at the maximum (ceiling) or minimum (floor) value throughout the measurement period. Initial investigations using censored baseline distributions were unsatisfactory as these occasions could not be used to estimate model parameters. These occasions were deemed to be indescribable [26]. To extract the maximum possible information from the data, mixture models were used to estimate describable data from the

Table 1.

Ceiling- and floor effect. Different scenarios present for data affected by ceiling- or floor effect and the number of subjects affected by the given scenario. Indescribable indicates that the maximum or minimum values were reached in all four tests (pre-burn and PB1-3) following administration of placebo, low-dose morphine and/or high-dose morphine. Describable indicates that at least one of the four tests reached a value not determined by the cut-off limit of the tests.

	Placebo	Morphine, low dose	Morphine, high dose	Number of Subjects
EPTo	Describable	Describable	Describable	26
	Describable	Indescribable	Indescribable	1
	Indescribable	Describable	Indescribable	1
2HA	Describable	Describable	Describable	19
	Indescribable	Describable	Describable	4
	Describable	Indescribable	Describable	1
	Describable	Indescribable	Indescribable	2
	Indescribable	Indescribable	Indescribable	2

different populations as summarized in table 1. Allocation into the different populations was based on the observed data and was not estimated. Model predictions in the describable data were constrained to be less than 100 for EPTo and > 12.5 for 2HA. The 2HA data were log-transformed to approximate a normal distribution.

Placebo time course. The models for the time course for the placebo response were systematically examined to evaluate whether there were changes over time from baseline, included no change, as well as linear and quadratic functions over time in the change from baseline. The following models were examined:

No change model: no change in effect,

$$\text{Effect}_{\text{placebo}} = \text{BASE}, \tag{4}$$

where BASE = baseline value. The no change model allowed baseline variation between subjects but with no change over time. Furthermore, genetics and sensitivity as covariates on baseline were tested.

Linear model: linear change in effect with time,

$$\text{Effect}_{\text{placebo}} = \text{BASE} + \text{SLP}_{\text{placebo}} \times \text{TIME}, \tag{5}$$

where $\text{SLP}_{\text{placebo}}$ = slope of the change in effect over time. The linear model tests whether time linearly influences the effect after placebo. The linear model was tested with or without population variability on the slope term. Importantly, a model was tested that fixed the slope to 0 (i.e., the population mean did not change over time) with normally distributed between-subject variability still present. This model tested the hypothesis that although, on average, there is no consistent change in the pain metric, it allows for subjects to have changes in pain metric over time in either positive or negative directions. Furthermore, genetics and sensitivity as covariates on baseline and slope were tested.

Quadratic model: quadratic change in effect with time,

$$\begin{aligned}\text{Effect}_{\text{placebo}} = \text{BASE} + \text{SLP}_{\text{placebo}} \times \text{TIME} \\ + \text{SLP}_{\text{placebo2}} \times \text{TIME}^2,\end{aligned} \tag{6}$$

where $\text{SLP}_{\text{placebo2}}$ = slope 2. The quadratic model tests whether there is a curvature in the effect–time relationship. Furthermore, the quadratic model was tested with or without population variability on the slope terms. Genetics and sensitivity as covariates on baseline and slopes and mixture models were tested.

PK/PD modelling.

The models best suited to describe the relationship of the placebo response for the two psychophysiological tests were used to examine the response of these tests parameters to the drug with the assumption that the drug effect was additive;

$$\text{Effect} = \text{Effect}_{\text{placebo}} + \text{Effect}_{\text{drug}}, \tag{7}$$

or proportional to the placebo response:

$$\text{Effect} = \text{Effect}_{\text{placebo}} \times (1 + \text{Effect}_{\text{drug}}). \tag{8}$$

Models were also tested with and without effect compartments to test the hypothesis that there was a delay in the response relative to changes in concentration:

$$dC'_{\text{eff}}dt = k_{e0} \times (C_{\text{plasma}} - C_{\text{eff}}), \tag{9}$$

where k_{e0} is a first-order distribution rate constant describing the rate of change of morphine concentration in the effect compartment (C_{eff}) which is assumed to represent the concentration of the drug at a hypothetical biophase site of action. In the case of models without an effect compartment, then C_{eff} is simply equal to the plasma morphine concentration (C_{plasma}).

In addition, as the placebo response is still present during morphine administration, BOV was also tested in parameters describing a change in effect in the placebo submodel.

The models for the time course for the drug response were systematically examined included linear, $E_{\max}$ and sigmoid $E_{\max}$ models, and models were tested with proportional and/or additive residual error models.

Linear model with effect compartment:

$$\text{Effect}_{\text{drug}} = \text{SLPmorphine} \times C_{\text{eff}}, \tag{10}$$

where $\text{SLP}_{\text{morphine}}$ = the slope of the morphine effect.

$E_{\max}$ model with effect compartment:

$$\text{Effect}_{\text{drug}} = [E_{\max} \times C_{\text{eff}}/(C_{\text{eff}} + EC_{50})], \tag{11}$$

where $E_{\max}$ = the maximum achievable effect and EC_{50} is the drug concentration associated with half the maximum effect.

The E_{max} model is used to determine whether the concentration–effect is nonlinear.

Sigmoid E_{max} model with effect compartment:

$$\text{Effectdrug} = [E_{max} \times C_{eff^n}/(C_{eff^n} + EC_{50^n})], \qquad (12)$$

where n = the Hill coefficient. The Sigmoid E_{max} model is used to determine whether the concentration–effect is sigmoidal in shape.

The following covariates were tested for their effect on model parameters for EPTo and 2HA; weight and BSA (body surface area) were tested using a power function, while the categorical covariates (genetics and sensitivity) were tested using a proportional change model.

Results

Subjects.

The intention-to-treat (ITT) and per-protocol (PP) groups were 32:32 (males:females) and 14:13, respectively (fig. 2).

Three subjects did not complete the study; one subject did not complete the last treatment (low-dose morphine) as he was excluded based on the study exclusion criteria of too high drug liking score [19], one subject withdrew due to feeling uncomfortable before drug infusion, and one subject due to adverse events. Data from these last two subjects were not included in the analyses. Thus, the analyses included 28 subjects for placebo and high-dose morphine and 27 subjects for low-dose morphine.

Significant sex differences were seen in regard to height and weight ($p < 0.0001$) and in age distribution ($p = 0.012$), but there were no significant sex differences in body mass index (BMI; $p = 0.328$, table 2).

In the genetic assays, (SNP) 118 in the OPRM1 gene, three subjects were heterozygote (AG). Data were not obtainable in one subject due to missing blood sample; this subject was assumed to be homozygote (AA).

Morphine pharmacokinetics.

A two-compartment linear model gave the best description of the plasma concentration–time profiles of morphine, compared to a one-compartment model, which failed to describe the terminal phase. A three-compartment model was rejected as it effectively collapsed back to two-compartment model with very low values for the intercompartmental clearance to the third compartment. Moreover, no significant difference in the residual plots between two- and three- compartment models was observed. The final two-compartment base model included a proportional residual error to the morphine plasma concentration, and random effects on CL, V_1 and Q. The inclusion of allometric scaling for weight provided an improvement over the base model (ΔOBJ = −14.9). The population pharmacokinetic parameter estimates of the final PK model are presented in table 3.

All population parameters were estimated within acceptable precision (3.1–27.4% S.E.) as were the estimates for random effects (26.1–57.7% S.E.). Interindividual variability ranged between 14.5% and 83.5% with the highest variability found for V_1. The model had a residual unexplained variability of 15.7%. The model had acceptable shrinkage values for CL and V_1 (4.04% and 24.2%, respectively), but slightly high values for Q (52.9%). Omitting interindividual variability on Q, however, gave a significantly worse model-fit to data (ΔOBJ = 3.9), and this random effect was therefore kept in the model in spite of the high shrinkage value. This was further justified as the PK model was mainly intended to provide MAP Bayes estimates for the PD modelling stage. There was an acceptable agreement between the observed and individually predicted concentrations (fig. 3).

The VPC based on 500 simulations of the final PK model suggested an acceptable fit of the model to the morphine plasma concentration–time data for both doses (fig. 4).

Placebo response.

Several subjects were affected by ceiling- or floor-effects (indescribable data): the tests were affected by these limitations in 11 of 56 cases (table 1).

The modellings of the EPTo and 2HA during the placebo treatment were best described by a linear model and quadratic model, respectively. The best fit for the model for EPTo was obtained with the numbers of non-responders (subjects not reaching their electrical pain tolerance due to ethical and

Table 2.

Anthropometric data. Age (years), height (cm), weight (kg) and BMI (kg/m^2) reported as mean, with standard deviation in brackets (n = 28). Abbreviation: BMI, body mass index.

Sex	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Females	23.2 (2.0)	168.3 (6.2)	64.7 (7.4)	22.9 (2.6)
Males	26.7 (4.3)	184.4 (7.5)	81.0 (9.4)	23.8 (2.2)
Females and males	25.1 (3.8)	176.9 (10.6)	73.4 (11.8)	23.4 (2.4)

Table 3.

Parameter estimates and standard error (%) of the final pharmacokinetic model. Abbreviations: V_1, central distribution volume; CL, apparent clearance; V_2, peripheral distribution volume; Q_2, intercompartmental clearance between V_1 and V_2; WT, subject's weight;% S.E., standard error the parameter estimates.

Model parameter	Estimates	% S.E.	% Shrinkage
CL	$\theta_1 \times$ (WT/70)^0.75		
V_1	$\theta_2 \times$ (WT/70)		
Q	$\theta_3 \times$ (WT/70)^0.75		
V_2	$\theta_4 \times$ (WT/70)		
Population estimates			
θ_1 (L/min)	1.37	3.1	
θ_2 (L)	19.4	27.4	
θ_3 (L/min)	2.73	5.3	
θ_4 (L)	134	6.0	
Interindividual variability (%CV)			
ωCL	14.5	26.1	4.04
ωV_1	83.5	41.3	24.2
ωQ	19.4	57.7	52.9
Residual variability			
ε (%CV)	15.7	22.1	6.2

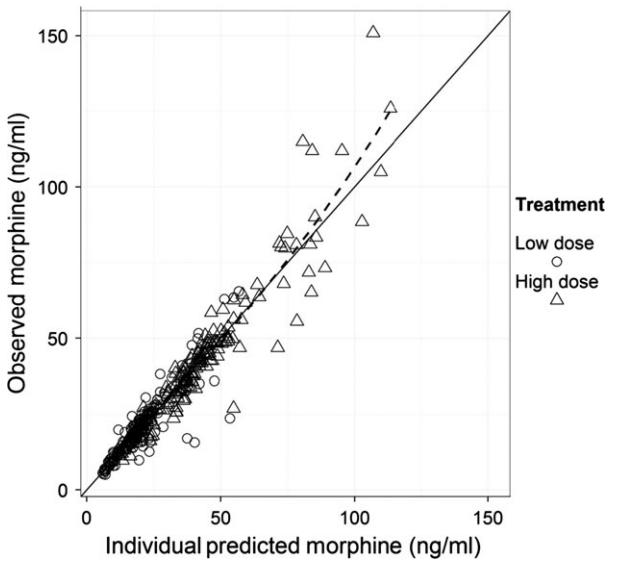

Fig. 3. Observed morphine *versus* individual predicted morphine concentrations

medical limitations of the testing procedure) fixed to that from the observed data, and with sensitivity (low- *versus* high pain sensitivity) included as a covariate on baseline. The best fit for the model for 2HA was obtained with the genetic variable included as a covariate on the slope of the placebo response.

Electrical pain tolerance model (EPTo).

The model discrimination process showed clear evidence for adding BOV on baseline (ΔAIC = −13.3) and an effect-delay compartment (k_{e0}; ΔAIC = −91.7). A linear drug effect to the final model for EPTo improved the fit compared to no drug effect (ΔAIC = −12.9). However, more complex drug effect models did not show further improvement (ΔAIC > 25), were unstable or did not converge. A drug effect proportional to the placebo effect was preferred over an additive relationship (ΔAIC = −27). The sensitivity covariate was significant on the baseline measurements (ΔAIC = −2.7), but not on the drug effect slope parameter (ΔAIC = −0.5). Population variability or a genetic covariate on the drug effect slope parameter failed to improve the fit of the model (ΔAIC = 5.3 and ΔAIC = 1.6, respectively). Box-Cox transformation was investigated to allow skewness in the baseline distribution; however, this improvement was not substantial (ΔAIC = −2.1).

Population parameters for the EPTo final model were estimated within acceptable precision (11.0–40.3%S.E.) as was the precision for interindividual variability (23.0–62.4%S.E.) (table 4). Interindividual variability ranged between 19% and 46% with the highest variability found for the slope for the morphine effect. The effect-delay rate constant, k_{e0}, (0.000243 min^{-1}) resulted in a relatively long equilibration $t_{1/2}$ (47.5 hr) but was estimated with a good precision (10.9% S.E.). Removing the effect-compartment from the final model gave a significantly worse fit of the model to the data (ΔOBJ = 98).

The baseline EPTo had a relatively high population parameter variability of 46%, of which BOV account for approximately 19%. Figure 5 shows the baseline distribution for the observed and individual predicted values for EPTo.

The model showed good agreement between the observed and individual predicted effects (fig. 6).

Secondary hyperalgesia model.

Based on the defined model development criteria, the best fit for 2HA was a linear concentration–effect model with an effect-compartment delay including BOV and genetics as a covariate on the placebo slope and baseline fixed to log (12.5 cm), which is the area of the thermode. The model had a significant linear drug effect with interindividual variability

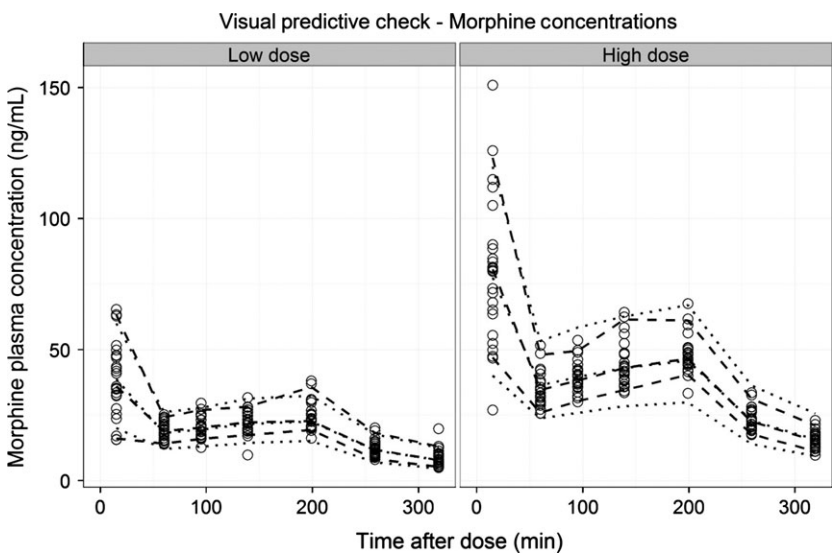

Fig. 4. Visual predictive check. Observed and predicted morphine plasma concentration–time profiles after i.v. infusion of a low- and high-dose of morphine, using the final PK model with weight as covariate. Dashed lines are median and the 5% (lower) and 95% (upper) confidence intervals for the observed data. Dotted lines are median and the 5% (lower) and 95% (upper) confidence intervals for the simulated data.

Table 4.
Population estimates and interindividual variability for the preferred model of EPTo. $t_{½,\ ke0}$ calculated as $\ln(2)/k_{e0}$. Abbreviations: EPTo: electrical pain tolerance, BASE: baseline, $SLP_{placebo}$: linear slope on placebo, SLP_{MOR}: slope on morphine, $Sens_{BASE}$: sensitivity covariate on baseline, k_{e0}: effect compartment rate constant, $t_{½,ke0}$: effect site equilibration half-life, half-time, and ε: proportional residual error.

Model parameter	Estimates	% S.E.
BASE	$\theta_1 \times (1 + Sens_{BASE})$	
$Sens_{BASE}$	θ_2	
$SLP_{placebo}$	θ_3	
SLP_{MOR}	θ_4	
k_{e0}	θ_5	
Population estimates		
θ_1	19.7	11.0
θ_2	0.784	40.3
θ_3	−0.00792	16.3
θ_4	0.213	28.5
θ_5 (min^{-1})	0.000243	10.9
$t_{½,\ ke0}$ (h)	47.5	
Population parameter variability (PPV)		
ω_{PPV}BASE (%CV)	45.6	23.0
ω_{BOV}BASE (%PPV)	19	57.9
ω SLP_{MOR} (S.D.)	0.145	62.4
Residual variability		
ε (S.D.)	0.172	21.3

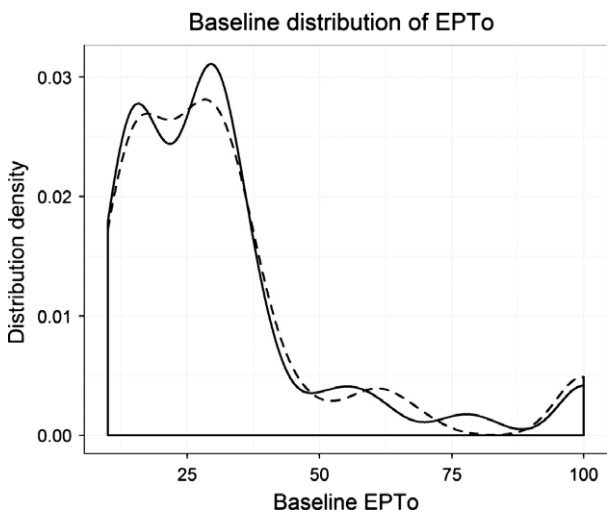

Fig. 5. Baseline distributions for EPTo. Observed (dashed) and individual predicted (solid) baseline data. Abbreviation: EPTo, electrical pain tolerance [arbitrary scale (0–100)].

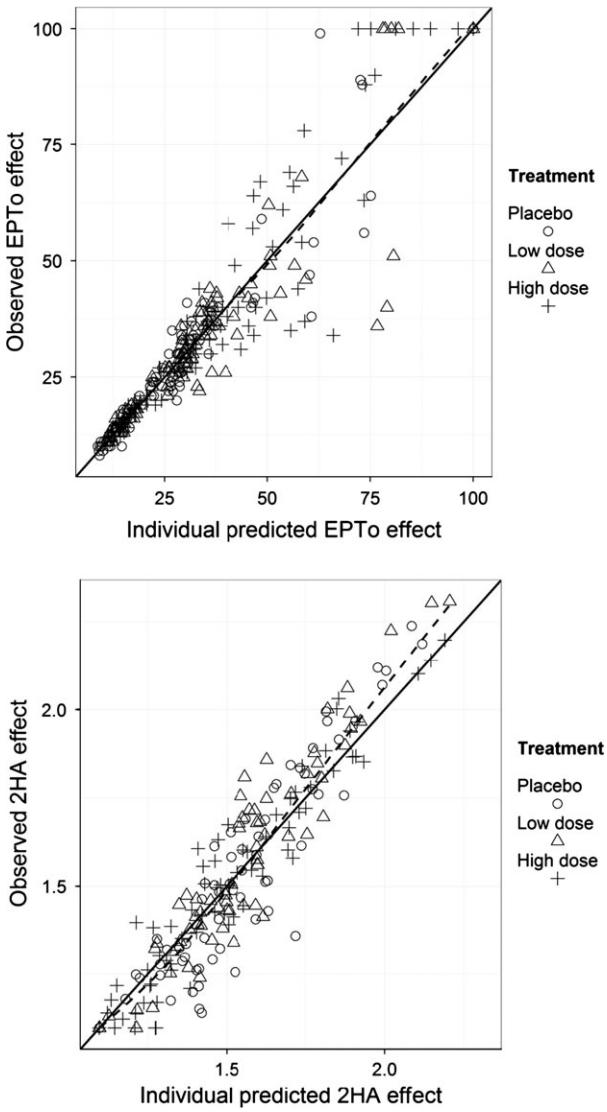

Fig. 6. Observed and individual predicted data for EPTo (top) and 2HA (bottom). Data from placebo (open circles), low dose (triangle) and high dose (cross) of morphine. The solid lines indicate a line of slope 1, and the dashed line is a less smoothed line. Abbreviations: EPTo, electrical pain tolerance; 2HA, secondary hyperalgesia area.

on this parameter, but more complex drug effect models did not show further improvement (ΔAIC > 20), were unstable or did not converge. A drug effect proportional to the placebo effect was preferred over an additive relationship (ΔAIC = −46.9). Compared to the final model, estimating baseline (ΔAIC = 2.2), or allowing PPV with or without BOV, on baseline worsened the fit (ΔAIC >200) and resulted in unstable models. Similarly when assuming a normally distributed PPV (ΔAIC = 569) or removing BOV on drug effect (ΔAIC = 119). Removing the effect of genetics on drug effect resulted in a marked worsening of model fit (ΔAIC = 51.6). Removal of the effect compartment delay (k_{e0}) resulted in a model which terminated abnormally over a wide range of initial estimates.

The population estimates and interindividual variability for the preferred model for 2HA are listed in table 5.

Population parameters for 2HA were estimated within the precision range of 1.9–65%, except genetics as a covariate on the placebo slope ($SLP_{placebo}$), which had a %S.E. of 148. However, removing this covariate from the model caused an increase of 49 in OBJ, and it was therefore kept in the final model. Interindividual variability ranged between 63% and 345%, with the highest variability found for the slope on baseline, and with interindividual variability estimates between 0.5% and 57.8% S.E. The distribution rate constant,

Table 5.
Population estimates and interindividual variability for the preferred model of 2HA. $t_{½,\ ke0}$ calculated as $\ln(2)/k_{e0}$. Abbreviations: 2HA, secondary hyperalgesia; BASE, baseline; $SLP_{placebo}$, linear slope on placebo; $SLP2_{placebo}$, quadratic slope on placebo; SLP_{MOR}, slope on morphine; Gen_{BASE}, genetic covariate on baseline; k_{e0}, effect compartment rate constant; $t_{½,ke0}$, effect site equilibration half-life; half-time, and ε: proportional residual error.

Model parameter	Estimates	% S.E.
BASE	θ_1	
Gen_{SLP}	θ_2	
$SLP_{placebo}$	θ_3	
$SLP2_{placebo}$	θ_4	
SLP_{MOR}	θ_5	
k_{e0}	θ_6	
Population estimates		
θ_1 (cm^2)	Log(12.5) = 1.1	8.8
θ_2	0.00453	11.0
θ_3	−0.000102	64.7
θ_4	−0.00266	147.7
θ_5	0.0853	1.9
θ_6 (min^{-1})	0.00756	
$t_{½,\ ke0}$ (h)	1.5	
Population parameter variability (PPV)		
$\omega_{PPV}SLP_{placebo}$ (%CV)	12.3	250
$\omega_{BOV}SLP_{placebo}$ (%PPV)	57.8	345
ω SLP_{MOR} (S.D.)	0.45	63.1
Residual variability		
ε (S.D.)	6.9	77.8

k_{e0}, was slightly high (0.00756 min^{-1}) but was estimated well (1.9%S.E.). The model had a proportional residual error of prediction of 6.9%. The model showed good agreement between the observed and individual predicted effects (fig. 6), although with a slight tendency towards under predictions at the high end of the scale for 2HA and the model failed to reach the non-responders.

Discussion

In the present study, population PK/PD models were developed to characterize the analgesic and antihyperalgesic effects of morphine, in addition to delineate a placebo-response model in healthy volunteers.

Morphine pharmacokinetics.
The population pharmacokinetics of morphine after intravenous administration was successfully described by a two-compartment distribution model with first-order elimination from the central compartment. Typical values and interindividual variability for the population PK parameters were estimated with acceptable precision and within the range of that reported in previous literature [23,27] for a 70-kg person. Unexplained interindividual variability was significantly reduced by inclusion of body-weight allometrically scaled to clearances and volumes, suggesting that a more uniform morphine exposure is achieved in healthy volunteers, when doses are weight-adjusted accordingly.

Analgesic versus *antihyperalgesic effect.*
One of the primary aims of the study was to evaluate the PK/PD relationship of morphine using an experimental model of analgesic (ETPo) and antihyperalgesic (2HA) responses. The results from the study corroborate the analgesic effects of morphine observed in a number of studies [28–30] using heat models (heat injury, heat-capsaicin, brief thermal stimulation), but the lack of antihyperalgesic effect of morphine was originally unexpected as up to 85% reductions in hyperalgesia areas has been reported after use of morphine [29]. However, the PK/PD analyses were in accordance with the findings from the statistical analyses reported earlier [19].

For ETPo, a linear model, and for 2HA, a quadratic model, were used to describe the time–response relationship for placebo treatment, signifying the importance of including this in the evaluation of the morphine response in the experimental models to avoid bias, as the responses to the stimuli were not constant over time.

A linear concentration–effect model incorporating an effect-compartment delay relative to changes in plasma concentration provided the best fit to both the ETPo- and 2HA response. Blood samples were taken at fixed time-points during each session; more individually scattered sample times would have been preferable as more modelling points would have been available. In spite of the sparse sampling times, acceptable parameter precision was obtained for the ETPo model. After accounting for the placebo response, a significant effect of morphine was found on the ETPo response in terms of higher pain threshold with increasing morphine concentration. However, compared to data from previous studies, a surprisingly long effect-compartment half-life was suggested for the analgesic response (47.5 hr). In healthy volunteers and patients, morphine has been reported to have a $t_{½,\ ke0}$ around 0.7–7 hr with various quantitative sensory testing (QST) response types [31], including electrical skin stimulation [32]. It could be speculated that the active metabolite M6G may contribute to the prolonged $t_{½,\ ke0}$ in the present study. However, given M6G's relatively low appearance rate [27] and long effect site equilibration with an equilibration half-life ($t_{½,\ ke0}$) in the range of 6–8 hr [31,33,34] combined with the condition that the last PD measurements and blood samples were taken at 5.5 hr, it is unlikely that a significant contribution from M6G, in relation to the analgesic and antihyperalgesic effects, was present. A study design with more response assessment times and longer study duration would likely have been beneficial if the scope was to determine the most accurate $t_{½,\ ke0}$ for morphine, in the electrical pain tolerance model. However, the large drop in OBJ signifies the importance of having an effect compartment in the model, to describe to present dataset.

Individual pain sensitivity (determined by the pain intensity during the burn injury) was significantly positively correlated with baseline score for ETPo, but did not appear to have a significant relationship with the drug response slope. This significant finding at baseline confirms the results from the previous study [20] in which EPTo was one of the predictive variables of the heat injury from which the subjects were categorized as either low- or high pain-sensitive subjects.

In relation to the antihyperalgesic response, no significant effect of morphine was suggested by the pharmacodynamics drug slope ($SLP_{morphine}$), with a 95% CI that entails zero [0.001; −0.006]. A significant impact of the examined SNP variants was found on $SLP_{placebo}$ (8% increase), suggesting that the AG variant causes increased hyperalgesia.

The EPTo and 2HA analyses of the present study were complicated by the maximum and minimum obtainable values. EPTo demonstrated ceiling effects and 2HA demonstrated floor effects. Hence, the data did not contain sufficient information to estimate the maximum analgesic and antihyperalgesic effects of morphine. However, the impact was limited as the data only were deemed indescribable in cases where all four measurements reached the maximum or minimum obtainable values (table 1).

Thus, in conclusion, a population PK/PD model has been developed for morphine which indicates that the analgesic and antihyperalgesic effects of morphine are pharmacologically distinct entities. Among the investigated covariates, bodyweight has been suggested as significantly associated with the pharmacokinetics of morphine, while pain sensitivity is significantly related to analgesic baseline score and the A118G SNP on the OPRM1 receptor-expressing gene is associated with an increased hyperalgesic area.

Using nonlinear mixed effect modelling, this study has successfully discriminated between analgesic and antihyperalgesic pharmacodynamics properties of morphine. Additionally, the population PK/PD modelling approach enabled identification of important factors explaining interindividual variability (weight for PK, subject sensitivity and genetics). The number of subjects was limited by the study design with the need to include the high and low pain-sensitive subjects from a previous study. Some of the parameters possessed relatively high standard errors, which may reflect the low number of subjects or model misspecification. Also, the relatively small number of heterozygotes should be taken into consideration when evaluating the genetic aspects and the clinical implication. The presented models may be useful for simulation and design of future trials involving experimental pain models using electric pain stimulation in healthy volunteers.

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