Web Page Supplements

Appendix 1. Determination of Serum Concentrations of Morphine, Morphine-3-glucuronide and Morphine-6-glucuronide.

Serum concentrations of morphine, M3G and M6G were determined using solid-phase extraction and reverse-phase high-performance liquid chromatography (HPLC) with spectrophotometric and electrochemical detection.

Ten microliters of the internal standard solution (5μg/ml hydromorphone in methanol) and 200 μL carbonate buffer solution (0.79 g/L NH_4HCO_3, adjusted to pH 9.3 with 4 M KOH) were added to 200 μl serum, followed by whirlmixing for 5 s. Solid phase extraction columns (Sep Pak Vac 100 mg C18, Waters, Milford, MA) were washed twice with 1 ml methanol and once with 1 mL water and 1 mL carbonate buffer (pH 9.3). Subsequently, samples were applied to the columns (rate 0.2–1 ml/min). After waiting 1 min, the columns were washed with 1 ml carbonate buffer and 1 ml water, and then dried for 5 min under vacuum. Morphine and its glucuronides were eluted with 0.5 mL 0.005 M acetic acid in methanol. The eluent was evaporated to dryness at 50 °C under a stream of dry nitrogen. The residue was then redissolved in 100 μl of the mobile phase, consisting of water/85% H_3PO_4 (940:1.2 v/v and adjusted to pH 4.0 with 4 M KOH)-methanol (941.2:60 v/v) and 30 μl were introduced in to the HPLC system.

The HPLC system consisted of a Model 300 HPLC, a Gina 50 automatic sample injector pump (both Gynkotek, Germering, Germany), a Spectroflow 773 variable wavelength detector (Kratos Analytical Instruments, Westwood, NJ) and an Antec CU-04AZ electrochemical detector (Antec, Leiden, The Netherlands), placed in series, and a Chromeleon chromatography data system (Softron, Gräfelfing, Germany). The column was a stainless steel ODS-AQ 3μm (100 $\times$ 4.6 mm i.d.) and was used together with a guard column (17 $\times$ 4.6 mm i.d.) with the same material (both YMC Co., Ltd, Japan). The column temperature was ambient. The flow rate of the mobile phase was 1.00 ml/min. Morphine, M3G and hydromorphone were assayed using spectrophotometric detection. The UV absorption wavelenth was set at 210 nm. M6G and hydromorphone were assayed using electrochemical detection. The voltage of the electrochemical detection was set at 0.90 V. The retention times of M3G, M6G, morphine and hydromorphone were 2.65, 5.15, 5.9 and 10.4 min, respectively, and the total run times were 38 min. Extraction recoveries were 85%, 83%, 80% and 75% for morphine, M3G, M6G and hydromorphone,

respectively. Calibration lines were linear in the investigated ranges (5–100 ng/ml morphine; 15-300 ng/ml M3G; 5–100 ng/ml M6G). Within-day coefficients of variation (7 determinations) were: 4.3% at 10 ng/ml and 0.9% at 75 ng/ml morphine; 9.4% at 30 ng/ml and 5.8% at 225 ng/ml M3G; 5.9% at 10 ng/ml and 3.9% at 75 ng/ml M6G. Inter-day coefficients of variation (41 determinations) were 6.5%, 12.5% and 14.5% at 19 ng/ml morphine, 104 ng/ml M3G and 31 ng/ml M6G, respectively. Quantification limits were set at 5 ng/ml morphine, 30 ng/ml M3G and 10 ng/ml M6G.

Appendix 2. Details of the Pharmacodynamic Analysis

Pharmacodynamic model

The relationship between the (estimated) effect-site morphine concentration and the electrical current necessary to exceed a pain response threshold, shown in the figure and described by equation 3 in the main text, is analyzed as follows. First, the perceived pain level at a certain stimulus was assessed in the form of binary response data (no response or pain/stop) for which logistic regression is the commonly used technique. Let X_n denote the event that a response threshold is exceeded at a stimulus current of n mA (with n = 10, 20, $\cdots$, cutoff [= 80]). If an arbitrary current was applied only once, the probability of X_n is given by

$$P\{X_n\} = \int_0^n \text{lpdf}(x;\mu,\sigma)dx \tag{4}$$

where lpdf is the log-logistic probability density function. For convenience, μ and σ denote the median and standard deviation of the underlying logistic probability density function, respectively. However, because n increased in steps of 10 mA, recording the fact "X_n" actually implies that the response threshold was not exceeded at lower imposed currents. Assuming that the probabilities of consecutive responses (for example: no response — no response — no response — pain, occurring at 10, 20, 30 and 40 mA) are independent, the probability of recording X_n is then given by

$$P\{\text{recording } X_n\} = P\{X_n\} \cdot \prod_{i=10}^{n-10} (1 - P\{X_i\}) \approx \int_{n-10}^{n} \text{lpdf}(x;\mu,\sigma)dx \text{ for small } \sigma \tag{5}$$

The effect-site concentration affects the the probability of response by assuming that

$$\mu(t) = \text{baseline} \cdot (1 + (C_e(t)/AC_{50})^{\gamma}) \tag{6}$$

The maximum likelihood approach comprises of maximizing the product of the probabilities of the occurrence of measurements, assuming that the measurements are independent. Moreover, the probability that X_n is not achieved at or below the cutoff is given by

$$P\{\text{no response at or below cutoff}\} = \int_{\text{cutoff}}^{\infty} \text{lpdf}(x;\mu,\sigma)dx \tag{7}$$

Note that σ determines how steep the response thresholds are in terms of the probability of response (see figure 1 in the main text).

Parameter estimation

Population parameters values of k_{e0}, baseline, AC_{50}, γ and σ were estimated using the maximum likelihood estimation method of NONMEM (NONMEM User's Guides, SL Beal and LB Sheiner (Eds.) NONMEM Project Group, University of California at San Francisco, San Francisco. 1999). The improvement of the model fit by inclusion of a linear dependence of the above parameters on each of the covariates sex, age, weight and lean body mass was tested using the likelihood ratio criterion: a decrease of the objective function value of more than 6.64 was considered significant ($P < 0.01$). For the covariate sex, this is equivalent to testing whether separate parameters for each gender improves the fit. Note that intraindividual variability is represented by parameter σ introduced above. Interindividual variability of the model parameters was assumed to be lognormally distributed and variance parameters were included until no significant decrease of the objective function was observed.

Goodness of fit

The goodness of the individual Bayesian fits was assessed by calculating the residual deviance (unit = bits, *i.e.*, binary digital representation of information), defined as twice the difference between the maximum achievable log likelihood and that attained under the fitted model. The deviance is proportional to the weighted residual sum of squares in ordinary regression with continuous data. To compare goodness of fits between data sets the deviance was normalized by dividing it by twice the number of measurements, resulting in a measure of the average amount of additional information necessary to achieve best fit. Thus, the smaller the normalized deviance the better the fit. For further explanation see McCullagh P, JA Nelder: Generalized linear models. 2nd edition. London, Chapman and Hall, 1990, p 118; Shannon CE, Weaver W: The Mathematical Theory of Communication. Urbana, Illinois, University of Illinois University Press, 1963.

Appendix 3. Population Pharmacokinetics of Morphine.

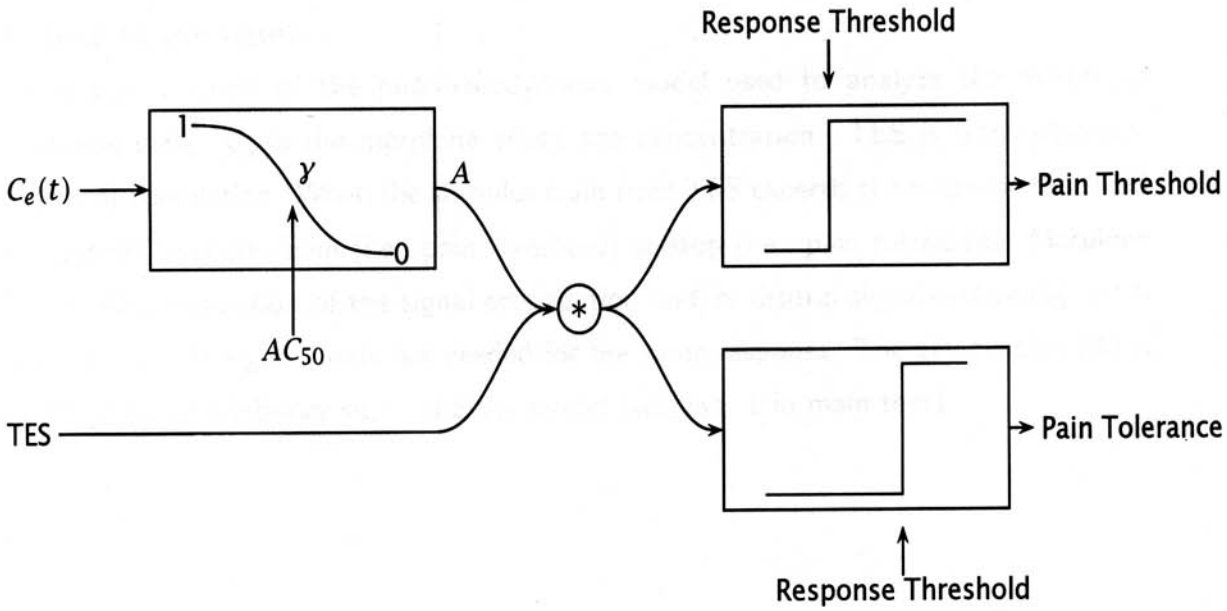
Although not designed for pharmacokinetic analysis, we did subject our morphine *vs.* time data set to population pharmacokinetic analysis using NONMEM. Two- and three-compartment pharmacokinetic models were fitted to the data. The improvement of the model fit by inclusion of covariates (sex, age, weight, and lean body mass) was tested using the likelihood ratio criterion (Rao CR: Linear Statistical Inference and its Application. 2nd edition. New York, John Wiley & Sons, 1973). Since these data may be of clinical importance (e.g. for the development of target controlled infusion systems) we present the results here.

The data was best described by a three-compartment model with rate constants k_{ij} and weight-normalized V_1. For none of the model parameters did the inclusion of the covariates improve the model fits ($P > 0.01$). The analysis resulted in the following population parameters, standard errors (SE) and measures of interindividual variability (%CV or percentage coefficient of variation):

	value	SE	%CV
V_1	0.075	0.034	13
k_{10}	0.300	0.115	12
k_{12}	0.183	0.098	11
k_{21}	0.087	0.055	20
k_{13}	0.290	0.144	14
k_{31}	0.013	0.0017	18

Units of V_1 and k are L/kg and min^{-1}, respectively.

The performance of the population model was assessed in terms of the ability to fit the measured blood concentrations. This was accomplished by calculating the median absolute weighted residual (MDAWR) and median weighted residual (MDWR) (Egan TD, Huizinga B, Gupta SK, Jaarsma RL, Sperry RJ, Yee JB, Muis KT: Remifentanil pharmacokinetics in obese versus lean patients. Anesthesiology 1998; 98: 562–73). The MDAWR (a measure of model accuracy) was 17%. The MDWR (a measure of model bias) was 2%.


Response Threshold
$C_e(t)$
1
γ
0
A
AC_{50}
*
TES
Pain Threshold
Pain Tolerance
Response Threshold

Legend to the figure.

Schematic diagram of the pharmacodynamic model used to analyze the morphine-analgesia data. C_e is the morphine effect site concentration. TES is transcutaneous electrical stimulation. When the stimulus train from TES exceeds the response threshold the subject indicates pain (i.e., pain threshold) or stop (i.e., pain tolerance). Morphine causes the attenuation of the signal propagation and/or central signal processing. As a consequence stronger stimuli are needed for the same response. The attenuation (A) is modeled by an inhibitory sigmoid E_{max} model (see eqn. 1 in main text).