Cyclosporine-inhibitable Blood-Brain Barrier Drug Transport Influences Clinical Morphine Pharmacodynamics

Konrad Meissner, M.D.¹, Michael J. Avram, Ph.D.², Viktar Yermolenka, Ph.D.³, Amber M. Francis, R.N., B.S.N.⁴, Jane Blood, R.N., B.S.N.⁵, and Evan D. Kharasch, M.D., Ph.D.⁶,*

Department of Anesthesiology, Universitätsmedizin Greifswald, the Departments of Anesthesiology and of Biochemistry and Molecular Biophysics, Washington University in St. Louis, and the Department of Anesthesiology, Northwestern University Feinberg School of Medicine

¹Associate Professor of Anesthesiology, Universitätsmedizin Greifswald, Klinik für Anästhesiologie und Intensivmedizin, Greifswald, Germany, Department of Anesthesiology, Division of Clinical and Translational Research, Washington University in St. Louis, St. Louis Missouri

²Professor of Anesthesiology, Department of Anesthesiology, Northwestern University Feinberg School of Medicine, Chicago, Illinois

³Research Technician, Department of Anesthesiology, Division of Clinical and Translational Research, Washington University in St. Louis, St. Louis, Missouri

⁴Clinical Research Coordinator, Department of Anesthesiology, Division of Clinical and Translational Research, Washington University in St. Louis, St. Louis, Missouri

⁵Head Research Nurse, Department of Anesthesiology, Division of Clinical and Translational Research, Washington University in St. Louis, St. Louis, Missouri

⁶Russell D. and Mary B. Shelden Professor of Anesthesiology, Professor of Biochemistry and Molecular Biophysics, Vice-Chancellor for Research, Department of Anesthesiology, Division of Clinical and Translational Research, Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis, St. Louis, Missouri

Abstract

Background—The blood-brain barrier is richly populated by active influx and efflux transporters influencing brain drug concentrations. Morphine, a drug with delayed clinical onset, is a substrate for the efflux transporter P-glycoprotein in vitro and in animals. This investigation tested whether morphine is a transporter substrate in humans.

Methods—Fourteen healthy volunteers received morphine (0.1 mg/kg, 1 h intravenous infusion) in a crossover study after nothing (control) or the validated P-glycoprotein inhibitor cyclosporine (5 mg/kg, 2 h infusion). Plasma and urine morphine and morphine glucuronide metabolite
concentrations were measured by mass spectrometry. Morphine effects were measured by miosis and analgesia.

**Results**—Cyclosporine minimally altered morphine disposition, increasing the area under the plasma morphine concentration versus time curve to 100 ± 21 versus 85 ± 24 ng/ml•hr (p < 0.05) without changing maximum plasma concentration. Cyclosporine enhanced (3.2 ± 0.9 vs. 2.5 ± 1.0 mm peak) and prolonged miosis, and increased the area under the miosis-time curve (18 ± 9 vs. 11 ± 5 mm•hr), plasma-effect site transfer rate constant (k$_{e0}$, median 0.27 vs. 0.17 hr$^{-1}$), and maximum calculated effect site morphine concentration (11.5 ± 3.7 vs. 7.6 ± 2.9 ng/ml) (all p < 0.05). Analgesia testing was confounded by cyclosporine-related pain.

**Conclusions**—Morphine is a transporter substrate at the human blood-brain barrier. Results suggest a role for P-glycoprotein or other efflux transporters in brain morphine access, although the magnitude of the effect is small, and unlikely to be a major determinant of morphine clinical effects. Efflux may explain some variability in clinical morphine effects.

**INTRODUCTION**

Morphine is a most peculiar and curiously-acting opioid. Although there is well-described inter- and intraindividual variability in morphine disposition and effects, more perplexing is the unexplained disparity between plasma concentrations and clinical effect. This disparity is exemplified by the long delay in onset of analgesia, miosis, and respiratory depression. Generally, delay between the time courses of plasma drug (opioid) concentration and clinical effect is explained by a hypothetical effect compartment with a half-life ($t_{1/2ke0}$) characterizing first order drug transfer from plasma to this compartment. Whereas the $t_{1/2ke0}$ is 1 min for ultrafast onset opioids (remifentanil, alfentanil) and 5-9 min for fast onset opioids (sufentanil, fentanyl, methadone), it is 2-4 h for morphine. Morphine effects and effect-site concentrations can increase while plasma concentrations decline. The mechanism for delayed morphine effects is unknown.

The human blood-brain barrier has a variety of protective mechanisms, including both a structural barrier (endothelial cells with tight junctions, astrocytes, pericytes), and a functional barrier (numerous influx or efflux active transport systems). Drug efflux transporters of the adenosine-triphosphate-binding cassette family are energy-dependent, membrane-bound proteins, including P-glycoprotein (P-gp, ABCB1, multidrug resistance protein 1, MDR1), breast cancer resistance protein (ABCG2), and multidrug resistance-related proteins (ABCC proteins). P-gp is expressed in brain capillary endothelial cells.

Considerable evidence suggests that morphine is a P-gp substrate *in vitro* and in animals. P-gp transported morphine out of bovine brain capillary endothelial cells, and P-gp inhibitors (cyclosporine, GF120918) impaired morphine efflux. P-gp involvement in brain morphine disposition in animals was shown using genetic knock-outs and chemical inhibitors. In mdr1a (murine MDR1 homologue) knockout mice, compared with wild-type mice, morphine brain uptake clearance was moderately (1.2-fold) increased, microdialysis and *in-situ* brain perfusion confirmed significant contribution of mdr1 to morphine uptake. Morphine antinociception was significantly greater, and the ED$_{50}$ for morphine antinociception was reduced in half. Among 16 strains of inbred mice, morphine hyperalgesia was most strongly associated with P-gp gene haplotype. Wild-type animals pre-treated with the P-gp inhibitor verapamil behaved like mdr1a-deficient animals. Cyclosporine markedly increased morphine analgesia in wild-type but not mdr1a knockouts. In rats, GF120918 increased cerebral morphine uptake, the area under the morphine concentration-time curve in brain extracellular fluid and tissue, and increased antinociception approximately 3-fold.
In contrast to cellular and animal studies, there is less information on P-gp and morphine brain access in humans. Some P-gp inhibitors have been tested. PSC833 had no effect on morphine-related respiratory depression. Quinidine did not alter intravenous morphine-dependent changes in pupil diameter (miosis) or respiratory depression, or morphine concentration-effect relationships. Although quinidine did increase miosis after oral morphine, this was attributed to intestinal P-gp inhibition, increased morphine absorption, and increased plasma concentrations rather than enhanced brain penetration and altered blood-brain barrier P-gp activity. Quinidine however is a comparatively nonpotent P-gp inhibitor, and plasma quinidine concentrations may not have been sufficient to inhibit brain P-gp activity and P-gp-mediated morphine transport (if present).

More recently, better clinical P-gp inhibitors have been identified. For example, cyclosporine has been shown to inhibit human blood-brain barrier P-gp activity. Specifically, intracerebral concentrations of the P-gp substrate verapamil, quantified using positron emission tomography imaging, were increased 79% by cyclosporine. This finding validated cyclosporine as an in vivo inhibitory P-gp probe in humans.

This investigation tested the hypothesis that morphine is a substrate for human blood-brain barrier drug transporters, such as P-glycoprotein, and that transport activity influences morphine plasma concentration-effect relationship (pharmacodynamics). Cyclosporine was used as a drug transport inhibitor. Morphine concentration-effect relationships were studied using pupil diameter as a primary measure of effect and analgesia as a secondary measure in a single-center, open-label, randomized crossover study in healthy volunteers.

**MATERIALS AND METHODS**

**Clinical Protocol**

The protocol was a sequential open-label crossover study in healthy volunteers. It was approved by the Institutional Review Board of Washington University in St. Louis, Missouri, and all subjects provided written informed consent to participate. Males and females, age 18-40 yr, in good general health with no remarkable medical conditions and a body mass index of 20-33 kg/m² were eligible for inclusion in this study. Exclusion criteria were a history of major medical problems, including a history of liver or kidney disease, use of prescription or non-prescription medications, herbals, or foods known to be substrates of P-glycoprotein or to affect its activity, pregnant or nursing females, and a known history of addiction to drugs or alcohol. Sixteen healthy volunteers (10 males and 6 females) were enrolled in the study. The subjects were asked to abstain from apples and citrus foods or juices for 5 days before the study day and on the study day, because these may influence transporter function, and from alcohol or caffeine for 1 day before the study and on the study day, and to not ingest any food or liquid after midnight before each study day. Intravenous catheters were placed in one arm for blood sampling and in the contralateral arm for drug administration, on each study day. Monitoring after opioid administration consisted of a standard electrocardiogram, blood pressure, and pulse oximetry. Subjects received supplemental oxygen if oxygen saturation decreased to less than 94%.

Subjects were familiarized with the test procedures, including drug administration, pupil measurements, and analgesia testing at a pre-study visit. The first study session (control) consisted of a morphine infusion (0.1 mg/kg over 1 h), given after baseline measurements. No placebo was infused in lieu of cyclosporine. Subjects were given antiemetic prophylaxis with ondansetron (4 mg IV) prior to morphine administration. Venous blood samples for morphine and morphine metabolite analysis were obtained just before and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, and 10 h after the start of the morphine infusion. Plasma was separated and stored at −20°C for later analysis. Subjects were instructed to
save all of their urine for 24 h after the morphine infusion. The volume returned was recorded, and an aliquot was stored at −20°C for later analysis. Subjects were given a standard breakfast 1 h after the end of the morphine administration and had free access to food and water thereafter. Subjects returned for a second (cyclosporine) study session after about 1 week. At this session subjects received an intravenous infusion of cyclosporine (2.5 mg/kg/h) (Bedford Laboratories, Bedford, OH) over 2 h, corresponding to the cyclosporine dose producing 79% P-gp inhibition for verapamil. A 1-h morphine infusion (0.1 mg/kg) was administered during the second hour of the cyclosporine infusion. Any physicochemical incompatibilities between morphine and cyclosporine have not been described. Venous blood samples for morphine and metabolite analysis were obtained at the times described above. Venous samples were also obtained 1 (mid-infusion), 2 (end-infusion), and 4 h after the start of the cyclosporine infusion for determination of cyclosporine concentration. Additional blood samples were obtained before and the day after cyclosporine for serum creatinine concentration measurement as a safety laboratory measure.

Morphine clinical effects were determined using miosis and response to thermal stimulus. Dark-adapted pupil diameters were measured in triplicate coincident with blood sampling using a handheld infrared-based pupillometer (Neuroptics, Irvine, CA), as described previously. Pupil diameter before the morphine infusion was the baseline value used to calculate pupil diameter change (miosis) at each time. Analgesia was assessed by response to thermal stimulus using both the maximum tolerated temperature and response to predetermined temperatures. A hot-water based 3 cm² computer-controlled Peltier-type thermal stimulator (Pathway, Medoc Advanced Medical Systems, Ramat Yishai, Israel) was applied to the volar side of the forearm in which study drugs were infused. The thermode was set to a baseline of 32°C, and programmed to gradually increase the stimulus (0.5°C/sec). Subjects pressed the button on a hand-held device when the thermode reached a maximum tolerable temperature to stop the temperature increase and initiate thermode cooling. Maximum thermode temperature was set to 52°C. If the subject tolerated this temperature without pressing the button, then 52°C was recorded as the maximum tolerable temperature. The maximum temperature testing was repeated twice, with the probe moved and cooled between stimuli, and the average of the three temperatures was recorded. Response to discrete heat stimuli was determined next. Probe temperature was set to a baseline of 32°C, and programmed to increase (0.5°C/s) to one of six predefined temperatures (41.0, 43.0, 44.8, 46.5, 48.2, and 50.0°C) in random order, with the probe cooled and moved between stimuli. Subjective pain intensity was rated by the subject on a verbal analog scale (VAS) ranging from 0 (no pain) to 100 (worst possible pain).

All 16 subjects completed the control arm of the study. Two subjects reported feelings of flushing and warmth after the beginning of the cyclosporine infusion and stopped the study session before the morphine infusion began. As a result, the final study cohort consisted of the 14 subjects completing both control and cyclosporine arms of the protocol (10 males and 4 females, 75 ± 13 kg, body mass index 24 ± 3 kg/m²). The average morphine dose was 7.5 ± 1.3 mg (range 5.8 to 10.2 mg) and that of cyclosporine was 420 ± 75 mg (range 326 to 574 mg). Samples were analyzed and data are reported for the 14 subjects completing both study arms.

**Analytical methods**

Plasma and urine concentrations of morphine, morphine-3-glucuronide, and morphine-6-glucuronide were determined by high-performance liquid chromatography (HPLC)-tandem mass spectrometry, using deuterated internal standards. Subject plasma, quality control, or calibration samples (250 μL) were diluted with 750 μL of freshly prepared 2% (v/v) aqueous ammonium hydroxide containing internal standards (15 ng/ml morphine-d3, 24 ng/ml morphine-6-glucuronide-d3, and 75 ng/ml morphine-3-glucuronide-d3), processed through a
96 well solid-phase extraction plate (Strata-X, 30-mg, Phenomenex, Torrance, CA) through which 1 ml methanol and 1 ml water had been passed sequentially, rinsed with 1 ml of water, dried under vacuum, and eluted with methanol (4 × 0.25 ml). The samples were evaporated to dryness under nitrogen at 60°C and reconstituted in 100 μL of 10 mM aqueous ammonium formate with 10% methanol.

The mass spectrometer (4000 QTRAP, ABI Inc., Foster City CA) was equipped with a Turbo Ion Spray ionization source operating in positive ionization mode and an 1100 series HPLC system (Agilent, Wilmington, DE). Chromatographic separation was performed on a T3 HPLC column (150 × 2.1 mm, 3.5 μm) (Waters Corp, Milford, MA). The injection volume was 10 μL and the oven temperature was 25°C. The HPLC mobile phase (0.3 ml/min) was (A) 10 mM ammonium formate in water and (B) 10 mM ammonium formate in methanol. The gradient program was 10% B for 0 min, linear gradient to 50% B between 0 and 1.0 min, linear gradient to 95% between 1.0 and 2.0 min, held at 95% B until 4 min, then re-equilibrated to initial conditions (10% B) between 4.01 and 8.0 min. Under these conditions, retention times were 2.5, 3.7, and 6.0 min, respectively, for morphine-3-glucuronide, morphine-6-glucuronide, and morphine. Both Q1 and Q3 quadrupoles were optimized to unit mass resolution, and the mass spectrometer conditions were optimized for each analyte. The instrument was operated in positive-ion mode with an ion spray voltage of 5500 V. The curtain gas was set at 20, ion source gas 1 was 40, ion source gas 2 was 40 and collision gas was set at high. Multiple reaction monitoring transitions for each analyte and internal standard were m/z 286.2 → 152.3 and m/z 289.2 → 152.3 for morphine and morphine-d3, and m/z 462.5 → 286.2 for morphine-3- and -6-glucuronides, and m/z 465.5 → 289.2 for morphine-3- and -6-glucuronides-d3. Analytes were quantified using peak area ratios and standard curves prepared using calibration standards in blank plasma. Calibration standards for morphine-3-glucuronide were 2, 4, 10, 20, 40, 200, 360, and 400 ng/ml, and morphine-6-glucuronide and morphine calibration standards were 0.5, 1, 2.5, 5, 10, 50, 90, and 100 ng/ml. Quality control samples for morphine-3-glucuronide were 10, 40, and 320 ng/ml, and morphine-6-glucuronide and morphine quality control samples were 2.5, 10, and 80 ng/ml. Interday variability of quality control samples (n = 22) for the entire study ranged from 2.9 to 8.4% (coefficient of variation) and accuracy ranged from 91 to 105%.

Morphine and morphine metabolite concentrations in urine were determined similarly. Calibration standards for morphine-3-glucuronide were 20, 100, 200, 1,000, 1,600, 2,000, 4,000, and 5,000 ng/ml, and morphine-6-glucuronide and morphine calibration standards were 5, 25, 50, 250, 400, 500, 1,000, and 5,000 ng/ml. All urine samples were evaluated in a single batch.

Cyclosporine blood concentrations were determined by the Barnes-Jewish Hospital Clinical Laboratory using HPLC-tandem mass spectrometry. Serum creatinine concentrations were quantified by the hospital laboratory using standard methods.

**Noncompartmental Pharmacokinetic-Pharmacodynamic Analysis**

Maximum plasma concentration (C_{max}), areas under the plasma concentration-time curve (AUCs) for morphine and morphine glucuronides during the measurement interval (0-10 h) and extrapolated to infinity, elimination clearance, and elimination half-life as well as maximum miosis, the area under the miosis-time curve (AUC), and time to maximum effect (t_{max}) were determined by noncompartmental analysis with WinNonlin (Pharsight, Palo Alto, CA). Urine morphine and metabolite clearance were determined as described previously.\textsuperscript{19}
Compartmental Pharmacokinetic-Pharmacodynamic Modeling

Data from both the control and cyclosporine arms of a subject were modeled simultaneously. A standard two-stage sequential pharmacokinetic and pharmacodynamic analysis was done using the SAAM II software system (SAAM Institute, Seattle, WA) implemented on a Windows-based PC (Microsoft, Redmond, WA). The SAAM II objective function used was the extended least-squares maximum likelihood function using data weighted with the inverse of the model-based variance of the data at the observation times. Systematic deviations of observed data from the calculated values were sought using the one-tailed one-sample runs test (results not shown), with $P < 0.05$, corrected for multiple applications of the runs test, as the criterion for rejection of the null hypothesis. Model misspecification was sought by visual inspection of the measured and predicted drug concentrations and effects versus time relationships.

Plasma morphine concentrations were first modeled with 2-compartment pharmacokinetic models of morphine disposition under control and experimental conditions. All of the pharmacokinetic parameters describing morphine disposition under the two conditions were determined independently except the initial volume of distribution or central volume ($V_C$). The initial volume of distribution was constrained to be the same for both models, so was codetermined by the data collected in both studies in a given individual. This was done because $V_C$ is difficult to determine accurately with limited data and misestimation of $V_C$ can lead to apparent differences in other model parameters.

After finalizing the fit of the pharmacokinetic models to the morphine data, the parameters of the models were fixed and pharmacokinetic models were fit to the morphine-3-glucuronide and morphine-6-glucuronide data simultaneously for both the control study and the cyclosporine study. The formations of both morphine-3-glucuronide and morphine-6-glucuronide were modeled as a fraction of the elimination clearance of morphine in those models (i.e., morphine elimination clearance was set equal to the sum of clearance by metabolism to morphine-3-glucuronide plus the clearance by metabolism to morphine-6-glucuronide plus the clearance by all other mechanisms). To model these data, the morphine dose was first converted to a molar amount and the plasma morphine, morphine-3-glucuronide, and morphine-6-glucuronide concentrations were converted to molar concentrations. Metabolite formation was described using tanks-in-series delay elements to characterize the non-instantaneous appearance of the metabolite in the plasma. The steady-state volumes of distribution ($V_{SS}$) of both morphine-3-glucuronide and morphine-6-glucuronide were fixed at 0.2 x body mass (in kg), to approximate a total distribution volume equal to extracellular fluid space for each subject during both control and cyclosporine treatments.

After finalizing the fit of the pharmacokinetic model to the morphine, morphine-3-glucuronide, and morphine-6-glucuronide data, all pharmacokinetic parameters were fixed and the pharmacodynamic model was fit to the effect data. The pupil diameter effect data were described as change from baseline (miosis) by a sigmoid $E_{\text{max}}$ model:

$$E(t) = \left( \frac{E_{\text{max}} \times C_{E}(t)^{\gamma}}{C_{E}(t)^{\gamma} + EC_{50}^{\gamma}} \right)$$

where: $E(t)$ is the effect at time $t$; $E_{\text{max}}$ is the maximum effect produced by morphine; $C_{E}(t)$ is the morphine concentration in the effect site at time $t$; $C_{50}$ is the morphine biophase concentration at which the effect is half of maximum; and $\gamma$ is the shape parameter that determines the steepness of the biophase concentration versus effect relationship. Because a maximum effect was not generally observed, it was assumed that the maximum observable morphine effect possible was a pupil diameter nadir of 2.5 mm, as described previously, hence the maximum possible miosis ($E_{\text{max}}$) was estimated as the difference between the

*Anesthesiology. Author manuscript; available in PMC 2014 October 01.*
baseline pupil diameter and 2.5 mm. EC$_{50}$ and $\gamma$ were constrained to be the same for both sessions, so were codetermined by the data collected in both studies in a given individual. This was done because we postulated cyclosporine-inhibitable transport plays a role in determining morphine access to the brain, which would be reflected in differences in $k_{e0}$ between control and cyclosporine studies in the same individuals.

The pharmacodynamic model did not include potential effects of morphine glucuronides. Morphine-3-glucuronide has no clinical effect and does not influence morphine effects. Morphine-6-glucuronide is an active metabolite, but crosses the blood–brain barrier slowly and is considered noncontributory to the short-term effects of morphine, and, the observed morphine-6-glucuronide concentrations were never more than 10% of the median EC$_{50}$ for miosis reported previously. Therefore, morphine-6-glucuronide was not included in the model, as described previously.

### Statistical analysis

Normally distributed continuous data are reported as mean ± SD. Continuous data found not to be normally distributed by the Shapiro-Wilk normality test are reported as median and range. Within subject differences in normally distributed pharmacokinetic and pharmacodynamic data were assessed using the paired $t$-test, while within subject differences in data that were not normally distributed were compared with the Wilcoxon signed rank test (SigmaPlot, Systat Software, San Jose, CA). Significance testing was two-tailed. The criterion for rejection of the null hypothesis was $P < 0.05$.

### RESULTS

#### Plasma concentrations and noncompartmental pharmacokinetics

Plasma morphine and morphine glucuronides concentrations versus time are illustrated in figure 1, and pharmacokinetic parameters provided in table 1. Morphine concentrations peaked at the end of the infusion and were comparable in controls and cyclosporine-treated subjects. Morphine 3- and 6-glucuronide concentrations peaked 0.2 and 0.5 h, respectively, after the end of the morphine infusion. Cyclosporine had no or negligible effects on peak morphine glucuronide concentrations, but significantly delayed the time to peak concentrations (by 0.4 h for both). After peaking, plasma concentrations of morphine and both glucuronides in the cyclosporine arm were consistently higher than in controls. Areas under the curve (AUC$_{0 \rightarrow \infty}$) of morphine, morphine-3-glucuronide, and morphine-6-glucuronide concentration versus time in cyclosporine-treated volunteers were 17%, 29%, and 16% higher than in controls, respectively (table 1, $P < 0.05$). The increased morphine AUC reflects an 18% decrease in morphine elimination clearance by cyclosporine (table 1, $P < 0.05$).

#### Compartmental Pharmacokinetic Modeling

The best, median, and worst fits of the compartmental model to the morphine and glucuronide data are illustrated in figure 1. Pharmacokinetic parameters are presented in table 2. The initial distribution volume ($V_C$) of morphine did not differ between control and cyclosporine treatments because it was constrained to be the same, as indicated in Methods. Despite having the same initial distribution volume, the average peripheral volume ($V_P$) during cyclosporine treatment was approximately 11% less than control and, as a result, the average total volume of distribution ($V_{SS}$) was 10% less than control, although these differences were not statistically significant. Average intercompartmental clearance ($CL_P$) differed less than 10% between the two experimental conditions but the average elimination clearance ($CL_E$) during the cyclosporine phase was 18% less than in controls ($P < 0.001$), similar to the noncompartmental analysis. The fraction of morphine elimination clearance.
represented by metabolism to morphine-3-glucuronide differed by less than 10% between control and cyclosporine sessions, but the fraction of morphine elimination clearance represented by metabolism to morphine-6-glucuronide during cyclosporine treatment was >25% less than in controls ($P < 0.001$). The average morphine elimination half-life differed by less than 5% between the experimental conditions.

Pharmacokinetic parameters for morphine glucuronides are presented in table 2. The volumes of distribution of these metabolites were fixed at a total distribution volume ($V_{SS}$) equal to extracellular fluid space for each subject during both control and cyclosporine treatments (see Methods). While the median fraction of morphine eliminated as morphine-3-glucuronide was approximately 40% and did not differ between treatments, the median fractions of morphine dose eliminated as morphine-6-glucuronide were 8.1% under control conditions and 7.4% during cyclosporine treatment ($P < 0.05$). Cyclosporine decreased the median elimination clearance of morphine-3-glucuronide by slightly more than 10% ($P < 0.05$) and it decreased the median elimination clearance of morphine-6-glucuronide by nearly 25% ($P < 0.01$).

Pupillometry

Pupillometry results are given in figure 2, shown as measured dark-adapted pupil diameter (fig. 2A) and miosis (difference vs. predrug baseline, fig. 2B), and miosis parameters are provided in table 3. It is notable that maximum miosis did not occur until an average of 1.6 h after the end of the morphine infusion ($T_{max}$). Cyclosporine did not change this delay. Maximum miosis ($E_{max \, \text{observed}}$) was significantly greater in cyclosporine-treated subjects (3.2 ± 0.9 vs. 2.5 ± 1.0 mm, $P < 0.05$), and this difference in the cyclosporine-treated subjects persisted for the remainder of the observation period. The AUC for miosis during the 10-h observation period was significantly greater in the cyclosporine-treated subjects (17.7 ± 8.9 vs. 10.9 ± 5.1 mm•hr). The relationship between miosis and morphine plasma concentration is shown in figure 2C. The delay in onset of morphine effect is apparent from the considerable and well-known hysteresis. Cyclosporine altered this hysteresis, specifically, increasing miosis on the portion of the curve representing the decline in plasma morphine concentrations.

Pharmacodynamic Modeling

Table 3 summarizes the pharmacodynamic parameters for pupillary effects for the effect model used in this study. The best, median, and worst fits of the $E_{max}$ models to the pupillometry data are illustrated in figure 2D-F. The median $k_{e0}$ of the cyclosporine sessions was 59% larger than that during the control sessions. ($P < 0.05$). Effect site modeling using miosis and Equation 1 were used to determine predicted biophase morphine concentrations, and the influence of cyclosporine (fig. 3). The predicted maximum biophase concentration (41 ± 13 vs. 27 ± 11 ng/ml) and the AUC$_{0-12}$ (87 ± 20 vs. 64 ± 20ng/ml•hr) were significantly greater after cyclosporine (both $p < 0.001$).

Analgesia

One testing paradigm evaluated maximally tolerated temperature on the volar side of the forearm. Maximally tolerated temperature before the start of the morphine infusion were comparable in control (48.8 ± 2.3°C) and cyclosporine (48.5 ± 2.6°C) sessions. Morphine increased the maximally tolerated temperature to only 49.1 ± 1.7°C after 1.5 h (0.5 h after the end of the infusion, which was not significantly different than baseline (fig. 4A). Cyclosporine actually decreased the maximally tolerated temperature to 47.5 ± 2.3°C 1.5 h after the start of the cyclosporine infusion, and this was not significantly modulated by morphine.
A second testing paradigm used VAS scores in response to discreet temperatures, applied in random order at each time (fig. 4B-E). There was a clear stimulus-response relationship between temperature and pain rating (D, E). This response in both groups was essentially unaffected by morphine. There were no time- and morphine-dependent changes in VAS scores at any temperature (B, C). Pain scores were higher in cyclosporine-treated subjects, and these also were not affected by morphine. Thus, morphine (0.1 mg/kg) had negligible analgesic effects in this population, and cyclosporine clearly decreased thermal pain tolerance.

**Cyclosporine**

Cyclosporine concentrations were 3411 ± 541 and 3808 ± 604 ng/ml after 1 and 2 h of infusion (at the beginning and end of the morphine infusion), respectively, and 748 ± 183 ng/ml 2 h after the cyclosporine infusion was stopped (4 h after starting cyclosporine).

**Adverse Events**

Two subjects aborted the study after the first 10 min of cyclosporine infusion due to uncomfortable feelings of warmth and flushing of the face and trunk, which stopped after the infusion was aborted, and required no treatment. Other subjects also reported these effects, but did not consider them intolerable. These side effects resolved after morphine administration.

Serum creatinine concentrations were monitored as a safety assessment of renal function after cyclosporine. Creatinine concentrations were 1.0 ± 0.1 and 0.9 ± 0.2 mg/dl, respectively, before and one day after the cyclosporine infusion. Three subjects had a postcyclosporine increase in creatinine of 0.1 mg/dl or less, and five subjects had a decrease. Cyclosporine was therefore considered to have had no significant effect on renal function.

**DISCUSSION**

This investigation tested the hypothesis that morphine is a substrate for drug transport at the human blood-brain barrier, and that transport activity influences morphine pharmacodynamics. More specifically, focus was on the efflux transporter, P-gp. Cyclosporine was used as a clinically validated inhibitory P-gp probe.\(^{20}\) Previously, 2.5 mg/kg/hr cyclosporine for 2 h (achieving a blood concentration of 2.8 ± 0.4 \(\mu\)M, or 0.2 \(\mu\)M unbound) increased human brain uptake of the P-gp substrate verapamil by 79%, measured using positron emission tomography.\(^{20,21}\) The blood EC\(_{50}\) for cyclosporine inhibition of P-gp was reported as 7 \(\mu\)M.\(^{22,37}\) In the present investigation, using the same cyclosporine dosing, cyclosporine blood concentrations were 2.8 ± 0.4 and 3.2 ± 0.5 \(\mu\)M after 1 and 2 h of infusion, respectively (beginning and end of the morphine infusion), and 0.6 ± 0.2 \(\mu\)M 2 h after cyclosporine was stopped. These concentrations were comparable to those previously shown to inhibit human brain P-gp activity by 79%, and were 40% of the EC\(_{50}\) for P-gp inhibition.\(^{20,22,37}\) Thus, cyclosporine concentrations apparently sufficient to achieve effective P-gp inhibition were attained in the present investigation.

After initiating this study, it became apparent that cyclosporine also inhibits other transporters (multidrug resistance-related protein 2 and breast cancer resistance protein), although shown only \textit{in vitro},\(^{38-41}\) and the cyclosporine IC\(_{50}\) for breast cancer resistance protein (26 \(\mu\)M)\(^{40}\) is far greater than concentrations achieved in the present study. Nevertheless, we refer more broadly to cyclosporine-inhibitable, rather than specifically to P-gp-dependent transport.

Morphine and morphine glucuronide pharmacokinetics in controls were similar to previous reports.\(^{17,18,30,36}\) Cyclosporine slightly increased morphine plasma concentrations, largely
due to an 18% decrease in morphine elimination clearance. One possible explanation is that cyclosporine inhibited morphine metabolism. A small fraction of morphine (~4%) is N-demethylated by cytochrome P4503A (CYP3A), and cyclosporine inhibits hepatic CYP3A activity. The \( \textit{in vitro} \) \( K_i \) for cyclosporine inhibition of hepatic microsomal CYP3A was 1.4 \( \mu M \), and clinically, 200 mg/day cyclosporine, which achieved a trough concentration of 119 ng/ml (0.1 \( \mu M \)), inhibited hepatic CYP3A activity by 24%. Thus, cyclosporine concentrations in the present investigation (>3 \( \mu M \) peak) would be expected to inhibit >90% of hepatic CYP3A activity. This might account for some, but not all, of the 17% increase in plasma morphine AUC. Cyclosporine may have also inhibited transporters mediating hepatic morphine uptake, as well as metabolism, further reducing morphine clearance.

Cyclosporine significantly increased plasma morphine glucuronide concentrations. Other P-gp inhibitors, quinidine and PSC833, also increased the AUC for morphine glucuronides. This may simply reflect slightly higher morphine plasma concentrations, as cyclosporine had no apparent effect on morphine glucuronidation (based on the fraction of morphine eliminated as glucuronides, morphine metabolic clearance to glucuronides, glucuronide formation clearance, and plasma glucuronide/morphine AUC ratios). However, cyclosporine did somewhat (10-25%) decrease glucuronide elimination clearance. Morphine glucuronides are eliminated renally. Cyclosporine may inhibit one or more renal tubular efflux transporters (P-gp, breast cancer resistance protein, multidrug resistance-related protein) mediating luminal excretion, to reduce morphine glucuronides clearance. Some evidence also suggests hepatic excretion of morphine glucuronides, by multidrug resistance-related protein 2 and 3, which may also be inhibitable by cyclosporine. In animals, absence of multidrug resistance-related protein 2 and 3 increased plasma morphine-3-glucuronide concentrations, potentially explained by reduced hepatic and renal efflux transport. Together these considerations suggest a role for cyclosporine-inhibitable transporters in morphine glucuronide elimination in humans.

Morphine pharmacodynamics in controls was similar to that reported previously. Miosis occurred after a substantial time delay, as observed previously. Cyclosporine enhanced morphine-induced miosis, increasing maximum miosis and nearly doubling the miosis AUC. This increase in miosis occurred during the post-infusion phase. The 18% decrease in morphine elimination clearance by cyclosporine, resulting in a 17% increase in average morphine AUC, might not entirely explain the 62% increase in miosis AUC. However, the 59% increase in median \( k_e \) produced by cyclosporine, together with the slightly increased plasma morphine concentrations, offers a ready explanation of the increased miosis observed during the cyclosporine sessions. This combined effect is clearly apparent in the increased predicted morphine biophase concentrations after \( T_{max} \) (fig. 3). Thus, cyclosporine increased morphine access to the brain. In theory, increased levels of intracerebral morphine could originate from enhanced uptake and/or delayed efflux. However, no uptake transporters for morphine have been identified, and cyclosporine has been shown to significantly inhibit morphine efflux \textit{in vitro}. Thus, the major conclusion of this investigation is that cyclosporine-inhibitable efflux transport at the blood brain barrier measurably influences morphine access to the brain in humans. Moreover, P-glycoprotein may be a key element of this phenomenon. This investigation is the first to demonstrate a clinically measurable influence of efflux transport inhibition on morphine effects and pharmacodynamics.

Results of this investigation can be compared with previous studies of P-gp inhibition and morphine, in both humans and animals. Quinidine did not change morphine effects (pupil diameter, respiratory depression, and subjective self-assessments or maximum miosis, AUC of miosis \textit{versus} time, and subjective self-assessments) in volunteers. Quinidine may
have insufficient inhibitory potency for human P-gp. Quinidine IC$_{50}$ was 34 μM in L-MDR1 cells expressing human P-gp, 36 μM in mouse brain,$^8$ and 3 μM in porcine brain capillary cells,$^4$ compared to 9 μM clinical plasma concentrations.$^{19}$ The P-gp inhibitor PSC833 did not significantly change morphine respiratory depression or drowsiness.$^{17}$ Therefore, differences between cyclosporine and other P-gp inhibitor effects on morphine pharmacodynamics may be attributable to differences in inhibitor concentrations relative to the $K_i$ for transporter inhibition.

Results of this clinical investigation can be compared with previous studies of morphine and transporter inhibition in cells and animals. In general, there are marked interspecies differences in P-gp inhibitor effects in vitro.$^{48,49}$ Inhibition of bovine brain capillary cell P-gp-mediated transport by GF120918 or cyclosporine abolished basolateral to apical flux of morphine.$^7$ In vivo, inhibition of P-gp transport by GF120918 tripled cerebral morphine uptake in rats.$^{15}$ Mdr1 knockout mice exhibited 5-fold increases in cerebral morphine concentration and analgesia.$^{12}$ Thus, effects of P-gp and transport inhibition on cerebral drug disposition appear greater in animals than humans.

Results of this investigation, showing a 60% increase in morphine effect AUC by cyclosporine, can also be compared with studies of other P-gp substrates. Cyclosporine (10 mg/kg IV, ~5.6 μM in blood) in volunteers increased brain uptake of the P-gp substrate loperamide (assessed by positron emission tomography) by 110%.$^{37,*}$ and, when corrected for loperamide metabolism, increased 457%. Loperamide respiratory depression was also increased by quinidine.$^{50}$ The most likely reason why quinidine influenced apparent brain uptake of loperamide,$^{50}$ but not morphine,$^{19}$ is that loperamide is a much better P-gp substrate, although there may also be differential sensitivity of the effect parameters (carbon dioxide response for loperamide and pupil diameter for morphine). Supporting this contention, loperamide has a higher efflux ratio than morphine (>20% vs. <10%) in P-gp-overexpressing cells,$^{47}$ and the transport ratio in mdr1-knockout mice vs control was 10.4 for loperamide and only 1.2 for morphine.$^8$ Thus, in humans, the quantitative consequence of transport inhibition was far less for morphine than for a much better P-gp substrate.

Although effects of transporter inhibition on morphine pharmacodynamics and clinical effects were statistically significant, the magnitude of effect was relatively small. Any clinical relevance to patients receiving cyclosporine therapeutically (where concentrations are well below 1 μM, and unlikely to influence morphine transport) is, therefore, probably negligible. More generally, given that cyclosporine concentrations were 40% of the EC$_{50}$ for P-gp inhibition and comparable to those previously shown to inhibit human brain P-gp activity by 79%,$^{20}$ and this degree of transporter inhibition had only minor consequences for morphine brain uptake and clinical effects, it is unlikely that other brain transporter inhibitors (unless substantially more effective) would have clinically meaningful consequences for morphine effects and use.

The small clinical effects of efflux transport (P-gp) inhibition on morphine pharmacodynamics complement previous pharmacogenetic studies of P-gp and morphine. Although one investigation found greater pain relief in cancer pain patients who were homozygous for the P-gp C3435T single nucleotide polymorphism,$^{51}$ and another reported lower oral morphine requirements in C3435T homozygotes in a mixed chronic pain population,$^{52}$ neither the C3435T nor G2677T/A polymorphisms had any significant association with morphine dose requirements for postoperative or cancer pain.$^{53,54}$

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*Anesthesiology. Author manuscript; available in PMC 2014 October 01.
results are consistent with only a minor role for brain efflux transport in clinical morphine analgesia.

A most unexpected finding in this investigation was that cyclosporine caused cutaneous sensitization to touch and heat. This differs from the previously well-described cyclosporine pain syndrome (bilateral bone pain in the lower extremities), and invalidated analgesia for measuring cyclosporine influence on morphine effects. Whether this sensitization in humans is analogous to reductions in thermal tail-flick latencies by cyclosporine in mice is unclear. This appears to be the first report of acute cyclosporine sensitization in humans, and was not described after previous cyclosporine use to inhibit P-gp.20 The mechanism of sensitization is unknown. Further investigation as a potential experimental pain model, or source of mechanistic insights for acute sensitization and pain in humans may be appropriate.

There are potential limitations to the present investigation. Miotic effects of morphine-6-glucuronide were not incorporated into the pharmacodynamic model, but this was because morphine-6-glucuronide does not materially contribute the effects of single-dose morphine.1 Previous studies characterized cyclosporine inhibitory potency using EC\textsubscript{50} values, determined with the substrate verapamil, rather than with K\textsubscript{i}, which might affect the estimate of P-gp inhibition used in this investigation, given that a different substrate was used.

In summary, this investigation showed that cyclosporine, used as an in vivo inhibitor probe for blood-brain barrier P-gp and other transporters, moderately enhanced morphine-induced miosis and nearly doubled the area under the miosis-time curve. Effects were mostly explained by an increase in morphine k\textsubscript{e0}, and apparent effect-site morphine concentrations. This suggests a role for P-gp or other efflux transporters in brain morphine access, although the magnitude of the effect is small, and unlikely to be a major determinant of morphine clinical effects.

Acknowledgments

We greatly appreciate the valuable insights of Thomas K. Henthorn, M.D., Professor and Chairman, Department of Anesthesiology, University of Colorado, Denver, Colorado, regarding the pharmacodynamic modeling. We also thank Daniel Brennan, M.D., Professor of Medicine (Nephrology), Washington University in St. Louis, St. Louis, Missouri, for valuable guidance regarding cyclosporine dosing, Mitch Scott, Ph.D., Professor of Pathology & Immunology, Washington University in St. Louis, St. Louis, Missouri, for cyclosporine analysis and interpretation of analytical methods, and Kathryn Vehe, R.Ph., Investigational Pharmacist, Barnes-Jewish Hospital, St. Louis Missouri, for drug preparation. The valuable technical assistance of Nichole Meier, R.N., and Patty Suntrup R.T., B.A., C.C.R.P., both Clinical Research Coordinators, Department of Anesthesiology, Washington University in St. Louis, St. Louis, Missouri is appreciated. We also thank Jason Maynes, M.D., Ph.D., Assistant Professor, Department of Anesthesiology, The Hospital for Sick Children, Toronto Ontario, Canada for valuable discussion.

FUNDING Clinical Scholar Research Award from the International Anesthesia Research Society (San Francisco, California) and Mentored Research Training Grant from the Foundation for Anesthesia Education and Research (Rochester, Minnesota, both to Konrad Meissner), and National Institutes of Health (Bethesda, Maryland) Grants K24-DA00417 and R01-DA14211 (to Evan D. Kharasch), and National Institutes of Health Grants UL1 RR024992 to the Washington University in St Louis Institute of Clinical and Translational Sciences.

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What we already know about this topic

*Considerable evidence suggests that morphine is a substrate for the blood-brain barrier efflux transporter P-glycoprotein \textit{in vitro} and in animals, but little is known about blood-brain barrier transporters and morphine brain access in humans

What this article tells us that is new

* In 14 healthy volunteers, cyclosporine, an inhibitor of blood-brain barrier transporters, had minimal effects on circulating morphine concentrations, but increased the centrally mediated effect of morphine (miosis)

* These results suggest that transporter-mediated removal of morphine from the brain plays a role in morphine’s pharmacodynamics after systemic administration
Figure 1.
Effects of cyclosporine on plasma concentrations of (A) morphine, (E) morphine-3-glucuronide (M-3-G), and (I) morphine-6-glucuronide (M-6-G) during and after a 1-h infusion of morphine (0.1 mg/kg) in subjects receiving nothing (controls, open circles) or a 2-h cyclosporine infusion (2.5 mg/kg/hr, begun 1 h before the morphine infusion, open triangles). Times are relative to the start of the morphine infusion. Results are shown as the mean ± SD (N = 14). Pharmacokinetic model fits (best-median-worst, respectively) of a 2-compartment pharmacokinetic model for are shown for (B-D) morphine, (F-H) morphine-3-glucuronide (M-3-G), and (J-L) morphine-6-glucuronide (M-6-G) concentrations.
Figure 2.
Morphine effects on dark-adapted pupil diameter, and influence of cyclosporine. Times are relative to the start of the morphine infusion. Symbols reflect controls (circles) and cyclosporine-treated subjects (triangles). Results are shown as the mean ± SD (N = 14) for (A) pupil diameter versus time, (B) pupil diameter change from baseline (miosis) versus time, and (C) miosis versus plasma morphine concentration (error bars omitted for clarity). Pharmacodynamic model fits for miosis using the $E_{\text{max}}$ model (Equation 1) are shown for the (D) best, (E) median, and (F) worst data fits.
Figure 3.
Model-predicted biophase morphine concentrations during and after a 1-h infusion of morphine (0.1 mg/kg) in subjects receiving nothing (controls, open circles) or a 2 h cyclosporine infusion (2.5 mg/kg/hr, begun 1 h before the morphine infusion, open triangles). Times are relative to the start of the morphine infusion. Results are shown as the mean ± SD (N = 14).
Figure 4.
Thermal pain perception before, during and after a one-hour infusion of morphine. Times are relative to the start of the morphine infusion. The 2-h cyclosporine infusion (−1 to 1 h) was started 1 h before morphine (−1 to 0 h). Results are shown as the mean ± SD (N = 14) with some error bars omitted for clarity. (A) Maximally tolerated temperatures in control (circles) and cyclosporine (triangles) sessions. No time-specific maximally tolerated temperature was significantly greater than predrug baseline, in either controls or cyclosporine-treated subjects. (B and C) Verbal analog scores in response to specific thermal stimuli applied in random order at each time. Temperatures were 41.0, 43.0, 44.8, 46.5, 48.2, and 50.0 °C. (B and C) Results are shown for selected temperatures in controls (circles) and cyclosporine-treated subjects (triangles) at (B) 44.8 °C and (C) 46.5 °C. (D and E) Results are shown for all temperatures at selected times in (D) controls and (E) cyclosporine-treated subjects.
Table 1
Morphine and Morphine Metabolite Noncompartmental Pharmacokinetic Parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cyclosporine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma $C_{\text{max}}$ (ng/ml)</td>
<td>46.4 ± 11.5</td>
<td>50.3 ± 11.4</td>
</tr>
<tr>
<td>Plasma AUC₂₅ (ng/ml•hr)</td>
<td>85 ± 24</td>
<td>100 ± 21 *†</td>
</tr>
<tr>
<td>Plasma Cl (ml/kg/min)</td>
<td>21.1 ± 6.9</td>
<td>17.4 ± 3.9 *</td>
</tr>
<tr>
<td>Plasma $t_{\frac{1}{2}}$ (hr)</td>
<td>2.2 ± 0.6</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>Urine %dose eliminated</td>
<td>6 ± 2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Urine clearance (ml/kg/min)</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td><strong>Morphine-3-glucuronide</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma $C_{\text{max}}$ (ng/ml)</td>
<td>123 ± 25</td>
<td>131 ± 25 *</td>
</tr>
<tr>
<td>Plasma $T_{\text{max}}$ (h)</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.5 *</td>
</tr>
<tr>
<td>Plasma AUC₂₅ (ng/ml•hr)</td>
<td>667 ± 179</td>
<td>838 ± 165 ‡</td>
</tr>
<tr>
<td>Plasma AUC ratio (morphine glucuronide/morphine)</td>
<td>5.0 ± 1.2</td>
<td>5.3 ± 1.1</td>
</tr>
<tr>
<td>Plasma $t_{\frac{1}{2}}$ (h)</td>
<td>3.3 ± 1.3</td>
<td>3.6 ± 1.0</td>
</tr>
<tr>
<td>Urine %dose eliminated</td>
<td>31 ± 4</td>
<td>35 ± 10</td>
</tr>
<tr>
<td>Urine formation clearance (ml/kg/min)</td>
<td>6.5 ± 1.8</td>
<td>6.2 ± 2.9</td>
</tr>
<tr>
<td><strong>Morphine-6-glucuronide</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma $C_{\text{max}}$ (ng/ml)</td>
<td>24.9 ± 5.2</td>
<td>23.6 ± 5.8</td>
</tr>
<tr>
<td>Plasma $T_{\text{max}}$ (h)</td>
<td>1.5 ± 0.2</td>
<td>1.9 ± 0.5 *</td>
</tr>
<tr>
<td>Plasma AUC₂₅ (ng/ml•hr)</td>
<td>117 ± 32</td>
<td>134 ± 32 ‡</td>
</tr>
<tr>
<td>Plasma AUC ratio (morphine glucuronide/morphine)</td>
<td>0.88 ± 0.20</td>
<td>0.85 ± 0.20</td>
</tr>
<tr>
<td>Plasma $t_{\frac{1}{2}}$ (h)</td>
<td>2.8 ± 1.2</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Urine %dose eliminated</td>
<td>7 ± 1</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Urine formation clearance (ml/kg/min)</td>
<td>1.5 ± 0.5</td>
<td>1.2 ± 0.5 *</td>
</tr>
</tbody>
</table>

Urine data were not available for one control subject. All data are reported as mean ± standard deviation except AUC ratios (cyclosporine/control), which are the geometric mean and 90% confidence interval (n = 14)

* $p<0.01$ versus control;
† AUC ratio (cyclosporine/control) geometric mean and 90% confidence interval was 1.19 (1.11, 1.27);
‡ AUC ratio (cyclosporine/control) geometric mean and 90% confidence interval was 1.28 (1.20, 1.36);
§ AUC ratio (cyclosporine/control) geometric mean and 90% confidence interval was 1.15 (1.08, 1.23).

AUC=area under the plasma concentration-time curve; $C_{\text{max}}$= peak plasma concentration; $T_{\text{max}}$=time to maximum concentration.
Table 2
Morphine and Morphine Metabolite Compartmental Pharmacokinetic Parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cyclosporine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_C$ (L) *</td>
<td>21.5 ± 4.7</td>
<td>21.5 ± 4.7</td>
</tr>
<tr>
<td>$V_P$ (L)</td>
<td>162 ± 28</td>
<td>143 ± 37</td>
</tr>
<tr>
<td>$V_{SS}$ (L)</td>
<td>183 ± 28</td>
<td>165 ± 36</td>
</tr>
<tr>
<td>$CL_P$ (L/h)</td>
<td>162 ± 82</td>
<td>149 ± 76</td>
</tr>
<tr>
<td>$CL_E$ (L/h)</td>
<td>93.6 ± 22.0</td>
<td>77.1 ± 17.4 **</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>2.2 (1.2 to 3.8)</td>
<td>2.2 (1.4 to 2.8)</td>
</tr>
<tr>
<td>Morphine-3-glucuronide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction eliminated (%) †</td>
<td>40.5 (27 to 52)</td>
<td>43.4 (21 to 73)</td>
</tr>
<tr>
<td>$CL_{EM-3-G}$ (L/h)‡</td>
<td>39.0 ± 14.8</td>
<td>35.3 ± 13.4</td>
</tr>
<tr>
<td>Delay time (h)§</td>
<td>0.010 (0.003 to 0.09)</td>
<td>0.022 (0.003 to 0.13) &amp;</td>
</tr>
<tr>
<td>$V_{SS}$ (L)</td>
<td>15.0 ± 2.5</td>
<td>15.0 ± 2.5</td>
</tr>
<tr>
<td>$CL_E$ (L/h)</td>
<td>8.4 (5.3 to 12.4)</td>
<td>7.5 (3.7 to 14.0) @</td>
</tr>
<tr>
<td>Morphine-6-glucuronide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction eliminated (%) †</td>
<td>8.1 (4.9 to 12.5)</td>
<td>7.4 (3.3 to 16.6) &amp;</td>
</tr>
<tr>
<td>$CL_{EM-6-G}$ (L/h)‡</td>
<td>8.3 ± 3.6</td>
<td>6.2 ± 3.1 **</td>
</tr>
<tr>
<td>Delay time (h)§</td>
<td>0.11 (0.04 to 0.34)</td>
<td>0.18 (0.06 to 0.37)</td>
</tr>
<tr>
<td>$V_{SS}$ (L)</td>
<td>15.0 ± 2.5</td>
<td>15.0 ± 2.5</td>
</tr>
<tr>
<td>$CL_E$ (L/h)</td>
<td>9.6 (5.7 to 14.7)</td>
<td>7.3 (3.7 to 17.3) @</td>
</tr>
</tbody>
</table>

All data are reported as mean ± standard deviation or median (range) (n = 14)

* Central volume (VC) was constrained to be the same for each subject during both control and cyclosporine treatments;
† Percent of morphine dose eliminated as the glucuronide metabolite;
‡ Elimination clearance represented by metabolism to morphine-glucuronide, calculated as $CL_E \times$ percent eliminated as glucuronide;
§ When delay time for M-3-G could not be estimated (9 times for controls, 6 times for cyclosporine treatments), it was arbitrarily set at 0.01 h;
# Steady-state volumes of distribution ($V_{SS}$) of both morphine-glucuronides were fixed at 0.2 × body mass (in kg), for each subject during both control and cyclosporine treatments, to approximate a total distribution volume equal to extracellular fluid space;
** $p < 0.001$ versus control;
& $p < 0.05$ versus control;
@ $p < 0.01$ versus control.

CL = clearance; $V_C$ = central volume of distribution, $V_P$ = peripheral volume of distribution, $V_{SS}$ = steady-state volume of distribution.
### Table 3

**Morphine Pharmacodynamic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cyclosporine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Noncompartmental</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}$ observed (mm)</td>
<td>2.5 ± 1.0</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>2.6 ± 1.8</td>
<td>2.7 ± 1.3</td>
</tr>
<tr>
<td>MiosisAUC$_{0-10}$ (mm•h)</td>
<td>10.9 ± 5.1</td>
<td>17.7 ± 8.9</td>
</tr>
<tr>
<td><strong>Pharmacokinetic-pharmacodynamic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_e$ (hr$^{-1}$)</td>
<td>0.17 (0.07, 0.57)</td>
<td>0.27 (0.12, 0.45)</td>
</tr>
<tr>
<td>$E_{\text{max}}$ maximum possible (mm)</td>
<td>4.4 ± 0.7</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>$E_{\text{C50}}$ (ng/ml or nM)</td>
<td>10.3 ± 5.6 ng/ml (36 ± 20 nM)</td>
<td>10.3 ± 5.6 ng/ml (36 ± 20 nM)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>2.0 ± 0.7</td>
<td>2.0 ± 0.7</td>
</tr>
</tbody>
</table>

All data are reported as mean ± standard deviation or median (range) except AUC ratios (cyclosporine/control), which are the geometric mean and 90% confidence interval (n = 14).

$E_{\text{max}}$ observed represents the maximum observed miosis due to morphine;

$^\dagger$p < 0.01 versus control;

$^\ddagger$AUC ratio (cyclosporine/control) geometric mean and 90% confidence interval was 1.60 (1.37, 1.87);

$^\S$Determined using the $E_{\text{max}}$ model for pupil diameter change from pre-drug baseline (miosis) (equation 1). $E_{\text{max}}$ maximum possible represents the maximum possible miosis (difference between resting baseline diameter and the theoretical nadir of 2.5 mm). $E_{\text{C50}}$ and $\gamma$ were codetermined for both control and cyclosporine sessions in a given individual; $p < 0.05$ versus control.

AUC = area under the curve.

*Anesthesiology. Author manuscript; available in PMC 2014 October 01.*