Cyclosporine-inhibitable Cerebral Drug Transport Does Not Influence Clinical Methadone Pharmacodynamics

Konrad Meissner, M.D., Jane Blood, R.N., B.S.N., Amber M. Francis, R.N., B.S.N., Viktar Yermolenka, Ph.D., Evan D. Kharasch, M.D., Ph.D.

ABSTRACT

Background: Interindividual variability and drug interaction studies suggest that blood–brain barrier drug transporters mediate human methadone brain biodistribution. In vitro and animal studies suggest that methadone is a substrate for the efflux transporter P-glycoprotein, and that P-glycoprotein–mediated transport influences brain access and pharmacologic effect. This investigation tested whether methadone is a transporter in humans sample contents.

Methods: Healthy volunteers received oral (N = 16) or IV (N = 12) methadone in different crossover protocols after nothing (control) or the validated P-glycoprotein inhibitor cyclosporine (4.5 mg/kg orally twice daily for 4 days, or 5 mg/kg IV over 2 h). Plasma and urine methadone and metabolite concentrations were measured by mass spectrometry. Methadone effects were measured by miosis and thermal analgesia (maximally tolerated temperature and verbal analog scale rating of discreet temperatures).

Results: Cyclosporine marginally but significantly decreased methadone plasma concentrations and apparent oral clearance, but had no effect on methadone renal clearance or on hepatic N-demethylation. Cyclosporine had no effect on miosis or on R-methadone concentration–miosis relationships after either oral or IV methadone. Peak miosis was similar in controls and cyclosporine-treated subjects after oral methadone (1.4 ± 0.4 and 1.3 ± 0.5 mm/mg, respectively) and IV methadone (3.1 ± 1.0 and 3.2 ± 0.8 mm, respectively). Methadone increased maximally tolerated temperature, but analgesia testing was confounded by cyclosporine-related pain.

Conclusions: Cyclosporine did not affect methadone pharmacodynamics. This result does not support a role for cyclosporine-inhibitable transporters mediating methadone brain access and biodistribution. (Anesthesiology 2014; 121:1281-91)

Methadone use for opioid addiction and analgesia is influenced by variable clinical effects and untoward side effects. Numerous investigations have focused on pharmacokinetic variability and drug interactions, yet less is known about pharmacodynamic-based variability and drug interactions, and their causes. For example, well-maintained methadone patients experience withdrawal symptoms, and toxicity may occur at seemingly therapeutic methadone plasma concentrations. Rifampin, ritonavir, nelfinavir, and efavirenz shifted methadone plasma concentration–effect (miosis) curves leftward and upward, increasing apparent potency and maximum effect. These latter findings provided insight into previously unexplained lack of opioid withdrawal despite methadone concentrations decreased by certain antiretrovirals. This implicated brain transport-mediated methadone drug interactions, hence suggesting that blood–brain barrier (BBB) efflux and/or influx proteins influence methadone clinical effects.

Drug transporters of the adenosine triphosphate-binding cassette (ABC) family, including the efflux transporters P-glycoprotein (P-gp, ABCB1, multidrug resistance protein 1), breast cancer resistance protein (BCRP, ABCG2), and multidrug resistance proteins (MRP, ABCC), are expressed in human brain capillary endothelial cells, and BBB ABC transporters have been implicated in brain opioid biodistribution, and some evidence in vitro suggests methadone is a substrate for blood–brain barrier efflux transporters.

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an ABC transporter substrate.\textsuperscript{10,11} Methadone did not accumulate in ABCB1-transfected pig kidney cells compared with controls, suggesting methadone was a P-gp substrate.\textsuperscript{12} In human P-gp-overexpressing cells, the P-gp inhibitors verapamil and GF120918 (elacridar) significantly decreased basal-to-apical methadone transport.\textsuperscript{13} In vivo, and consistent with these data, methadone brain uptake clearance or concentrations were approximately three-fold higher in multidrug-resistant (mdr)-deficient mdr1a/b(−/−) mice relative to wild-type mdr1a/b(+/+ ) mice, and methadone produced greater analgesia.\textsuperscript{13–15} Cerebral methadone concentrations were substantially greater in mdr1a (−/−) compared with wild-type mice.\textsuperscript{16} Upregulation of BBB P-gp activity in wild-type mice reduced methadone antinociception.\textsuperscript{17} In rats, methadone coadministration with the ABC transport inhibitor PSC833 (valspodar) increased methadone brain concentrations and antinociception, and reduced the dose for half-maximal effect (ED\textsubscript{50}).\textsuperscript{18} Together, these studies suggest that methadone is a substrate for P-gp, and brain P-gp-mediated transport influences brain access and pharmacologic effect.

In contrast to cellular and animal studies, little information exists on the role of P-gp in determining methadone brain access in humans. Indirect evidence from a pharmacogenetic study of P-gp genetic variants and dose requirements in methadone-maintained patients suggested P-gp substrate potential for methadone.\textsuperscript{19} In contrast, the P-gp inhibitor quinidine did not alter IV methadone-dependent changes in pupil diameter (miosis) or methadone concentration–effect relationships.\textsuperscript{20} Although quinidine did increase miosis after oral methadone, this was attributed to intestinal P-gp inhibition, increased methadone absorption, and increased plasma concentrations rather than enhanced brain penetration and altered BBB P-gp activity.\textsuperscript{20} It was recognized that quinidine is a nonpotent P-gp inhibitor, and plasma quinidine concentrations possibly insufficient to inhibit brain P-gp and P-gp-mediated methadone transport (if present).\textsuperscript{20} Therefore, the potential role of BBB P-gp in influencing human methadone brain penetration is unknown.

A recent study in human volunteers, conducted because in vitro and animal studies implicated P-gp in morphine transport, suggested a role for P-gp or other efflux transporters in morphine brain access and pharmacodynamics.\textsuperscript{21} Specifically, morphine miosis was more pronounced and prolonged in subjects pretreated with cyclosporine, reported to be an effective inhibitor of human BBB P-gp activity.\textsuperscript{21–23}

The current study, therefore, tested the hypothesis that methadone is a substrate for human BBB drug transporters, such as P-gp, and that transport activity influences methadone plasma concentration–effect relationships (pharmacodynamics). The secondary aim was to evaluate the role of intestinal and renal transporters in the oral absorption and renal excretion of methadone. Cyclosporine was used as a drug transport inhibitor. Methadone concentration–effect relationships were studied using pupil diameter and analgesia as primary and secondary effect measures, in a single-center, open-label, crossover study in healthy volunteers.

**Materials and Methods**

**Clinical Protocol**

The clinical investigation comprised two separate protocols, for oral and IV drug administration, in healthy volunteers (fig. 1). Both were approved by the Institutional Review Board of Washington University in St. Louis. The protocols were two-period sequential crossovers in healthy volunteers (control session first, for logistical considerations) with each subject as their own control. All subjects provided written informed consent. Healthy males and females, aged 18 to 40 yr and body mass index 20 to 33 kg/m\textsuperscript{2}, were eligible. Exclusion criteria were a history of major medical problems, including a history of liver or kidney disease, use of prescription or nonprescription medications, herbal, or foods known to be substrates of P-gp or to affect its activity, pregnant or nursing females, and a known history of addiction to drugs or alcohol. For both protocols, IV catheters were inserted for drug administration and blood sampling, and subjects received IV ondansetron (4 mg) for antiemetic prophylaxis. Subjects were monitored with a pulse oximeter and automated blood pressure cuff, and received supplemental oxygen for saturations less than 94%. Subjects were fed a standard breakfast 2 h after drug dosing and had free access to food and water thereafter. Methadone doses were chosen to target a small change (2 to 3 mm) in pupil diameter based on previous studies.

Protocol 1 (oral methadone) consisted of two sessions at least 10 days apart, the second of which was preceded by oral cyclosporine 4.5 mg/kg twice per day (maximally used therapeutic dose) (Gengraf; Abbott, Abbott Park, IL) for 4 days before and on the morning of the study day. The first four subjects were given 10 or 8 mg of racemic methadone hydrochloride orally for the control (session 1) or cyclosporine (session 2) sessions, respectively, in anticipation of

<table>
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<tr>
<th>ORAL METHADONE PROTOCOL</th>
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<tr>
<td>Oral Cyclosporine (4.5 mg/kg) am X X X X X X X X</td>
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<td>pm X X X X X X X X</td>
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<tr>
<td>Oral methadone HCl Controls: 10 mg or 0.175 mg/kg Cyclosporine: 9 mg or 0.14 mg/kg</td>
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<tr>
<td>Blood sampling X X X X</td>
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<td>Pupil measurements, pain assessments X X X X</td>
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<td>Day</td>
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|| INTRAVENOUS METHADONE PROTOCOL |
|-----------------------------|
| IV Cyclosporine (2.5 mg/kg/hr) |
| IV methadone HCl infusion 0.1 mg/kg |
| Blood sampling |
| Pupil measurements, pain assessments |
| Hour | -1 0 1 ... 24 |

**Fig. 1.** Protocol scheme. IV = intravenous.
a potentially increased methadone effect when coadministered with cyclosporine. The 10 mg dose was chosen to target a small change (2 to 3 mm) in pupil diameter. Methadone was administered 2 h after the final oral cyclosporine dose. Because of greater than anticipated intersubject variability in weight, the remaining 12 subjects received weight-based dosing (0.175 and 0.14 mg/kg methadone hydrochloride, respectively, in control and cyclosporine sessions) to diminish potential interindividual variability in plasma concentrations.

For protocol 2 (IV methadone), also on two occasions at least a week apart, 12 subjects received 0.1 mg/kg methadone as a 1 h IV infusion for both control (session 1) and cyclosporine (session 2) sessions. In session 2, subjects received an IV infusion of 2.5 mg kg⁻¹ h⁻¹ cyclosporine (Bedford Laboratories, Bedford, OH) for 2 h. This cyclosporine dose produced a 79% increase in intracerebral concentrations of the P-gp substrate verapamil, and was used in the previous investigation of morphine pharmacodynamics. Methadone was administered starting at the beginning of the second hour of the cyclosporine infusion.

Dark-adapted pupil diameter was measured in triplicate coincident with blood sampling using a handheld infrared pupillometer (Neuroptics, Irvine, CA). Pupil diameter coincident with blood sampling using a handheld infrared the cyclosporine infusion.

**Data and Statistical Analysis**

The intended primary outcome measure was the EC₅₀ (plasma concentration causing 50% attenuation of response to thermal stimulation) and secondarily, EC₉₀ for miosis, determined using a standard sigmoid Eₘₐₓ model, where Eₘₐₓ is the maximum possible effect (e.g., pupil diameter change, miosis) and C is plasma methadone concentration:

\[
\text{Effect} = \frac{E_{\text{max}} \cdot C^\gamma}{C^\gamma + EC_{50}^\gamma}
\]

Because methadone concentrations high enough to cause maximum miosis (Eₘₐₓ) were not attempted or achieved, Eₘₐₓ was fixed at 7 mm for the modeling, assuming typical minimum and maximum pupil diameters of 2.5 and 9.5 mm.

Methadone metabolism and clearance were assessed, and standard pharmacokinetic parameters were determined by noncompartmental analysis, as described previously. Pharmacokinetic data were assessed using paired t tests and effect data were analyzed by two-way repeated measure ANOVA with Student–Neumann–Keuls post hoc analysis, with two-tailed hypothesis testing (SigmaPlot; Systat Software Inc., San Jose, CA). P value less than 0.05 was considered statistically significant.

Sample size was based on a secondary outcome (area under the plasma methadone concentration vs. time curve, AUC), because intradividual variability in methadone analgesia was not known a priori. Based on prior 22 and 33% interday–intrasubject variability in IV and oral methadone AUC, respectively, to detect a 25% change using a paired t test (1-β = 0.8, α = 0.05) would require 9 and 16 subjects.

**Results**

**Oral Methadone**

Cyclosporine blood concentrations after 4 days of oral administration were 451 ± 158 ng/ml (trough) and
1,163 ± 248 ng/ml (peak). Dose-adjusted plasma methadone and EDDP concentrations versus time are shown in figure 2, and pharmacokinetic parameters in table 1. In cyclosporine-treated subjects, compared with untreated controls, dose-adjusted R- and S-methadone C_{max} was slightly (approximately 10%) albeit significantly lower, T_{max} was delayed, and dose-adjusted methadone enantiomer concentrations were lower between 1 and 3 h after dosing, yet AUC_{0-24}/dose (during and most immediately after cyclosporine dosing) was not different between groups. Together this suggests marginally impaired oral methadone absorption by cyclosporine. In contrast, methadone enantiomers AUC_{0-∞}/dose and AUC_{0-∞}/dose were somewhat (approximately 10%) but significantly greater in the cyclosporine-treated subjects. Cyclosporine marginally (approximately 10%) but significantly decreased methadone apparent oral clearance, without affecting methadone renal clearance or the fraction eliminated in urine, either in the 24 h most immediately after cyclosporine dosing or throughout the 96 h follow-up period. Cyclosporine had no effect on EDDP apparent formation clearance, but did diminish EDDP elimination, evidenced by a greater elimination half-life, and thereby somewhat (11 to 15%) increased the EDDP/methadone AUC ratio.

Oral methadone effects are shown in figure 3. Dose-adjusted dark-adapted pupil diameter difference versus pre-drug baseline (miosis) was not different between control and cyclosporine-treated subjects (fig. 3A). Miotic effects coincided with peak plasma methadone concentrations. Peak miosis was not different in controls (1.4 ± 0.4 mm/mg) and cyclosporine-treated subjects (1.3 ± 0.5 mm/mg). R-methadone (the active enantiomer) concentration–effect relationships (hysteresis curves) showed no difference between controls and cyclosporine-treated subjects (fig. 3B). However, at the early times and highest plasma concentrations after methadone dosing (0.5 to 5 h), the lack of cyclosporine effects on miosis (fig. 3A) despite slightly lower plasma concentrations (fig. 2A), and the apparently minor leftward shift of the mean concentration–effect curve (fig. 3B), prompted closer examination. Individual concentration–effect data for the 0.5 to 5 h time period showed no differences between control and cyclosporine-treated subjects (fig. 3C), and modeling of the data using a sigmoid E_{max} model showed no significant differences between control and cyclosporine-treated subjects in R-methadone EC_{50} concentrations (29 ± 5 and 23 ± 3 ng/ml, respectively) or γ (1.0 ± 0.2 and 1.1 ± 0.2). Methadone increased the maximally tolerated temperature in the method of limits paradigm, with the time of maximum analgesia coinciding with peak plasma methadone concentrations (fig. 3D). Maximally tolerated temperature was lower in the cyclosporine-treated subjects. In the paradigm using verbal analog ratings to discrete temperatures, there was a small and brief analgesic effect of methadone (fig. 3E). However, VAS scores were elevated in cyclosporine-treated subjects. Cyclosporine hyperalgesia in both pain paradigms was similar to that reported previously.²³

**Intravenous Methadone**

A second protocol using IV methadone and IV cyclosporine was performed, to achieve higher plasma cyclosporine concentrations than achievable after oral dosing, and to eliminate potential effects of cyclosporine on intestinal methadone absorption. Based on the results of protocol 1 with oral methadone, the same IV methadone dose was used in both the control and the cyclosporine sessions. Cyclosporine blood concentrations were 321 ± 809 and 3,764 ± 1,277 ng/ml after 1 (at the start of the methadone infusion) and 2 h (at the end of the cyclosporine and methadone infusions) of cyclosporine, respectively, and 750 ± 146 ng/ml 2 h after the cyclosporine infusion was stopped. R- and S-methadone C_{max} were somewhat but significantly lower in the cyclosporine-treated subjects compared with controls, as were methadone enantiomer concentrations between 0.25 and 2 h after the start of the methadone infusion (fig. 4). Nevertheless, AUC_{0-24} was not different between groups and cyclosporine had no effect on methadone elimination in urine (table 2). Because the focus of this experiment was on methadone pharmacodynamics, plasma concentrations were...
Miosis was not different between control and cyclosporine-treated subjects (fig. 5A). Peak miosis was 3.1 ± 1.0 and 3.2 ± 0.8 mm in controls and cyclosporine-treated subjects. Hysteresis curves showing the relationship between miosis and plasma R-methadone concentrations (fig. 5B) were similar in controls and cyclosporine-treated subjects. A small degree of thermal analgesia compared with baseline was observed at the IV methadone doses used (data given from 11 subjects, due to technical problems). The maximum-tolerated thermal stimulus increased in both groups, peaked at the end of the methadone infusion, and abated after 3 to 4 h (fig. 5C). However, there were no statistically significant differences between control and cyclosporine-treated subjects. In the ramp and hold paradigm of discreet thermal stimuli, there was a clear relationship between temperature and VAS pain rating (fig. 5D), but VAS scores at peak methadone concentrations (end of the methadone infusion) were not different between controls and cyclosporine-treated subjects. Time-specific verbal pain ratings to discrete thermal stimuli were higher in the cyclosporine-treated subjects (fig. 5D). Thus, methadone had minimal analgesic effects in this experiment, and analgesia was not affected by cyclosporine pretreatment. Cyclosporine itself did decrease thermal pain tolerance.

### Adverse Events

During the IV cyclosporine infusion, some subjects reported uncomfortable feelings of warmth, which were not considered intolerable, stopped after the infusion was ended, and required no treatment. These side effects resolved after methadone administration. Serum creatinine concentrations were monitored as a safety assessment of renal function after cyclosporine. Creatinine concentrations were 0.9 ± 0.2 and 1.0 ± 0.2 mg/dl, respectively, before and after the cyclosporine session in protocol 1, and 0.9 ± 0.1 and 1.0 ± 0.1 mg/dl, respectively, before and after the cyclosporine session in protocol 2. One subject had a creatinine of 1.5 mg/dl post-cyclosporine, which had normalized when rechecked. Cyclosporine was therefore considered to have had no significant effect on renal function.

### Discussion

This investigation tested the hypothesis that methadone is subject to drug transport processes in humans. The primary focus was the BBB, and the hypothesis that transport activity, specifically the efflux transporter P-gp, influences

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**Table 1.** Oral Methadone Pharmacokinetic Parameters

<table>
<thead>
<tr>
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<th>Control</th>
<th>Cyclosporine</th>
<th>Control</th>
<th>Cyclosporine</th>
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<tr>
<td>R-Methadone</td>
<td></td>
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<tr>
<td>Plasma C&lt;sub&gt;max&lt;/sub&gt;/dose (ng ml&lt;sup&gt;-1&lt;/sup&gt; g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>2.8 ± 0.5</td>
<td>2.5 ± 0.6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.6 ± 0.8</td>
<td>4.0 ± 0.9&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>2.6 ± 0.8</td>
<td>5.1 ± 2.2&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.0 ± 0.5</td>
<td>4.6 ± 2.3&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>Plasma AUC&lt;sub&gt;0-24&lt;/sub&gt;/dose (ng ml&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;/mg)</td>
<td>38 ± 7</td>
<td>39 ± 8</td>
<td>59 ± 13</td>
<td>61 ± 17</td>
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<tr>
<td>Plasma AUC&lt;sub&gt;0-96&lt;/sub&gt;/dose (ng ml&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;/mg)</td>
<td>100 ± 22</td>
<td>112 ± 26&lt;sup&gt;*&lt;/sup&gt;</td>
<td>142 ± 42</td>
<td>156 ± 54&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>Plasma AUC&lt;sub&gt;0-∞&lt;/sub&gt;/dose (ng ml&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;/mg)</td>
<td>161 ± 64</td>
<td>193 ± 71&lt;sup&gt;*&lt;/sup&gt;</td>
<td>192 ± 82</td>
<td>211 ± 89</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt;/dose ratio (cyclosporine/control)</td>
<td>1.10 (1.10, 1.30)</td>
<td>1.10 (1.03, 1.18)</td>
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<td>Plasma Cl/F (ml kg&lt;sup&gt;-1&lt;/sup&gt; min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.64 ± 0.91</td>
<td>1.40 ± 0.88&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.41 ± 0.79</td>
<td>1.28 ± 0.77&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>Plasma V&lt;sub&gt;Z&lt;/sub&gt;/F (l/kg)</td>
<td>7.7 ± 2.0</td>
<td>7.3 ± 2.6</td>
<td>4.7 ± 1.4</td>
<td>4.5 ± 1.6</td>
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<tr>
<td>Plasma t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>66 ± 28</td>
<td>73 ± 32</td>
<td>46 ± 18</td>
<td>46 ± 15</td>
</tr>
<tr>
<td>Urine %dose eliminated (0–24 h)</td>
<td>1.6 ± 0.7</td>
<td>2.6 ± 1.8</td>
<td>1.2 ± 0.6</td>
<td>2.0 ± 1.5</td>
</tr>
<tr>
<td>Urine %dose eliminated (0–96 h)</td>
<td>4 ± 2</td>
<td>5 ± 2</td>
<td>3 ± 2</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Urine clearance (ml kg&lt;sup&gt;-1&lt;/sup&gt; min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.08 ± 0.05</td>
<td>0.08 ± 0.08</td>
<td>0.04 ± 0.03</td>
<td>0.05 ± 0.05</td>
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<tr>
<td>S-Methadone</td>
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<tr>
<td>Plasma C&lt;sub&gt;max&lt;/sub&gt;/dose (ng ml&lt;sup&gt;-1&lt;/sup&gt; mg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.24 ± 0.09</td>
<td>0.22 ± 0.09</td>
<td>0.35 ± 0.17</td>
<td>0.36 ± 0.14</td>
</tr>
<tr>
<td>Plasma T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>2.5 ± 0.5</td>
<td>4.1 ± 1.1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.3 ± 0.7</td>
<td>4.0 ± 1.6&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma AUC&lt;sub&gt;0-96&lt;/sub&gt;/dose (ng ml&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;/mg)</td>
<td>6.7 ± 1.6</td>
<td>8.2 ± 1.9&lt;sup&gt;*&lt;/sup&gt;</td>
<td>11.6 ± 3.2</td>
<td>14.4 ± 3.5&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>57 ± 22</td>
<td>73 ± 38&lt;sup&gt;*&lt;/sup&gt;</td>
<td>87 ± 25</td>
<td>102 ± 39&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma AUC&lt;sub&gt;0-96&lt;/sub&gt; (EDDP/methadone)</td>
<td>0.07 ± 0.02</td>
<td>0.08 ± 0.02&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.09 ± 0.04</td>
<td>0.12 ± 0.05&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma AUC&lt;sub&gt;0-96&lt;/sub&gt; (EDDP/methadone) ratio (cyclosporine/control)</td>
<td>1.11 (1.04, 1.08)</td>
<td>1.15 (1.04, 1.27)</td>
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<tr>
<td>Urine %dose eliminated (0–24 h)</td>
<td>4.2 ± 2.3</td>
<td>4.2 ± 1.8</td>
<td>7.2 ± 3.8</td>
<td>7.4 ± 3.7</td>
</tr>
<tr>
<td>Urine %dose eliminated (0–96 h)</td>
<td>9 ± 4</td>
<td>10 ± 4</td>
<td>15 ± 6</td>
<td>16 ± 8</td>
</tr>
<tr>
<td>Urine formation clearance (ml kg&lt;sup&gt;-1&lt;/sup&gt; min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.18 ± 0.15</td>
<td>0.15 ± 0.12</td>
<td>0.26 ± 0.23</td>
<td>0.24 ± 0.20</td>
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All data are reported as mean ± SD except AUC ratios (cyclosporine/control), which are the geometric mean and 90% CI (n = 16). Urine data were not available for five subjects.

<sup>*</sup>P < 0.05 vs. control.

AUC = area under the plasma concentration–time curve; C<sub>max</sub> = peak plasma concentration; EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; T<sub>max</sub> = time to maximum concentration, Cl/F = apparent oral clearance.

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not measured after 24 h, and hence formal pharmacokinetics parameters not determined.

Miosis was not different between control and cyclosporine-treated subjects (fig. 5A). Peak miosis was 3.1 ± 1.0 and 3.2 ± 0.8 mm in controls and cyclosporine-treated subjects. Hysteresis curves showing the relationship between miosis and plasma R-methadone concentrations (fig. 5B) were similar in controls and cyclosporine-treated subjects. A small degree of thermal analgesia compared with baseline was observed at the IV methadone doses used (data given from 11 subjects, due to technical problems). The maximum-tolerated thermal stimulus increased in both groups, peaked at the end of the methadone infusion, and abated after 3 to 4 h (fig. 5C). However, there were no statistically significant differences between control and cyclosporine-treated subjects. In the ramp and hold paradigm of discreet thermal stimuli, there was a clear relationship between temperature and VAS pain rating (fig. 5D), but VAS scores at peak methadone concentrations (end of the methadone infusion) were not different between controls and cyclosporine-treated subjects. Time-specific verbal pain ratings to discrete thermal stimuli were higher in the cyclosporine-treated subjects (fig. 5D). Thus, methadone had minimal analgesic effects in this experiment, and analgesia was not affected by cyclosporine pretreatment. Cyclosporine itself did decrease thermal pain tolerance.

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During the IV cyclosporine infusion, some subjects reported uncomfortable feelings of warmth, which were not considered intolerable, stopped after the infusion was ended, and required no treatment. These side effects resolved after methadone administration. Serum creatinine concentrations were monitored as a safety assessment of renal function after cyclosporine. Creatinine concentrations were 0.9 ± 0.2 and 1.0 ± 0.2 mg/dl, respectively, before and after the cyclosporine session in protocol 1, and 0.9 ± 0.1 and 1.0 ± 0.1 mg/dl, respectively, before and after the cyclosporine session in protocol 2. One subject had a creatinine of 1.5 mg/dl post-cyclosporine, which had normalized when rechecked. Cyclosporine was therefore considered to have had no significant effect on renal function.

### Discussion

This investigation tested the hypothesis that methadone is subject to drug transport processes in humans. The primary focus was the BBB, and the hypothesis that transport activity, specifically the efflux transporter P-gp, influences

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methadone pharmacodynamics. Cyclosporine was used as a previously validated inhibitory P-gp probe.\textsuperscript{22} Cyclosporine was previously found to enhance clinical effects (miosis) and pharmacodynamics of morphine, showing it to be a weak transporter substrate.\textsuperscript{21,22} In the current investigation, neither oral nor IV cyclosporine had a significant influence on oral or IV methadone plasma concentration–effect relationships, measured primarily as miosis. Since the investigation was initiated with the idea that cyclosporine was a selective inhibitor of P-gp, one conclusion would be to reject the hypothesis that methadone brain access and pharmacodynamics are mediated by P-gp. Nevertheless, it has become apparent that cyclosporine also inhibits other efflux transporters (vide infra). Therefore, the primary conclusion is to reject the hypothesis that methadone brain access and pharmacodynamics are mediated by cyclosporine-inhibitable transport processes.

Support for this conclusion is greater from the IV than the oral methadone protocol, because blood cyclosporine concentrations were higher. The EC\textsubscript{50} for cyclosporine inhibition of P-gp was previously reported in rats as 7 μM.\textsuperscript{26} In the current investigation, oral cyclosporine for 4 days achieved peak blood concentrations of 1.0 ± 0.2 μM. IV infusion (5 mg/kg over 2 h) achieved blood cyclosporine concentrations of 2.7 ± 0.7 and 3.1 ± 1.1 μM after 1 and 2 h, respectively, comparable to those previously shown to inhibit human brain P-gp activity.\textsuperscript{22,23,26} Specifically, intracerebral concentrations of the P-gp substrate verapamil, quantified using positron emission tomography imaging, were increased 79% by 2.8 μM cyclosporine (5 mg/kg over 2 h).\textsuperscript{23}

Accumulated evidence demonstrates, however, that cyclosporine is a nonselective inhibitor of several transport proteins, including the efflux transporters MRP2 and BCRP,\textsuperscript{27-30} and several uptake transporters. However, the cyclosporine IC\textsubscript{50} for BCRP (26 μM)\textsuperscript{29} is far greater than systemic concentrations, and, at relevant concentrations, cyclosporine had no effect on OAT1 or OAT3 or MRP4, and only moderate inhibitory activity toward MRP2 in vitro, and is only considered to have significant inhibitory effects on intestinal (but not hepatic) MRP2 activity.\textsuperscript{31,32} Thus, we refer more broadly to cyclosporine-inhibitable transport rather than to specific inhibition of P-gp.

The human BBB constitutes a formidable defensive bulwark designed to restrict xenobiotic penetration. The most...
Cyclosporine abundantly expressed human BBB ABC efflux transporters are P-gp, BCRP, and MRP4.33–35 These BBB efflux transporters collaborate to exclude drugs and prevent brain accumulation. Many drugs are substrates for more than one efflux transporter, and several murine studies have shown that for these polytransporter substrates, selective chemical inhibition or genetic knock-out of only one transporter has minimal effect on brain accumulation, but simultaneous inhibition or genetic knock-out of all members of the relevant transporter suite markedly increases brain biodistribution.36–38 Thus, because BBB efflux transporters work in concert, unless cyclosporine were to inhibit all BBB transporters responsible for methadone efflux (assuming methadone is actually an efflux transporter substrate in vivo), inhibiting less than the full efflux transporter suite might not alter methadone pharmacodynamics. Therefore, the present results do not rule out the possibility that methadone is a substrate for one or more (non-cyclosporine inhibitable) efflux transporters at the human BBB. In addition, because cyclosporine can also inhibit uptake transporters such as OATPs, simultaneous inhibition of BBB uptake and efflux by cyclosporine might be offsetting. These considerations compel the need to identify more precisely which BBB transporters are responsible for methadone influx and/or efflux.

Results of this investigation can be compared with previous studies of P-gp-mediated methadone transport, both in vitro and animals. Early in vitro data said to support the P-gp substrate potential of methadone (i.e., inhibition of rhodamine-123 transport in Caco-2 cells) found an IC50 of 7.5 μM, which was far above clinical methadone concentrations, and caused only a 25% reduction of rhodamine transport.39 Similarly, methadone transport in P-gp overexpressing cells was modest (transport efflux ratio of approximately 1.9)10,12 or absent.39 A transport ratio greater than 2 is generally accepted as denoting a substrate, and, for comparison, the transport ratio of the well-known P-gp substrate loperamide generally exceeds 10. Although verapamil and GF120918 inhibited methadone transport in Caco-2 cells,13 they may also inhibit other transporters. Thus, the present results are consistent with in vitro studies suggesting that methadone is a poor P-gp substrate. In contrast, animal studies in vivo, both with P-gp genetic knock-outs and cyclosporine inhibition, provided strong evidence for methadone brain efflux transport.13–15 Thus, cyclosporine effects on methadone brain transport in humans are less than that predicted using cell systems, and much less than that by genetic knock-out or cyclosporine experiments in mice. It is now well-recognized that there are species differences in BBB P-gp expression and function, and that rodent models are over-predictive of human P-gp transport.40

Results of this investigation can also be compared with previous studies of BBB methadone transport in humans. They are consistent with the inability of the P-gp inhibitor quinidine to alter methadone miosis and concentration–effect relationships.20 They are not consistent with a report that methadone dose requirements were influenced by P-gp genetic polymorphisms.19

Cyclosporine influence on methadone miosis and pharmacodynamics was less than that observed previously for other opioids. The same cyclosporine regimen (5 mg/kg over 2 h) increased and prolonged morphine miosis, increased the area under the miosis–time curve, plasma effect-site transfer rate constant, and calculated effect-site morphine concentrations, although the magnitude of the effects was small.21 Cyclosporine more markedly (110%) increased brain uptake of the known P-gp substrate loperamide (assessed by positron emission tomography) in volunteers,41,42 which, when corrected for loperamide metabolism, was even greater (457%).42 Thus, cyclosporine-inhibitable BBB transporters play a greater role in brain access, pharmacodynamics, and clinical effects of morphine, and certainly loperamide, than methadone.

A second conclusion of this investigation was that cyclosporine minimally altered the pharmacokinetics of oral and IV methadone. For both oral and IV protocols, plasma methadone enantiomer concentrations were slightly lower in the cyclosporine-treated subjects in the period immediately after methadone dosing. The mechanism for this effect on apparent methadone distribution is not evident, but appears unrelated to methadone elimination. The more mechanistically and clinically relevant observation is that cyclosporine had no significant effect on either methadone hepatic

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**Table 2. Intravenous Methadone Pharmacokinetic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cyclosporine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-Methadone</td>
<td>S-Methadone</td>
</tr>
<tr>
<td>Plasma Cmax (ng/ml)</td>
<td>22.6 ± 6.4</td>
<td>18.8 ± 5.5*</td>
</tr>
<tr>
<td>Plasma AUC0–24 (ng/ml•h)</td>
<td>174 ± 31</td>
<td>180 ± 41</td>
</tr>
<tr>
<td>Plasma AUC0–24/dose ratio (cyclosporine/control)</td>
<td>1.03 (0.97, 1.09)</td>
<td>1.00 (0.94, 1.08)</td>
</tr>
<tr>
<td>Urine %dose eliminated (0–24h)</td>
<td>6.3 ± 2.8</td>
<td>7.8 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Cyclosporine</td>
</tr>
<tr>
<td></td>
<td>5.1 ± 2.4</td>
<td>6.1 ± 2.5</td>
</tr>
</tbody>
</table>

All data are reported as mean ± SD except AUC ratios (cyclosporine/control), which are the geometric mean and 90% CI (n = 12).

*P < 0.05 vs. control.

AUC = area under the plasma concentration–time curve; Cmax = peak plasma concentration.
That methadone is also a CYP2B6 substrate, therefore to be cleared by CYP3A4, it is now clear in vivo in humans and cleared predominantly if not exclusively by CYP2B6 in vitro for cytochrome P4503A4. Although methadone was originally identified as a substrate for N-demethylation to EDDP or renal clearance. Metabolism and clearance, which can account for up to 25% of total systemic methadone clearance, was not mediated by cyclosporine. This may be consistent with observations that EDDP is a substrate for BCRP, OATP1A2, OCT1, and OCT3 (E. Kharasch, unpublished results) and that cyclosporine can affect these transporters.

Another investigational aim was to evaluate whether methadone is subject to intestinal and/or renal drug transport processes. Cyclosporine delayed methadone absorption and slightly reduced Cmax, but this is more consistent with inhibition of an uptake than an efflux transporter. Renal methadone clearance, which can account for up to 25% of total systemic methadone clearance, was not mediated by cyclosporine-inhibitable renal transporters. EDDP elimination did appear slightly reduced by cyclosporine. This may be consistent with observations that EDDP is a substrate for the method of limits (maximally tolerated temperature in an ascending temperature paradigm) or the ramp-and-hold method (VAS scores to specific temperatures). Miosis was detectable at plasma R-methadone concentrations averaging 5 ng/ml. In comparison, the median minimal effective (postoperative) analgesia threshold for (racemic) methadone was 31 ng/ml. Both IV and oral cyclosporine caused cutaneous sensitization to heat, similar to that reported previously for IV cyclosporine. This sensitization differs from the well-described pain syndrome (bilateral bone pain in the lower extremities) caused by cyclosporine. Cyclosporine sensitization confounded the use of analgesia as a metric of cyclosporine infusion. The last conclusion of this investigation was that miosis was a much more sensitive measure than thermal analgesia of methadone clinical effects and pharmacodynamics. Plasma R-methadone Cmax in controls averaged 23 and 16 ng/ml after IV and oral administration, respectively. Miotic response was greater and more sustained (average 2.5 and 2 mm, respectively) than thermal analgesia, using either the method of limits (maximally tolerated temperature in an ascending temperature paradigm) or the ramp-and-hold method (VAS scores to specific temperatures). Miosis was detectable at plasma R-methadone concentrations averaging 5 ng/ml. In comparison, the median minimal effective (postoperative) analgesia threshold for (racemic) methadone was 31 ng/ml. The lack of CYP3A inhibition of methadone metabolism to EDDP by cyclosporine is inconsistent with a role for CYP3A in clinical methadone metabolism and clearance, but is consistent with previous studies in which other strong CYP3A inhibitors also had no influence on (or sometimes even increased) methadone N-demethylation and clearance. and reinforces the predominant role of CYP2B6 in methadone metabolism and clearance.

Fig. 5. Influence of cyclosporine on intravenous methadone effects and pharmacodynamics. Effects were evaluated using dark-adapted pupil diameter and response to thermal stimulus. Time is relative to the start of the methadone infusion. The 2-h cyclosporine infusion (−1 to 1 h) was started 1 h before methadone (0−1 h). [A] Miosis (time-specific pupil diameter minus predrug pupil diameter) versus time after start of infusion. Results are the mean ± SD (N = 12). (B) Miosis versus plasma R-methadone concentrations. Results are the mean (SD omitted for clarity, N = 12). (C) Maximalia temperature difference compared with predrug baseline using the methods of limits over the first 5 h after the start of the methadone infusion. Results are the averages of differences (SD omitted for clarity, N = 11, asterisks denote significant differences vs. baseline). (D) Stimulus–response relationship (verbal analog scale pain ratings) in response to discreet thermal stimuli, at the end of the 1 h methadone infusion, and the influence of cyclosporine. Results are the mean (SD omitted for clarity, N = 12). (E) Pain ratings (verbal analog scale) in response to thermal stimulus (shown for 44.8° and 46.5°C). Results are the mean (SD omitted for clarity, N = 12).

Metabolism (N-demethylation to EDDP) or renal clearance. Although methadone was originally identified as a substrate in vitro for cytochrome P4503A4 (CYP3A4), and assumed therefore to be cleared in vivo by CYP3A4, it is now clear that methadone is also a CYP2B6 substrate in vitro, and cleared predominantly if not exclusively by CYP2B6 in humans in vivo. Cyclosporine inhibits hepatic and intestinal CYP3A4 activity and the clearance of CYP3A substrates. Based on the in vitro Ki for cyclosporine inhibition of CYP3A (1.4 μM), and clinical effects of 200 mg/day cyclosporine (24 to 31% inhibition of CYP3A activity at a trough concentration of 119 ng/ml [0.1 μM]), and blood cyclosporine concentrations in the present investigation (≥3 μM peak), substantial inhibition of CYP3A activity in the present investigation (approximately 720 mg/day oral cyclosporine) would be expected. Another investigational aim was to evaluate whether methadone is subject to intestinal and/or renal drug transport processes. Cyclosporine delayed methadone absorption and slightly reduced Cmax, but this is more consistent with inhibition of an uptake than an efflux transporter. Renal methadone clearance, which can account for up to 25% of total systemic methadone clearance, was not mediated by cyclosporine-inhibitable renal transporters. EDDP elimination did appear slightly reduced by cyclosporine. This may be consistent with observations that EDDP is a substrate for BCRP, OATP1A2, OCT1, and OCT3 (E. Kharasch, unpublished results) and that cyclosporine can affect these transporters.

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One limitation of this investigation is that cyclosporine is only a partial BBB P-gp inhibitor in humans. For example,
the third-generation P-gp inhibitor tariquidar (6 mg/kg) in humans increased brain concentrations of the P-gp substrates \(^{[11]}\)C]-N-desmethyl-loperamide and (R)-\(^{[11]}\)C]-verapamil 4- and 2.5-fold, respectively, whereas cyclosporine (2.5 mg kg\(^{-1}\) h\(^{-1}\) for 2 h) increased (RS)-\(^{[11]}\)C]-verapamil by only 88.9%.\(^{22}\) Nevertheless, third-generation P-gp inhibitors were not available when the present investigation was performed.

In summary, this investigation showed that cyclosporine, used as an inhibitory in vivo probe for BBB P-glycoprotein and other transporters, had no influence on methadone miosis, or on R-methadone plasma concentration–miosis relationships, for either IV or oral methadone. This suggests little or no role for cyclosporine-inhibitable transporters in methadone brain access and pharmacodynamics.

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Competing Interests

The authors declare no competing interests.

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