

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

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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 discrete 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,¹ and toxicity may occur at seemingly therapeutic methadone plasma concentrations.² Rifampin, ritonavir, nelfinavir, and efavirenz shifted methadone plasma concentration–effect (miosis) curves leftward and upward, increasing apparent potency and maximum effect.^{3–6} 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

What We Already Know about This Topic

- Pretreatment with the blood–brain barrier efflux transport inhibitor cyclosporine resulted in more pronounced and more prolonged morphine-induced miosis in healthy volunteers
- *In vitro* and animal studies have suggested methadone may also be a substrate for blood–brain barrier efflux transport

What This Article Tells Us That Is New

- Pretreatment of healthy volunteers with either oral or intravenous cyclosporine had no effect on the methadone concentration *versus* miosis relationship, suggesting there is no role for *P*-glycoprotein or other cyclosporine-inhibitable transporters in methadone brain access or pharmacodynamics in humans

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.^{7,8} BBB ABC transporters have been implicated in brain opioid biodistribution,⁹ and some evidence *in vitro* suggests methadone is

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an ABC transporter substrate.^{10,11} Methadone did not accumulate in ABCB1-transfected pig kidney cells compared with controls, suggesting methadone was a P-gp substrate.¹² In human P-gp-overexpressing cells, the P-gp inhibitors verapamil and GF120918 (elacridar) significantly decreased basal-to-apical methadone transport.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.^{13–15} Cerebral methadone concentrations were substantially greater in *mdr1a* (-/-) compared with wild-type mice.¹⁶ Upregulation of BBB P-gp activity in wild-type mice reduced methadone antinociception.¹⁷ In rats, methadone coadministration with the ABC transport inhibitor PSC833 (valsopodar) increased methadone brain concentrations and antinociception, and reduced the dose for half-maximal effect (ED₅₀).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.¹⁹ In contrast, the P-gp inhibitor quinidine did not alter IV methadone-dependent changes in pupil diameter (miosis) or methadone concentration–effect relationships.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.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).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.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.^{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/m², 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, herbals, 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

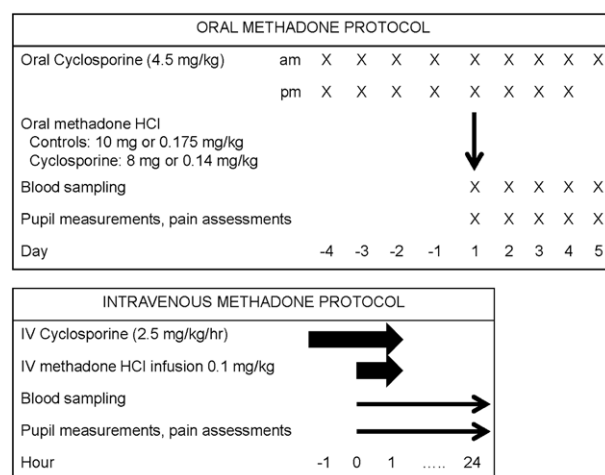


Fig. 1. Protocol scheme. IV = intravenous.

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,²³ and was used in the previous investigation of morphine pharmacodynamics.²¹ 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).²⁴ Pupil diameter change from predrug baseline (miosis) was determined at each time. Analgesia was assessed by response to thermal stimulus (Pathway; Medoc Advanced Medical Systems, Ramat Yishai, Israel) using both the maximum-tolerated temperature (method of limits) and the verbal analog pain rating of several predetermined temperatures (ramp-and-hold method). Thermode temperature started at 36°C and increased 0.5°C/s, and subjects pressed a button when the maximum tolerable temperature was reached. The average result of three stimuli was recorded in degree Celsius. Subjects then rated pain intensity on a verbal analog scale (VAS, 0 to 100) in response to discrete stimuli (41.0°, 43.0°, 44.8°, 46.5°, 48.2°, and 50.0°C in random order). The probe was moved to a different region for each thermal stimulus.

Sixteen subjects (eight males, eight females, 79 ± 14 kg, body mass index 27 ± 4) completed both arms of protocol 1. The average oral racemic methadone hydrochloride doses were 13 ± 3 and 10 ± 2 mg (control and cyclosporine sessions), corresponding to 5.8 ± 1.2 and 4.7 ± 1.0 mg of each methadone enantiomer base, and the cyclosporine dose was 361 ± 64 mg two times per day for 4 days. Twelve subjects (eight males, four females, 75 ± 14 kg, body mass index 25 ± 4) completed both arms of protocol 2. The average IV methadone hydrochloride dose was 7.5 ± 1.5 mg for control and cyclosporine sessions, corresponding to 3.4 ± 0.7 mg of each methadone enantiomer base. The cyclosporine dose was 424 ± 84 mg IV over 2 h.

Analytical Methods

Venous blood samples were obtained before and periodically for 96 (protocol 1) and 24 h (protocol 2) after methadone administration, and all urine was collected during these

times. Plasma was separated and stored at -20°C for later analysis. Cyclosporine blood concentrations were determined by the clinical laboratory of Barnes-Jewish Hospital as trough (predose) and peak (2 h after oral cyclosporine) concentrations on the methadone study day (protocol 1), and 1 (mid-infusion), 2 (end-infusion), and 4 h after starting the IV cyclosporine infusion (protocol 2). Serum creatinine was determined before and after the study.

Methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in plasma and urine were quantified by solid-phase extraction and stereoselective high-pressure liquid chromatography-mass spectrometry using a previous method.²⁵ Interday coefficients of variation in plasma averaged 6 and 6% for EDDP enantiomers (0.2, 2 ng/ml) and 7, 5, and 4% for methadone enantiomers (1, 10, and 80 ng/ml).

Data and Statistical Analysis

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

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

Because methadone concentrations high enough to cause maximum miosis (E_{max}) were not attempted or achieved, E_{max} was fixed at 7 mm for the modeling, assuming typical minimum and maximum pupil diameters of 2.5 and 9.5 mm.²¹

Methadone metabolism and clearance were assessed, and standard pharmacokinetic parameters were determined by noncompartmental analysis, as described previously.³⁻⁶ 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 intraindividual 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,³⁻⁶ 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 $AUC_{0-96}/dose$ and $AUC_{0-\infty}/dose$ 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.

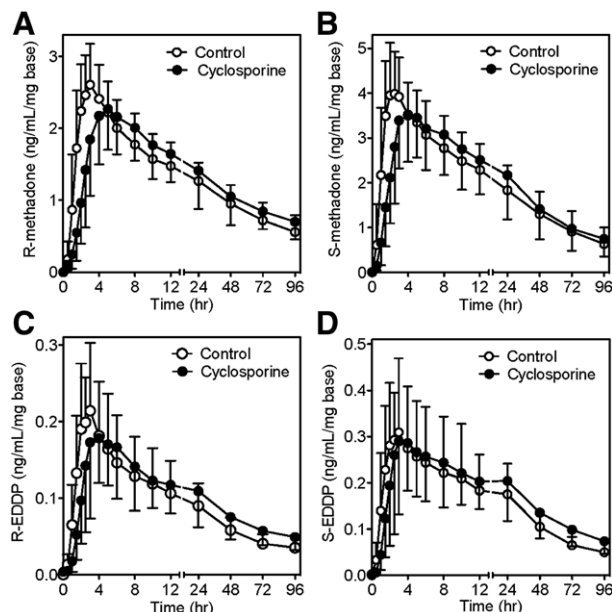


Fig. 2. Effects of cyclosporine on dose-normalized methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) enantiomer plasma concentrations after oral methadone in subjects receiving nothing (controls, 0.175 mg/kg methadone hydrochloride, open circles) or 4 days of cyclosporine (4.5 mg/kg twice per day), where the methadone hydrochloride dose was 0.14 mg/kg (closed circles). (A) R-methadone, (B) S-methadone, (C) R-EDDP, and (D) S-EDDP. Results are the mean ± SD (N = 16). Methadone and EDDP dose (enantiomer base)-normalized concentrations were significantly lower in cyclosporine-treated subjects between 1 and 3 h after dosing ($P < 0.05$).

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.²¹

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 $3,764 \pm 1,277$ 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

Table 1. Oral Methadone Pharmacokinetic Parameters

	Control	Cyclosporine	Control	Cyclosporine
	R-Methadone		S-Methadone	
Plasma C _{max} /dose (ng ml ⁻¹ g ⁻¹)	2.8 ± 0.5	2.5 ± 0.6*	4.6 ± 0.8	4.0 ± 0.9*
Plasma T _{max} (h)	2.6 ± 0.8	5.1 ± 2.2*	2.0 ± 0.5	4.6 ± 2.3*
Plasma AUC ₀₋₂₄ /dose (ng ml•h/mg)	38 ± 7	39 ± 8	59 ± 13	61 ± 17
Plasma AUC ₀₋₉₆ /dose (ng/ml•h/mg)	100 ± 22	112 ± 26*	142 ± 42	156 ± 54*
Plasma AUC _{0-∞} /dose (ng/ml•h/mg)	161 ± 64	193 ± 71*	192 ± 82	211 ± 89
AUC _{0-∞} /dose ratio (cyclosporine/control)		1.10 (1.10, 1.30)		1.10 (1.03, 1.18)
Plasma Cl/F (ml kg ⁻¹ min ⁻¹)	1.64 ± 0.91	1.40 ± 0.88*	1.41 ± 0.79	1.28 ± 0.77*
Vz/F (l/kg)	7.7 ± 2.0	7.3 ± 2.6	4.7 ± 1.4	4.5 ± 1.6
Plasma t _{1/2} (h)	66 ± 28	73 ± 32	46 ± 18	46 ± 15
Urine %dose eliminated (0–24 h)	1.6 ± 0.7	2.6 ± 1.8	1.2 ± 0.6	2.0 ± 1.5
Urine %dose eliminated (0–96 h)	4 ± 2	5 ± 2	3 ± 2	3 ± 2
Urine clearance (ml kg ⁻¹ min ⁻¹)	0.08 ± 0.05	0.08 ± 0.08	0.04 ± 0.03	0.05 ± 0.05
	R-EDDP		S-EDDP	
Plasma C _{max} /dose (ng ml ⁻¹ mg ⁻¹)	0.24 ± 0.09	0.22 ± 0.09	0.35 ± 0.17	0.36 ± 0.14
Plasma T _{max} (h)	2.5 ± 0.5	4.1 ± 1.1*	2.3 ± 0.7	4.0 ± 1.6*
Plasma AUC ₀₋₉₆ /dose (ng/ml•h/mg)	6.7 ± 1.6	8.2 ± 1.9*	11.6 ± 3.2	14.4 ± 3.5*
Plasma t _{1/2} (h)	57 ± 22	73 ± 38*	87 ± 25	102 ± 39*
Plasma AUC ₀₋₉₆ (EDDP/methadone)	0.07 ± 0.02	0.08 ± 0.02*	0.09 ± 0.04	0.12 ± 0.05*
Plasma AUC ₀₋₉₆ (EDDP/methadone) ratio (cyclosporine/control)		1.11 (1.04, 1.08)		1.15 (1.04, 1.27)
Urine %dose eliminated (0–24 h)	4.2 ± 2.3	4.2 ± 1.8	7.2 ± 3.8	7.4 ± 3.7
Urine %dose eliminated (0–96 h)	9 ± 4	10 ± 4	15 ± 6	16 ± 8
Urine formation clearance (ml kg ⁻¹ min ⁻¹)	0.18 ± 0.15	0.15 ± 0.12	0.26 ± 0.23	0.24 ± 0.20

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.

*P < 0.05 vs. control.

AUC = area under the plasma concentration–time curve; C_{max} = peak plasma concentration; EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; T_{max} = time to maximum concentration, Cl/F = apparent oral clearance.

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 discrete 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

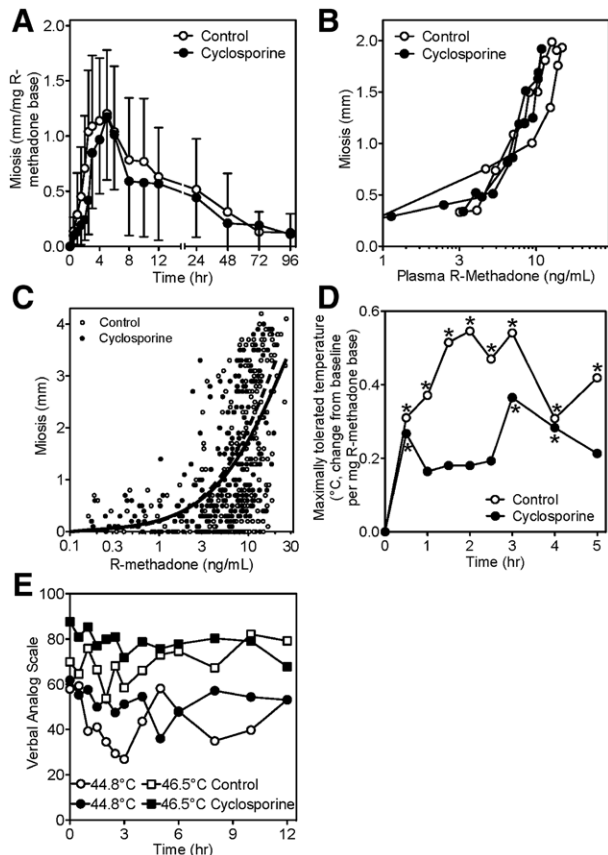


Fig. 3. Influence of cyclosporine on oral methadone effects and pharmacodynamics. Effects were evaluated using dark-adapted pupil diameter and response to thermal stimulus. *Open and solid symbols* reflect controls and cyclosporine-treated subjects, respectively. (A) R-methadone dose-normalized miosis (time-specific pupil diameter minus predrug pupil diameter). Results are the mean \pm SD (N = 16). (B) Miosis versus plasma R-methadone concentrations. Results are the mean (SD omitted for clarity) (N = 16). (C) Miosis versus plasma R-methadone concentrations for 0.5–5 h after methadone dosing. Data points are individual concentrations. *Solid lines* for controls and *dashed lines* for cyclosporine show results predicted by fitting the data using a sigmoid E_{\max} model. (D) R-methadone dose-normalized maximally tolerated temperature difference versus predrug baseline using the methods of limits. Results are the mean (SD omitted for clarity, N = 16, asterisks denote significant differences vs. baseline). (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 = 16).

methadone pharmacodynamics. Cyclosporine was used as a previously validated inhibitory P-gp probe.²² Cyclosporine was previously found to enhance clinical effects (miosis) and pharmacodynamics of morphine, showing it to be a weak transporter substrate.^{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

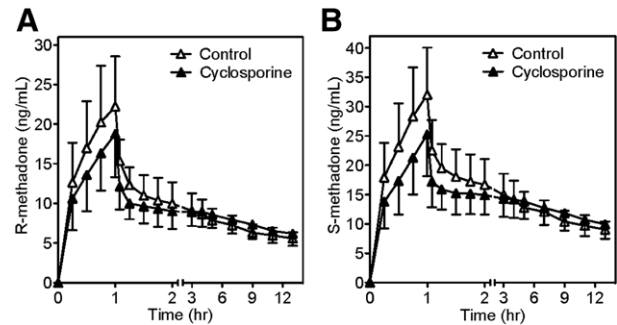


Fig. 4. Effects of cyclosporine on plasma concentrations of (A) R-methadone, (B) S-methadone, after a 1 h intravenous infusion of methadone hydrochloride (0.1 mg/kg) in subjects receiving nothing (controls, *open triangles*) or a 2 h infusion of cyclosporine (2.5 mg $\text{kg}^{-1} \text{ h}^{-1}$, *closed triangles*). Results are shown as the mean \pm SD (N = 12). Methadone concentrations were significantly lower in cyclosporine-treated subjects between 0.25 and 2 h after the start of the methadone infusion ($P < 0.05$). Times are relative to the start of the methadone infusion.

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_{50} for cyclosporine inhibition of P-gp was previously reported in rats as 7 μM .²⁶ In the current investigation, oral cyclosporine for 4 days achieved peak blood concentrations of $1.0 \pm 0.2 \mu\text{M}$. IV infusion (5 mg/kg over 2 h) achieved blood cyclosporine concentrations of 2.7 ± 0.7 and $3.1 \pm 1.1 \mu\text{M}$ after 1 and 2 h, respectively, comparable to those previously shown to inhibit human brain P-gp activity.^{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).²³

Accumulated evidence demonstrates, however, that cyclosporine is a nonselective inhibitor of several transport proteins, including the efflux transporters MRP2 and BCRP,^{27–30} and several uptake transporters. However, the cyclosporine IC_{50} for BCRP (26 μM)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.^{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

Table 2. Intravenous Methadone Pharmacokinetic Parameters

	Control	Cyclosporine	Control	Cyclosporine
	R-Methadone		S-Methadone	
Plasma C _{max} (ng/ml)	22.6 ± 6.4	18.8 ± 5.5*	32.2 ± 7.9	25.3 ± 7.2*
Plasma AUC ₀₋₂₄ (ng/ml•h)	174 ± 31	180 ± 41	276 ± 47	282 ± 68
Plasma AUC ₀₋₂₄ /dose ratio (cyclosporine/control)		1.03 (0.97, 1.09)		1.00 (0.94, 1.08)
Urine %dose eliminated (0–24 h)	6.3 ± 2.8	7.8 ± 3.2	5.1 ± 2.4	6.1 ± 2.5

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; C_{max} = peak plasma concentration.

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 exclude the possibility that methadone is a substrate for one or more (noncyclosporine 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

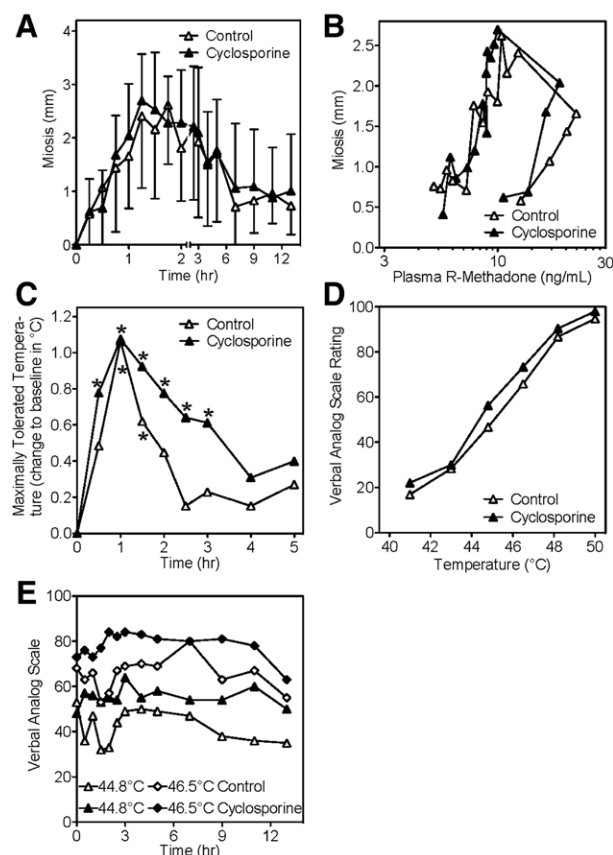


Fig. 5. Influence of cyclosporine on intravenous methadone effects and pharmacodynamics. Effects were evaluated using dark-adapted pupil diameter and response to thermal stimulus. Time is relative to the start of the methadone infusion. The 2-h cyclosporine infusion (–1 to 1 h) was started 1 h before methadone (0–1 h). *Open and solid symbols* reflect controls and cyclosporine-treated subjects, respectively. (A) Miosis (time-specific pupil diameter minus predrug pupil diameter) *versus* time after start of infusion. Results are the mean \pm SD ($N = 12$). (B) Miosis *versus* plasma R-methadone concentrations. Results are the mean (SD omitted for clarity, $N = 12$). (C) Maximally tolerated 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),⁴³ and assumed therefore to be cleared *in vivo* by CYP3A4, it is now clear that methadone is also a CYP2B6 substrate *in vitro*,^{3,44–47} and cleared predominantly if not exclusively by CYP2B6 in humans *in vivo*.^{3,5,25,48–51} Cyclosporine inhibits hepatic and

intestinal CYP3A activity and the clearance of CYP3A substrates.^{32,52} Based on the *in vitro* K_i for cyclosporine inhibition of CYP3A (1.4 μ M),⁵³ 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 μ M]),⁵² and blood cyclosporine concentrations in the present investigation ($>3 \mu$ M peak), substantial inhibition of CYP3A activity in the present investigation (approximately 720 mg/day oral cyclosporine) would be expected. 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,^{3,6,48,51,54} and CYP3A induction also had no effect.⁵⁵ This further reinforces the predominant role of CYP2B6 in methadone metabolism and clearance.^{3,5,25,48–51}

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 C_{max} , 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.⁵⁶

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 C_{max} 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.⁵⁷

Both IV and oral cyclosporine caused cutaneous sensitization to heat, similar to that reported previously for IV cyclosporine.²¹ This sensitization differs from the well-described pain syndrome (bilateral bone pain in the lower extremities) caused by cyclosporine.⁵⁸ Cyclosporine sensitization confounded the use of analgesia as a metric of cyclosporine influence on methadone effects and pharmacodynamics, and reinforces the value of pupillometry for evaluating these outcomes.

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,^{40,59} whereas cyclosporine (2.5 mg kg⁻¹ h⁻¹ for 2 h) increased (RS)-[^{11}C]verapamil by only 88%.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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References

1. Dyer KR, Foster DJ, White JM, Somogyi AA, Menelaou A, Bochner F: Steady-state pharmacokinetics and pharmacodynamics in methadone maintenance patients: Comparison of those who do and do not experience withdrawal and concentration-effect relationships. *Clin Pharmacol Ther* 1999; 65:685–94
2. Chugh SS, Socoteanu C, Reinier K, Waltz J, Jui J, Gunson K: A community-based evaluation of sudden death associated with therapeutic levels of methadone. *Am J Med* 2008; 121:66–71
3. Kharasch ED, Hoffer C, Whittington D, Sheffels P: Role of hepatic and intestinal cytochrome P450 3A and 2B6 in the metabolism, disposition, and mitotic effects of methadone. *Clin Pharmacol Ther* 2004; 76:250–69
4. Kharasch ED, Bedynek PS, Walker A, Whittington D, Hoffer C: Mechanism of ritonavir changes in methadone pharmacokinetics and pharmacodynamics: II. Ritonavir effects on CYP3A and P-glycoprotein activities. *Clin Pharmacol Ther* 2008; 84:506–12
5. Kharasch ED, Walker A, Whittington D, Hoffer C, Bedynek PS: Methadone metabolism and clearance are induced by nelfinavir despite inhibition of cytochrome P4503A (CYP3A) activity. *Drug Alcohol Depend* 2009; 101:158–68
6. Kharasch ED, Whittington D, Ensign D, Hoffer C, Bedynek PS, Campbell S, Stubbart K, Crafford A, London A, Kim T: Mechanism of efavirenz influence on methadone pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* 2012; 91:673–84
7. Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, Bertino JR: Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci U S A* 1989; 86:695–8
8. Bendayan R, Ronaldson PT, Gingras D, Bendayan M: *In situ* localization of P-glycoprotein (ABCB1) in human and rat brain. *J Histochem Cytochem* 2006; 54:1159–67
9. Wandel C, Kim R, Wood M, Wood A: Interaction of morphine, fentanyl, sufentanil, alfentanil, and loperamide with the efflux drug transporter P-glycoprotein. *ANESTHESIOLOGY* 2002; 96:913–20
10. Tournier N, Chevillard L, Megarbane B, Pirnay S, Scherrmann JM, Declèves X: Interaction of drugs of abuse and maintenance treatments with human P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2). *Int J Neuropsychopharmacol* 2010; 13:905–15
11. Tournier N, Declèves X, Saubaméa B, Scherrmann JM, Cisternino S: Opioid transport by ATP-binding cassette transporters at the blood-brain barrier: Implications for neuropsychopharmacology. *Curr Pharm Des* 2011; 17:2829–42
12. Crettol S, Digon P, Golay KP, Brawand M, Eap CB: *In vitro* P-glycoprotein-mediated transport of $^{\oplus}$ -, (S)-, (R,S)-methadone, LAAM and their main metabolites. *Pharmacology* 2007; 80:304–11
13. Hassan HE, Myers AL, Coop A, Eddington ND: Differential involvement of P-glycoprotein (ABCB1) in permeability, tissue distribution, and antinociceptive activity of methadone, buprenorphine, and diprenorphine: *In vitro* and *in vivo* evaluation. *J Pharm Sci* 2009; 98:4928–40
14. Thompson SJ, Koszdzin K, Bernards CM: Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. *ANESTHESIOLOGY* 2000; 92:1392–9
15. Dagenais C, Graff CL, Pollack GM: Variable modulation of opioid brain uptake by P-glycoprotein in mice. *Biochem Pharmacol* 2004; 67:269–76
16. Wang JS, Ruan Y, Taylor RM, Donovan JL, Markowitz JS, DeVane CL: Brain penetration of methadone $^{\oplus}$ - and (S)-enantiomers is greatly increased by P-glycoprotein deficiency in the blood-brain barrier of Abcb1a gene knockout mice. *Psychopharmacology (Berl)* 2004; 173:132–8
17. Bauer B, Yang X, Hartz AM, Olson ER, Zhao R, Kalvass JC, Pollack GM, Miller DS: *In vivo* activation of human pregnane X receptor tightens the blood-brain barrier to methadone through P-glycoprotein up-regulation. *Mol Pharmacol* 2006; 70:1212–9
18. Rodriguez M, Ortega I, Soengas I, Suarez E, Lukas JC, Calvo R: Effect of P-glycoprotein inhibition on methadone analgesia and brain distribution in the rat. *J Pharm Pharmacol* 2004; 56:367–74

19. Levran O, O'Hara K, Peles E, Li D, Barral S, Ray B, Borg L, Ott J, Adelson M, Kreek MJ: ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. *Hum Mol Genet* 2008; 17:2219–27
20. Kharasch ED, Hoffer C, Whittington D: The effect of quinidine, used as a probe for the involvement of P-glycoprotein, on the intestinal absorption and pharmacodynamics of methadone. *Br J Clin Pharmacol* 2004; 57:600–10
21. Meissner K, Avram MJ, Yermolenka V, Francis AM, Blood J, Kharasch ED: Cyclosporine-inhibitable blood-brain barrier drug transport influences clinical morphine pharmacodynamics. *ANESTHESIOLOGY* 2013; 119:941–53
22. Sasongko L, Link JM, Muzi M, Mankoff DA, Yang X, Collier AC, Shoner SC, Unadkat JD: Imaging P-glycoprotein transport activity at the human blood-brain barrier with positron emission tomography. *Clin Pharmacol Ther* 2005; 77:503–14
23. Hsiao P, Bui T, Ho RJ, Unadkat JD: *In vitro*-to-*in vivo* prediction of P-glycoprotein-based drug interactions at the human and rodent blood-brain barrier. *Drug Metab Dispos* 2008; 36:481–4
24. Kharasch ED, Walker A, Hoffer C, Sheffels P: Intravenous and oral alfentanil as *in vivo* probes for hepatic and first-pass cytochrome P450 3A activity: Noninvasive assessment by use of pupillary miosis. *Clin Pharmacol Ther* 2004; 76:452–66
25. Kharasch ED, Stubbert K: Role of cytochrome P4502B6 in methadone metabolism and clearance. *J Clin Pharmacol* 2013; 53:305–13
26. Hsiao P, Sasongko L, Link JM, Mankoff DA, Muzi M, Collier AC, Unadkat JD: Verapamil P-glycoprotein transport across the rat blood-brain barrier: Cyclosporine, a concentration inhibition analysis, and comparison with human data. *J Pharmacol Exp Ther* 2006; 317:704–10
27. Kobayashi M, Saitoh H, Kobayashi M, Tadano K, Takahashi Y, Hirano T: Cyclosporin A, but not tacrolimus, inhibits the biliary excretion of mycophenolic acid glucuronide possibly mediated by multidrug resistance-associated protein 2 in rats. *J Pharmacol Exp Ther* 2004; 309:1029–35
28. Qadir M, O'Loughlin KL, Fricke SM, Williamson NA, Greco WR, Minderman H, Baer MR: Cyclosporin A is a broad-spectrum multidrug resistance modulator. *Clin Cancer Res* 2005; 11:2320–6
29. Xia CQ, Liu N, Miwa GT, Gan LS: Interactions of cyclosporin a with breast cancer resistance protein. *Drug Metab Dispos* 2007; 35:576–82
30. Westley IS, Brogan LR, Morris RG, Evans AM, Sallustio BC: Role of Mrp2 in the hepatic disposition of mycophenolic acid and its glucuronide metabolites: Effect of cyclosporine. *Drug Metab Dispos* 2006; 34:261–6
31. El-Sheikh AA, Greupink R, Wortelboer HM, van den Heuvel JJ, Schreurs M, Koenderink JB, Masereeuw R, Russel FG: Interaction of immunosuppressive drugs with human organic anion transporter (OAT) 1 and OAT3, and multidrug resistance-associated protein (MRP) 2 and MRP4. *Transl Res* 2013; 162:398–409
32. Gertz M, Cartwright CM, Hobbs MJ, Kenworthy KE, Rowland M, Houston JB, Galetin A: Cyclosporine inhibition of hepatic and intestinal CYP3A4, uptake and efflux transporters: Application of PBPK modeling in the assessment of drug-drug interaction potential. *Pharm Res* 2013; 30:761–80
33. Shawahna R, Uchida Y, Declèves X, Ohtsuki S, Yousif S, Dauchy S, Jacob A, Chassoux F, Daumas-Duport C, Couraud PO, Terasaki T, Scherrmann JM: Transcriptomic and quantitative proteomic analysis of transporters and drug metabolizing enzymes in freshly isolated human brain microvessels. *Mol Pharm* 2011; 8:1332–41
34. Declèves X, Jacob A, Yousif S, Shawahna R, Potin S, Scherrmann JM: Interplay of drug metabolizing CYP450 enzymes and ABC transporters in the blood-brain barrier. *Curr Drug Metab* 2011; 12:732–41
35. Sane R, Agarwal S, Mittapalli RK, Elmquist WF: Saturable active efflux by p-glycoprotein and breast cancer resistance protein at the blood-brain barrier leads to nonlinear distribution of elacridar to the central nervous system. *J Pharmacol Exp Ther* 2013; 345:111–24
36. Tang SC, Lagas JS, Lankheet NA, Poller B, Hillebrand MJ, Rosing H, Beijnen JH, Schinkel AH: Brain accumulation of sunitinib is restricted by P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) and can be enhanced by oral elacridar and sunitinib coadministration. *Int J Cancer* 2012; 130:223–33
37. Lin F, Marchetti S, Plum D, Iusuf D, Mazzanti R, Schellens JH, Beijnen JH, van Tellingen O: Abcc4 together with abcb1 and abcg2 form a robust cooperative drug efflux system that restricts the brain entry of camptothecin analogues. *Clin Cancer Res* 2013; 19:2084–95
38. Oberoi RK, Mittapalli RK, Elmquist WF: Pharmacokinetic assessment of efflux transport in sunitinib distribution to the brain. *J Pharmacol Exp Ther* 2013; 347:755–64
39. Störmer E, Perloff MD, von Moltke LL, Greenblatt DJ: Methadone inhibits rhodamine123 transport in Caco-2 cells. *Drug Metab Dispos* 2001; 29:954–6
40. Bauer M, Zeitlinger M, Karch R, Matzneller P, Stanek J, Jäger W, Böhmendorfer M, Wadsak W, Mitterhauser M, Bankstahl JP, Löscher W, Koepp M, Kuntner C, Müller M, Langer O: Pgp-mediated interaction between @-11C]loperamide and tariquidar at the human blood-brain barrier: A comparison with rat data. *Clin Pharmacol Ther* 2012; 91:227–33
41. Passchier J, Comley R, Salinas C, Rabiner E, Gunn R, Cunningham V, Wilson A, Houle S, Gee A, Laruelle M: Blood brain barrier permeability of [¹¹C]loperamide in humans under normal and impaired P-glycoprotein function. *J Nucl Med* 2008; 49:211P
42. Hsiao P, Unadkat JD: P-glycoprotein-based looperamide-cyclosporine drug interaction at the rat blood-brain barrier: Prediction from *in vitro* studies and extrapolation to humans. *Mol Pharm* 2012; 9:629–33
43. Iribarne C, Berthou F, Baird S, Dréano Y, Picart D, Bail JP, Beaune P, Ménéz JF: Involvement of cytochrome P450 3A4 enzyme in the N-demethylation of methadone in human liver microsomes. *Chem Res Toxicol* 1996; 9:365–73
44. Gerber JG, Rhodes RJ, Gal J: Stereoselective metabolism of methadone N-demethylation by cytochrome P4502B6 and 2C19. *Chirality* 2004; 16:36–44
45. Totah RA, Allen KE, Sheffels P, Whittington D, Kharasch ED: Enantiomeric metabolic interactions and stereoselective human methadone metabolism. *J Pharmacol Exp Ther* 2007; 321:389–99
46. Totah RA, Sheffels P, Roberts T, Whittington D, Thummel K, Kharasch ED: Role of CYP2B6 in stereoselective human methadone metabolism. *ANESTHESIOLOGY* 2008; 108:363–74
47. Gadel S, Craford A, Regina K, Kharasch ED: Methadone N-demethylation by the common CYP2B6 allelic variant CYP2B6.6. *Drug Metab Dispos* 2013; 41:709–13
48. Kharasch ED, Bedynek PS, Park S, Whittington D, Walker A, Hoffer C: Mechanism of ritonavir changes in methadone pharmacokinetics and pharmacodynamics: I. Evidence against CYP3A mediation of methadone clearance. *Clin Pharmacol Ther* 2008; 84:497–505
49. Kharasch ED, Hoffer C, Whittington D, Walker A, Bedynek PS: Methadone pharmacokinetics are independent of cytochrome P4503A (CYP3A) activity and gastrointestinal drug transport: Insights from methadone interactions with ritonavir/indinavir. *ANESTHESIOLOGY* 2009; 110:660–72
50. Kharasch ED, Bedynek PS, Hoffer C, Walker A, Whittington D: Lack of indinavir effects on methadone disposition despite inhibition of hepatic and intestinal cytochrome P4503A (CYP3A). *ANESTHESIOLOGY* 2012; 116:432–47
51. Kharasch ED, Stubbert K: Cytochrome P4503A does not mediate the interaction between methadone and ritonavir-lopinavir. *Drug Metab Dispos* 2013; 41:2166–74

52. de Jonge H, de Loo H, Verbeke K, Vanrenterghem Y, Kuypers DR: *In vivo* CYP3A activity is significantly lower in cyclosporine-treated as compared with tacrolimus-treated renal allograft recipients. *Clin Pharmacol Ther* 2011; 90:414–22
53. Niwa T, Yamamoto S, Saito M, Shiraga T, Takagi A: Effect of cyclosporine and tacrolimus on cytochrome p450 activities in human liver microsomes. *Yakugaku Zasshi* 2007; 127:209–16
54. van Heeswijk R, Vandevoorde A, Verboren P, Boogaerts G, De Paepe E, van Solingen-Ristea R, Garg V, Beumont M: Pharmacokinetic interaction between methadone and the investigational HCV protease inhibitor telaprevir. *J Hepatology* 2011; 54: S491–492
55. Vourvahis M, Wang R, Gruener DM, Bruce RD, Haider S, Tawadrous M: Effect of lersivirine co-administration on pharmacokinetics of methadone in healthy volunteers. *Drug Alcohol Depend* 2012; 126:183–8
56. Picard N, Levoir L, Lamoureux F, Yee SW, Giacomini KM, Marquet P: Interaction of sirolimus and everolimus with hepatic and intestinal organic anion-transporting polypeptide transporters. *Xenobiotica* 2011; 41:752–7
57. Gourlay GK, Wilson PR, Glynn CJ: Pharmacodynamics and pharmacokinetics of methadone during the perioperative period. *ANESTHESIOLOGY* 1982; 57:458–67
58. Prommer E: Calcineurin-inhibitor pain syndrome. *Clin J Pain* 2012; 28:556–9
59. Kreisl WC, Liow JS, Kimura N, Seneca N, Zoghbi SS, Morse CL, Herscovitch P, Pike VW, Innis RB: P-glycoprotein function at the blood-brain barrier in humans can be quantified with the substrate radiotracer 11C-N-desmethyl-loperamide. *J Nucl Med* 2010; 51:559–66