

The Pronociceptive Effect of Ondansetron in the Setting of P-Glycoprotein Inhibition

Jason A. Scott, MD
Margaret Wood, MD
Pamela Flood, MD

Ondansetron is a potent antiemetic drug that acts through inhibition of the 5HT₃ receptors for serotonin. Minimum alveolar concentration (MAC) for isoflurane is not affected by systemic ondansetron; however ondansetron is a substrate of P-glycoprotein, a transport pump expressed in the blood-brain barrier. Thus, we hypothesized that central nervous system concentrations of ondansetron might be reduced by the P-gp protein. As potent inhibitors of P-gp are in clinical trials to improve access of desirable chemotherapeutic and antibiotic drugs to the central nervous system, we studied the effect of ondansetron in the absence of extrusion by P-gp. Normal rats were given lumbar intrathecal ondansetron or vehicle. P-gp knockout mice and wild-type controls were treated with systemic ondansetron in the presence and absence of clinically used P-gp inhibitors. Nociception was assessed as thermal hindpaw withdrawal latency and immobility was assessed as isoflurane MAC. In rats, intrathecal ondansetron (20 g) increased thermal pain sensitivity by 20.0% ± 5.8% ($P < 0.01$). Systemic ondansetron (2 mg/kg) increased pain sensitivity in P-gp knockout mice but had no effect in wild-type mice ($P < 0.01$). Systemic ondansetron had a small but statistically significant pronociceptive effect after treatment of wild-type mice with the P-gp inhibitor quinidine but not with cyclosporine or verapamil. Isoflurane MAC was not changed by intrathecal ondansetron in rats or systemically administered ondansetron in P-gp knockout mice. Intrathecal ondansetron can enhance thermal pain sensitivity. In the absence of P-gp protein, ondansetron can reach concentrations sufficient to increase pain sensitivity. Even with direct spinal application, ondansetron does not alter isoflurane MAC, supporting the idea that 5HT₃ modulation does not play a role in general anesthetic immobility.

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Ondansetron is a substrate of the multidrug resistance protein-1 p-glycoprotein (P-gp) (1). The MDR-1 protein is an efflux transport protein that is highly expressed in the blood-brain barrier, gut endothelium, and placenta. P-gp actively transports its substrates across cell membranes against concentration gradients. Thus, in normal circumstances ondansetron is actively pumped out of the central nervous system (CNS) and other P-gp expressing tissues. There are several inhibitors of the P-gp transporter in common clinical use, including verapamil, probenecid, quinidine, and cyclosporin A (2). New, more potent, and selective inhibitors such as PSC833 are in clinical trials both to allow increased permeation of antimicrobial drugs and anti-neoplastic drugs into the CNS in

settings such as human immunodeficiency virus infection, where the infective agent is protected by the blood-brain barrier (3,4).

Ondansetron, an inhibitor of the 5HT₃ receptor for serotonin, has become popular as a potent, effective prophylactic and therapeutic drug for the treatment of nausea and vomiting associated with anesthesia. Used alone, ondansetron is remarkable for its lack of side effects. Thus, it is used commonly in the post-chemotherapy setting in single adult doses as high as 32 mg. Ondansetron may be administered as part of a clinical anesthetic that uses combinations of several drugs combined to maximize their desirable effects and minimize unintended side effects. Notably, ondansetron does not alter the analgesic or respiratory depressant effects of alfentanil (5,6) and when given systemically to rats it does not affect the minimum alveolar concentration (MAC) concentration of isoflurane (7).

We hypothesized that in the absence of the active P-gp transporter, ondansetron could accumulate in the CNS in sufficient quantity to produce side effects relevant to anesthesia, including pain and immobility. The 5HT₃ receptor for serotonin is expressed in the dorsal horn of the spinal cord; their activation is antinociceptive (8). As a proof of concept, we tested the effect of intrathecal ondansetron in rats on MAC

From the Department of Anesthesiology, Columbia University, New York, New York.

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Address correspondence and reprint requests to Pamela Flood, MD, Assistant Professor, Department of Anesthesiology, Columbia University, 630 West 168th Street, New York, NY 10032. Address e-mail to pdf3@columbia.edu.

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and pain sensitivity. With the finding of a pronociceptive effect of intrathecal ondansetron, we studied the effect of systemic ondansetron on pain sensitivity in mice genetically altered to lack the MDR-1 gene.

METHODS

With approval of the Columbia Committee on Animal Research, we studied 24 adult female Sprague-Dawley rats weighing 250–400 g (Taconic Farms, Germantown, NY), 5 male P-gp knockout mice, and 10 male generation matched controls.

At least 2 days before experimentation, a polyethylene catheter (CS-1 Intrathecal Catheter System 32G; ReCathCo LLC, Allison Park, PA) was inserted through the atlanto-occipital membrane and advanced approximately 7.5 cm, such that its tip lay in the lumbar intrathecal space as previously described (9). Rats that showed persistent neurological deficits after awakening were killed. Correct catheter placement was confirmed by direct visualization, passage of catheter with only light fingertip pressure, and twitching of successive myotomes as the catheter was advanced. In the development of our technique, under isoflurane anesthesia we injected 60 μ L of methylene blue through the indwelling intrathecal catheter of a rat. The rat was then killed and dissected to demonstrate intrathecal spread of the drug with no intracranial extension. After implantation of the intrathecal catheters, rats were housed individually with free access to food and water and allowed to recover for at least 2 days before experimentation. Each animal was studied 2–3 times for nociceptive testing, with at least 2 days between tests. Ten animals were MAC tested. Animals were exposed to isoflurane and/or ondansetron.

Sterile clinical grade ondansetron solution (Zofran; GlaxoSmithKline, Philadelphia, PA), at a concentration of 2 mg/mL, was used for intrathecal injections. Ondansetron carrier solution (9.0 mg/mL NaCl, 0.5 mg/mL citric acid monohydrate, 0.25 mg sodium citrate) was tested in equal volumes to those in the experimental conditions in 4 rats. There was no evidence of neurological injury in either group.

MAC was determined as previously described (10). Animals received either 120 μ g of ondansetron or an equivalent volume of the ondansetron carrier solution (60 μ L) as a slow manual injection through the intrathecal catheter. Each animal was placed in a 30-cm-long transparent cylinder with an ID of 6.4 cm. The head end stopper contained a gas inflow port through which isoflurane was delivered in a fresh gas flow rate of 1 L/min oxygen. The tail end stopper had ports for gas effluent, the rat's tail, a temperature probe, and a gas analyzer probe (RGM, Datex-Ohmeda, Madison, WI). The temperature of each animal was determined rectally and maintained between 36° and 38°C using heating blankets. The MAC of isoflurane was measured as the mean of partial pressures bracketing the

animal's response and lack of response to a modified alligator clip. The clip was applied to the proximal tail for 60 s and vigorously manually rotated $\pm 45^\circ$ at approximately 1 Hz. After assessing the first response, the inspired isoflurane concentration was adjusted in 0.2vol% changes, depending on the animal's response, until each animal showed a move:no-move cross-over. Thirty minutes was allowed for isoflurane equilibration at each concentration step. The isoflurane partial pressure was measured continuously by gas analyzer calibrated with gas chromatography. MAC was determined for each group as the average MAC for each animal \pm SE.

Hindpaw withdrawal latency (HPWL) was measured with a modification of the device described by Hargreaves et al. (11) (Harvard Apparatus, Holliston, MA) in up to 4 unrestrained rats (per study) housed in individual plastic cubicles. The cubicles rested on a clear glass plate. Over the chambers we placed a clear plexiglas enclosure that rested on a silicone rubber gasket that produced a seal to the glass plate. Gas-tight fittings at either end permitted delivery and scavenging of gases. Oxygen was delivered from an oxygen tank. Heating strips warmed the glass plate to minimize body heat loss. To diminish exploratory activity, the rats were acclimated to this environment for at least 30 min before testing. After acclimation, a radiant heat source was focused on the plantar surface of the hindpaw. The stimulus intensity was controlled by a constant voltage source and was adjusted so that baseline latency to paw withdrawal from the heat source was 10–20 s. Both paws were tested in random order 1–2 min apart. In all experiments HPWL measurement was made for each hindpaw 5 times (total of 10 measurements). Measurements on each paw were made at approximately 5-min intervals. The 10 readings were averaged to produce a value for each test condition.

Before cumulative dose testing, intrathecal catheters were connected to adapters for multiple dosing. Rats were then placed in the plastic cubicles on the glass plate and baseline HPWLs were obtained. Cumulative doses of ondansetron (20, 40, 60, 80, 100, 120, 140, 160, and 180 μ g) were administered and HPWL testing was performed 30 min after each dose. We tested the duration of action of ondansetron's pronociceptive effect after a single dose of 80 μ g by testing HPWL every 30 min subsequently.

For P-gp knockout mice, MAC was determined using the same protocol as described for the rats. P-gp knockout mice and generation-matched controls were tested before and 30 min after 2 mg/kg intraperitoneal ondansetron.

HPWL testing was performed with up to 10 mice per study, housed in pairs in 5 plastic chambers. The protocol used was identical to that described for the rats. P-gp knockout mice and generation-matched controls were tested before, and 30 min after, intraperitoneal

Table 1. Minimum Alveolar Concentration (MAC) of Isoflurane (%) Required to Prevent a Response to Tail Clamp

	<i>n</i>	MAC
P-gp Knockout Mice		
Control	4	1.35 ± 0.20
2 mg/kg ondansetron	4	1.30 ± 0.24
Rat		
Control	10	1.33 ± 0.25
100 μg ondansetron	10	1.30 ± 0.22

P-glycoprotein knockout mice were tested before and after the administration of 2 mg/kg intraperitoneal ondansetron. There was no significant difference in MAC between the two groups. Rats were tested before and after the administration of 120 μg intrathecal ondansetron. There was no statistically significant difference in MAC between the two groups.

injection of 2 mg/kg of ondansetron. Generation-matched wild-type controls were also tested before and 30 min after intraperitoneal injection of ondansetron 2 mg/kg, in the presence of a clinical dose of a P-gp inhibitor (cyclosporin A 200 mg/kg, verapamil 25 mg/kg, or quinidine 50 mg/kg administered 45 min before testing). We chose maximal sublethal doses of P-gp inhibitors as previously studied (2). We tested the duration of action of ondansetron's pronociceptive effect after a single intraperitoneal dose of 2 mg/kg by testing HPWL every 30 min subsequently. All drugs not otherwise mentioned were purchased from Sigma Chemicals (St. Louis, MO).

The values for MAC are expressed as mean and se and are compared with an unpaired Student's *t*-test for significant difference. The effect of cumulative doses over time is determined with analysis of variance. A *P* value <0.05 is considered significant. Data for HPWL are expressed as % of baseline ± se.

RESULTS

The MAC for isoflurane before and after 120 μg intrathecal ondansetron in rats was 1.33 ± 0.08 and 1.30 ± 0.07, respectively. There was no significant difference in MAC before and after this dose of intrathecal ondansetron in rats (Student's *t*-test >0.05) (Table 1).

In rats, with cumulative doses of intrathecal ondansetron sensitivity to a noxious thermal stimulus increased. After receiving 20 μg intrathecal ondansetron a marked hyperalgesic effect was observed with HPWL reduced by 20% ± 5.8% (Fig. 1; analysis of variance, *P* < 0.01). This effect was maintained across the dose range (up to 160 μg intrathecal ondansetron) with no significant further increase in hyperalgesia. After a single intrathecal dose of 80 μg ondansetron there was a decrease of 23% ± 7.8% in HPWL with duration of effect of 4 h (Fig. 2) (Student's *t*-test, *P* < 0.05).

In P-gp knockout mice, the MAC for isoflurane before and after 2 mg/kg intrathecal ondansetron was 1.35 ± 0.10 and 1.3 ± 0.12, respectively. There was no significant difference in MAC before and after this dose of intraperitoneal ondansetron in P-gp knockout mice (Student's *t*-test *P* > 0.05) (Table 1).

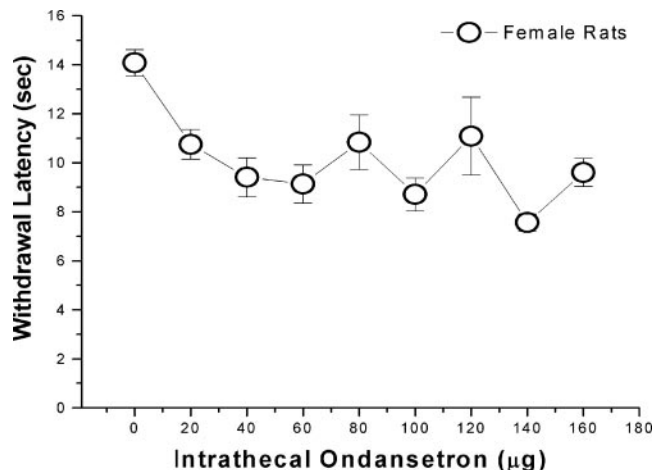


Figure 1. Rats received doses of intrathecal ondansetron to a cumulative dose of 160 μg (at a concentration of 2 μg/μL). After each dose they were tested for withdrawal latency to a heat stimulus. Latency was significantly reduced by treatment with ondansetron (analysis of variance, *P* < 0.01).

Withdrawal latency was reduced in P-gp knockout mice by 2 mg/kg intraperitoneal ondansetron (15.7 ± 1.3 and 9.7 ± 1.2 KO). This represented a significant hyperalgesic effect of systemic ondansetron in P-gp knockout mice (Student's *t*-test, *P* < 0.05) (Table 2). Thus there was a statistically significant decrease in HPWL of 18% ± 8.2% in P-gp knockout mice after a single intraperitoneal dose of 2 mg/kg with a duration of effect of 1½ h (*t*-test *P* < 0.01). Withdrawal latency before and after 2 mg/kg intraperitoneal ondansetron in generation-matched control mice was not different (13.6 ± 0.8 and 13.1 ± 0.8 respectively; Student's *t*-test *P* > 0.05) (Table 2).

To determine whether drugs in current use that exhibit P-gp inhibition predispose to ondansetron's hyperalgesic effect, we pretreated control mice with three P-gp inhibitors, cyclosporin A, verapamil, and quinidine, at doses known to cause inhibition of P-gp.

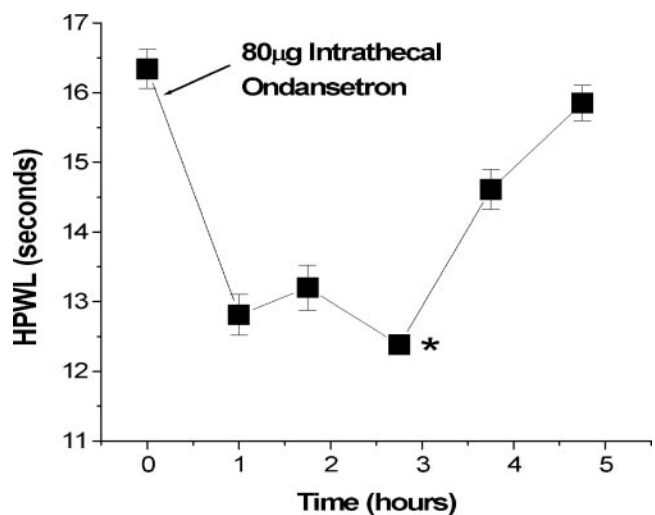


Figure 2. After a single intrathecal dose of 80 μg ondansetron there was a 23% ± 7.8% reduction in latency for heat withdrawal that persisted for 4 h (Student's *t*-test, *P* < 0.05).

Table 2. Hindpaw Withdrawal Latency in Both P-Glycoprotein (P-gp) Knockout Mice and Wild-Type Controls, Before and After the Administration of 2 mg/kg Intraperitoneal Ondansetron

	Latency (s)	P value
P-gp knockout mice (<i>n</i> = 5)		
Baseline	15.7 ± 2.9	<0.01
2 mg/kg ondansetron	9.7 ± 2.7	
Wild-type mice (<i>n</i> = 10)		
Baseline	13.6 ± 2.5	NS
2 mg/kg ondansetron	13.1 ± 2.5	

There was a significant reduction in latency in P-gp knockout mice after systemic ondansetron (paired Student's *t*-test *P* < 0.01) but no change in latency in the generation-matched controls. Values are mean ± sb.

Although pretreatment with each drug resulted in a small reduction in HPWL, only treatment with quinine was statistically different (Student's *t*-test *P* > 0.05) (Table 3).

DISCUSSION

We found that the intrathecal administration of ondansetron produced an increased sensitivity to pain in rats. The effects of intrathecal 5HT₃ antagonists are controversial. Serotonergic neurons from the medullary nucleus raphe magnus and adjacent nucleus reticularis project to the spinal cord and release serotonin, which acts on many different metabotropic receptors and one ionotropic serotonin receptor (12). Serotonin has both excitatory and inhibitory effects on pain at the level of the spinal cord. However its inhibitory actions are predominant (13).

In support of our findings, Alhaider et al. (8) found that intrathecal 5-HT₃ inhibition produced an increase in pain sensitivity. They hypothesized the involvement of GABAergic interneurons. However, the nociceptive response to 5HT₃ antagonism may depend on the pain modality studied and the location of 5HT₃ receptors. Zeitz et al. (14) have shown that intrathecal ondansetron decreases sensitivity to chronic inflammatory pain using the formalin test. However, in their studies intrathecal ondansetron did not change sensitivity in the acute phase after formalin injection. They did observe a small but not statistically significant increase in acute phase neuronal firing in lamina 5 that could represent increased nociceptive input that was not detectable in their behavioral assay. Giordano and Dyche (15) found no effect on thermal, mechanical, and chemical pain testing after administration of intracerebroventricular 5-HT₃ antagonists.

Ondansetron is a substrate of the phosphoglycoprotein transport pump encoded by the MDR1a gene. As such, the P-gp protein acts to actively pump ondansetron out of the blood-brain barrier across a concentration gradient. Schinkel et al. (1) showed a fourfold increase in brain concentration of ondansetron in mice deficient in the MDR1a P-gp, demonstrating ondansetron to be a substrate of this protein. We found systemically administered ondansetron produced an increased sensitivity to pain in MDR1a knockout mice. Although the increase in pain sensitivity parallels our findings in rats receiving intrathecal ondansetron, these mice had permeability of the blood-brain barrier throughout the nervous system and, therefore, inhibition of cerebral 5-HT₃ receptors may have influenced behavior as well. Several drugs in current clinical use act as inhibitors of the P-gp protein, although more potent inhibitors are in clinical trials to increase access of the normally cloistered CNS to antibiotic and chemotherapeutic drugs. In our studies of the less potent, currently available P-gp inhibitors cyclosporin A, quinidine, and verapamil, only quinidine enhanced pain sensitivity as we observed in the MDR1a knockout mice that received systemic ondansetron. The level of inhibition produced by the other drugs is not adequate to raise spinal cord levels of ondansetron enough to produce the hyperalgesic effect.

Rampil et al. (7) have shown that systemic ondansetron has no effect on MAC. We considered the possibility that this finding may have been a result of a failure of ondansetron to accumulate in the CNS in sufficient concentration because of extrusion by the P-gp protein. Therefore we studied the effect of intrathecal ondansetron on MAC in rats and the effect of intraperitoneal ondansetron in MDR1a knockout mice. As neither treatment affected the minimum concentration of isoflurane required for immobility, we concur with their conclusion that 5HT₃ modulation by isoflurane does not contribute to MAC.

Our studies suggest that there is a tonic activation of 5HT₃ receptors in the spinal cord that constrains the response to a noxious heat stimulus. Direct application of ondansetron can remove that tonic inhibitory tone and increase pain sensitivity. Systemic treatment of an animal with intact MDR1 protein does not cause this phenotype, likely because of insufficient CNS levels. The total removal of MDR1 in the knockout mouse or the bypass of the blood-brain barrier with

Table 3. Hindpaw Withdrawal Latency Was Measured in Wild-type Mice Treated with Clinically Available P-glycoprotein Inhibitors (Intraperitoneal Verapamil 25 mg/kg, Quinidine 50 mg/kg or Cyclosporin A 200 mg/kg), Before and After the Administration of 2 mg/kg Intraperitoneal Ondansetron 45 Minutes After Treatment with P-gp Inhibitor

P-gp inhibitor (<i>n</i> = 7)	<i>n</i>	Baseline with inhibitor	Post-ondansetron 2 mg/kg	P value
Verapamil (25 mg/kg)	5	9.9 ± 2.4	9.0 ± 0.8	NS
Quinidine (50 mg/kg)	5	11.1 ± 1.3	9.6 ± 1.6	<0.05
Cyclosporin A (200 mg/kg)	5	14.6 ± 1.1	13.6 ± 2.7	NS

Values are mean ± sb. There was statistically significant pain enhancement only after treatment with quinidine.

intrathecal application resulted in mild hyperalgesia in response to noxious heat. Ondansetron is a commonly used drug with minimal neurological side effects in the setting of intact MDR1 protein. However, when active extrusion of ondansetron from the CNS does not occur, it has pronociceptive effects. Because there are many substrates of the MDR1 protein, when potent MDR1 antagonists are introduced clinically it will be important to test for interaction with CNS targets that were previously protected.

REFERENCES

1. Schinkel AH, Wagenaar E, Mol CA, van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J Clin Invest* 1996;97:2517-24.
2. Arboix M, Paz OG, Colombo T, D'Incalci M. Multidrug resistance-reversing agents increase vinblastine distribution in normal tissues expressing the P-glycoprotein but do not enhance drug penetration in brain and testis. *J Pharmacol Exp Ther* 1997;281:1226-30.
3. Boesch D, Gaveriaux C, Jachez B, et al. *In vivo* circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. *Cancer Res* 1991;51:4226-33.
4. Choo EF, Leake B, Wandel C, et al. Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. *Drug Metab Dispos* 2000;28:655-60.
5. Dershwitz M, DiBiase PM, Rosow CE, et al. Ondansetron does not affect alfentanil-induced ventilatory depression or sedation. *Anesthesiology* 1992;77:447-52.
6. Petersen-Felix S, Arendt-Nielsen L, Bak P, et al. Ondansetron does not inhibit the analgesic effect of alfentanil. *Br J Anaesth* 1994;73:326-30.
7. Rampil IJ, Laster MJ, Eger EI II. Antagonism of the 5-HT(3) receptor does not alter isoflurane MAC in rats. *Anesthesiology* 2001;95:562-4.
8. Alhaider AA, Lei SZ, Wilcox GL. Spinal 5-HT3 receptor-mediated antinociception: possible release of GABA. *J Neurosci* 1991;11:1881-8.
9. LoPachin RM, Rudy TA, Yaksh TL. An improved method for chronic catheterization of the rat spinal subarachnoid space. *Physiol Behav* 1981;27:559-61.
10. Eger EI II, Zhang Y, Laster M, et al. Acetylcholine receptors do not mediate the immobilization produced by inhaled anesthetics. *Anesth Analg* 2002;94:1500-4.
11. Hargreaves KM, Mueller GP, Dubner R, et al. Corticotropin-releasing factor (CRF) produces analgesia in humans and rats. *Brain Res* 1987;422:154-7.
12. Furst S. Transmitters involved in antinociception in the spinal cord. *Brain Res Bull* 1999;48:129-41.
13. Li P, Zhuo M. Cholinergic, noradrenergic, and serotonergic inhibition of fast synaptic transmission in spinal lumbar dorsal horn of rat. *Brain Res Bull* 2001;54:639-47.
14. Zeitz KP, Guy N, Malmberg AB, et al. The 5-HT3 subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. *J Neurosci* 2002;22:1010-9.
15. Giordano J, Dyché J. Differential analgesic actions of serotonin 5-HT3 receptor antagonists in the mouse. *Neuropharmacology* 1989;28:423-7.