Regional Anesthesia and Pain

Analgesic effects of systemic midazolam: comparison with intrathecal administration

[Les effets analgésiques du midazolam à action générale : comparaison avec l'administration intrathécale]

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Purpose: Midazolam has antinociceptive effects when administered intrathecally, while its effects associated with systemic administration remain controversial. In the present study, the antinociceptive properties of systemically *vs* intrathecally administered midazolam were investigated in a rat model of thermal and inflammatory pain.

Methods: One hundred seventy-six ($n = 8$ animals per dose escalation) male Sprague-Dawley rats were instrumented with lumbar intrathecal catheters. Tail withdrawal in response to thermal stimulation, or paw flinching and shaking in response to *sc* hind paw formalin injection were compared following intrathecal injection of midazolam (1, 3, 10, 30, or 100 μg in 10 μL) or *ip* administration (3, 30, 300, or 3,000 μg in 300 μL). Saline 10 μL or 300 μL was used as a control. Behavioural side effects and motor disturbance were also examined.

Results: Intrathecal administration of midazolam increased tail flick latency dose dependently ($P < 0.05$) with a 50% effective dose (ED50) of 1.60 μg, whereas ip administration did not increase latency. Both intrathecal and ip routes of administration decreased the number of paw flinches in both phases 1 and 2 of the formalin test ($P < 0.05$). The ED50s were 1.26 μg [confidence interval (CI), 0.35–3.18 μg], (phase 1) and 1.20 μg (CI, 0.29–3.71 μg), (phase 2) with intrathecal administration, and 11.6 μg (CI, 2.5–19.3 μg), (phase 1) and 52.2 μg (CI, 18.3–102.7 μg), (phase 2) with ip administration.

Conclusion: Systemically administered midazolam induced antinociception for inflammatory pain only, while intrathecal administration elicited antinociceptive effects on both acute thermal and inflammatory-induced pain.

Objectif *: Administré par voie intrathécale, le midazolam a des effets antinociceptifs, mais les effets d'une administration intrapéritonéale (ip) demeurent controversés. Dans la présente étude, nous avons vérifié les propriétés antinociceptives de l'administration générale vs intrathécale du midazolam chez un modèle expérimental de douleur thermique et inflammatoire chez le rat.*

Méthode *: Un cathéter intrathécal lombaire a été mis en place chez 176 (n = 8 animaux par dose croissante) rats mâles Sprague-Dawley. Le retrait de la queue, en réaction à la stimulation thermique, ou le tressaillement et le tremblement de la patte en réaction à l'injection sc de formaline dans la patte arrière, ont été comparés à la suite d'une injection intrathécale de midazolam (1, 3, 10, 30, or 100 µg dans 10 µL) ou l'administration ip (3, 30, 300, ou 3 000 µg dans 300 µL). Une solution salée, 10 µL ou* 300 µL, *a servi de solution témoin. Les effets secondaires comportementaux et les troubles moteurs ont été aussi examinés.*

Résultats *: L'administration intrathécale de midazolam a augmenté la latence de la rétraction de la queue en fonction de la dose (P < 0,05) avec une dose efficace moyenne (ED_{50}) de 1,60 µg, tandis que l'administration ip n'a pas augmenté la latence. Les voies d'administration intrathécale et ip ont réduit le nombre de retraits de la patte au cours des phases 1 et 2 du test à la formaline (P < 0,05). Les ED_{50} ont été de 1,26 µg [intervalle de confiance (IC), 0,35–3,18 µg], (phase 1) et de 1,20 µg (IC, 0,29–3,71 µg), (phase 2) avec l'administration intrathécale et de 11,6 µg (IC, 2,5–19,3 µg), (phase 1) et de 52,2 µg (IC, 18,3–102,7 µg), (phase 2) avec l'administration ip.*

Conclusion *: L'administration intrapéritonéale de midazolam a induit une antinociception pour la douleur inflammatoire seulement alors que l'administration intrathécale a produit des effets antinociceptifs sur la douleur thermique aiguë et la douleur induite par l'inflammation.*

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A part of this study was supported by grant C-16591527 from the Ministry of Health, Labour and Welfare.
Accepted for publication June 12, 2006.
Revision accepted July 10, 2006.
Competing interests: None declared.

INTRAVENOUSLY administered benzodiazepines are not generally considered to have analgesic properties. When midazolam is administered intravenously, it is difficult to demonstrate analgesic effects over and above the drug's effect on consciousness.

Recent studies have shown that midazolam produces an analgesic action through the benzodiazepine/γ-aminobutyric acid $(GABA)_A$ receptor complex in the spinal cord.[1,2] However, intraperitoneally administered midazolam may be associated with hyperalgesia,[3] while it potentiates isoflurane-induced antinociception at doses where no effect is seen with midazolam alone.[4] Therefore, it is uncertain as to whether systemically administered midazolam is analgesic or hyperalgesic. As a result of this controversy, the antinociceptive effects of systemically and intrathecally administered midazolam were investigated on two different types of pain in a rat model. We hypothesized that systemic administration of midazolam would elicit analgesic effects, while the response would be less intense in comparison with intrathecal administration of this relatively short-acting benzodiazepine.

Methods

After obtaining the approval of the Research Committee of the University of Tokyo, male Sprague-Dawley rats (280–300 g; Nippon Bio-Supply, Tokyo, Japan) were implanted with lumbar intrathecal catheters under halothane (2%) anesthesia in 100% oxygen. An 8.5-cm polyethylene catheter (PE-10; Clay Adams, Parsippany, NJ, USA) was inserted caudally and advanced to the thoracolumbar level in the intrathecal space through the atlanto-occipital membrane. The rostral part of the catheter was plugged with a 28-G steel wire and advanced to the top of the skull. Only rats with normal motor function and normal behaviour seven days after the instrumentation procedure were used for the study. After each experiment, the location of the catheter was confirmed anatomically, and the data from any rat with incorrect catheter placement was excluded from the analysis. At each dose escalation, eight randomly selected rats whose correct catheter placement was confirmed, were enrolled (n = 176).

Midazolam (Sigma, St. Louis, MO, USA) was dissolved in normal saline to achieve solutions of 1, 3, 10, 30, and 100 μg in 10 μL for intrathecal administration, or 3, 30, 300, and 3,000 μg in 300 μL for *ip* administration. Normal saline was used as a control. A microsyringe was used for intrathecal injection and after each injection, the catheter was flushed with normal saline 10 μL to clear the catheter dead space (8.5 ± 0.6 μL). A 1-mL syringe with a 23-G needle was used for *ip* injection.

The tail flick test was performed with the Tail-Flick Analgesia Meter (MK-330A; Muromachi Kikai Co. Ltd., Tokyo, Japan). Rats were placed in a clear plastic cage with their tails extending through a slot located at the rear of the cage. Thermal stimulation was given by a beam of high-intensity light focused on the tail 2 to 3 cm proximal to the end. The time between the start of the stimulation and tail withdrawal was measured as the tail flick latency. The cut-off time in the absence of a response was set to 14 sec to prevent tissue injury. The tests were performed at five, ten, 15, 30, 60, 90, and 120 min after drug administration in the *ip* group, adding 180 and 240 min in the intrathecal group.

The formalin test was performed ten minutes after drug administration. Fifty microlitres of 5% formalin was injected subcutaneously into the dorsal surface of the right hind paw with a 30-G needle. Immediately after injection, the rat was placed in an open clear plastic chamber, and its flinching or shaking paw response was observed at five minutes intervals for a period of one hour. The number of flinches was counted for one minute. Usually two phases were observed: phase 1 for the first six minutes after injection, and phase 2 beginning after about ten minutes, with an interval of no flinches between phases.

Behavioural side effects were examined and judged as present or absent in rats for the tail flick test, measured at the same time as the tail flick assessment. Agitation was judged as spontaneous irritable movement, vocalization, or both. Allodynia was judged as escape, vocalization or both, induced by lightly stroking the flank of the rat with a small probe. The placing or stepping reflex was evoked by drawing the dorsum of either hind paw across the edge of the table. Normally, rats try to put the paw ahead into a position to walk. The righting reflex was assessed by placing the rat horizontally with its back on the table. Normally, rats twist their bodies to an upright position immediately. Flaccidity was judged as muscle weakness by placing the forepaw 3 to 5 cm higher than the hind paw. Normally, rats will walk up using the hind paw. The pinna and corneal reflexes were examined with a paper string. When a string is placed in the auditory canal or gently applied to the cornea, rats normally shake their heads. Abnormal ambulation was judged as asymmetrical movement in walking.

The tail flick data are presented as the percentage of maximum possible effect, shown as (post-drug latency – pre-drug latency at time 0) × 100 / (cut-off time – pre-drug latency at time 0). The effective dose in 50% of animals (ED50) was calculated using the maximum effects in the tail flick test and the area under the curve

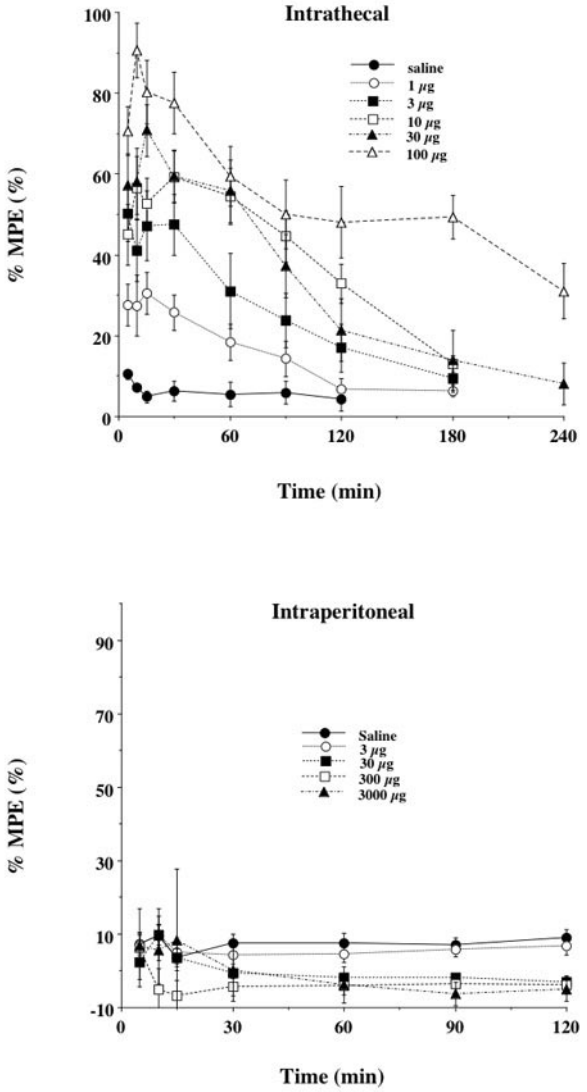

FIGURE 1 Tail flick latency in response to intrathecal (upper panel) and *ip* (lower panel) administration of midazolam. Data are presented as mean ± SD (8 rats at each dose).

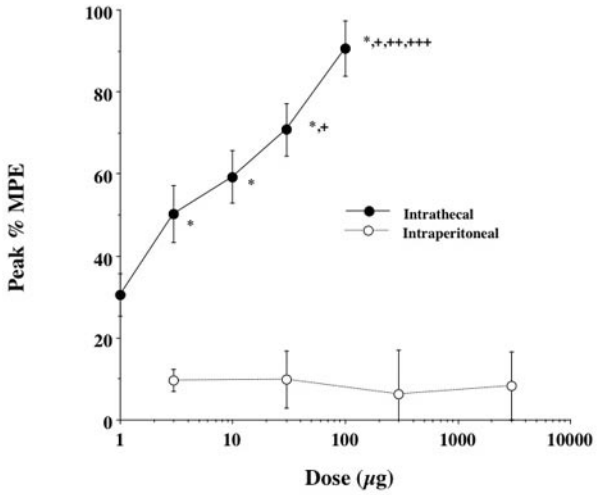

FIGURE 2 Dose-response curves of peak % MPE following intrathecal and *ip* administration of midazolam in the tail flick test. Data are presented as mean ± SD (8 rats at each dose), % MPE = % maximum possible effect; **P* < 0.05 *vs* 1 μg; +*P* < 0.05 *vs* 3 μg; ++*P* < 0.05 *vs* 10 μg, +++*P* < 0.05 *vs* 30 μg.

in the formalin test. Other data are shown as mean ± SD or 95% confidence interval (CI). Statistical analysis was performed with one way factorial analysis of variance followed by the Student Neuman Keuls test as a post hoc analysis for the dose response data. A *P* value < 0.05 was considered to be statistically significant.

Results

Intrathecal administration of midazolam increased tail flick latency dose dependently, while *ip* administration of midazolam did not increase the latency with the maximum allowed dose in 300 μL (3,000 μg), (Figures 1, 2). The ED50 as determined by tail flick latency was 1.60 μg (CI, 0.45–3.02 μg) with intrathecal administration, but the ED50 was not obtained by tail flick latency following *ip* administration.

Both intrathecal and *ip* administration of midazolam decreased the number of paw flinches in both phases of the formalin test (Figures 3, 4). The ED50s were 1.26 μg (CI, 0.35–3.18 μg), (phase 1) and 1.20 μg (CI, 0.29–3.71 μg), (phase 2) with intrathecal administration, and 11.6 μg (CI, 2.5–19.3 μg), (phase 1) and 52.2 μg (CI, 18.3–102.7 μg), (phase 2) with *ip* administration.

Motor disturbance and some behavioural side effects were observed with doses larger than the ED50s in association with both intrathecal and *ip* administration (Table I). No sedative effects were observed in any animal.

Discussion

This study shows that intrathecally administered midazolam mediates antinociceptive effects on both acute thermally and inflammatory induced pain, whereas *ip* midazolam has antinociceptive effects on inflammatory induced pain only.

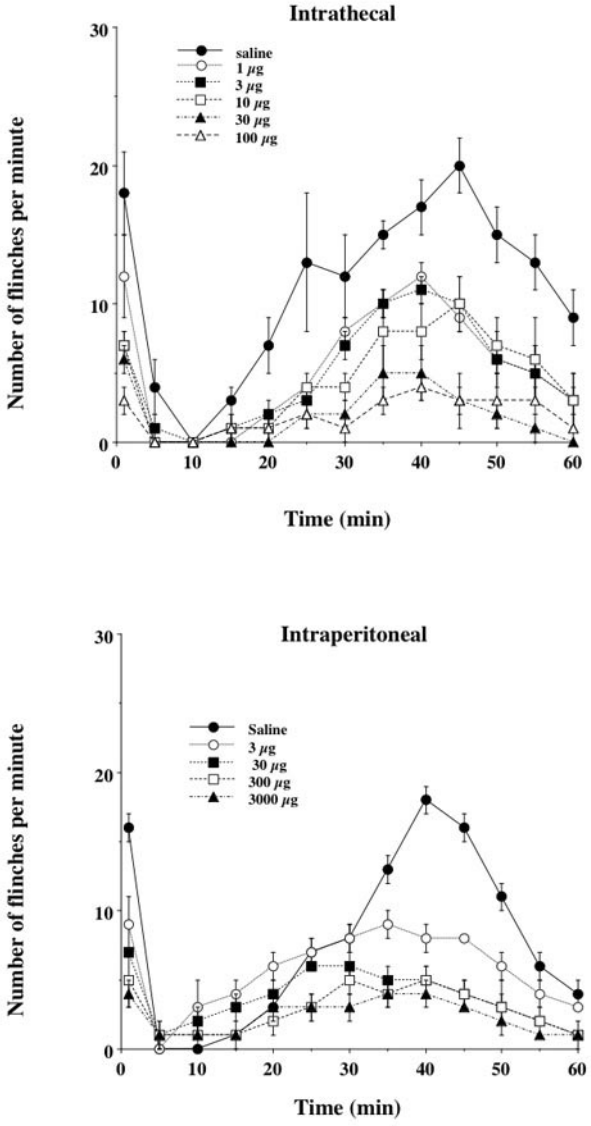

FIGURE 3 Number of flinches in the formalin test in response to intrathecal (upper panel) and *ip* (lower panel) administration of midazolam. Data are presented as mean ± SD (8 rats at each dose).

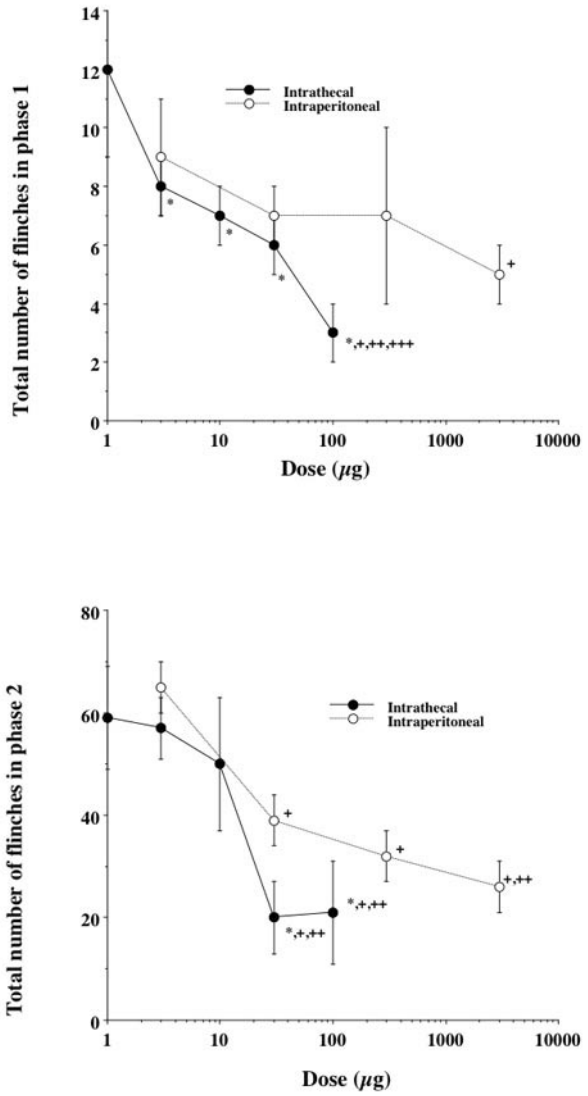

FIGURE 4 Dose-response curves associated with intrathecal and *ip* administration of midazolam in the formalin test phase 1 (upper panel) and phase 2 (lower panel). Data are presented as mean ± SD (8 rats at each dose). **P* < 0.05 *vs* 1 μg; +*P* < 0.05 *vs* 3 μg; ++*P* < 0.05 *vs* 10 μg, +++*P* < 0.05 *vs* 30 μg.

Several issues are raised by these interesting observations. Although motor disturbance was evident with both intrathecal and *ip* administration of high dose midazolam, the rats were still able to produce vigorous tail flick responses and paw flinches, indicating that the motor disturbance did not interfere with the animal's ability to respond to a noxious stimulus, while some evidence of muscle weakness was apparent.

The tail flick response is mediated by Aδ and C fibres at the level of the spinal cord, whereas behavioural responses in the formalin test are mediated by both the spinal and supraspinal sites. The phase 1 response of the formalin test is caused by the direct stimulation of nociceptors by formalin or tissue damage, and is thought to be an acute pain reaction.[5] This reflects activity that is prominent in Aβ, Aδ and high-threshold C nociceptor afferent fibres. The phase 2

TABLE Behavioural side effects and motor disturbance

	IT				*IP*	
	3	*10*	*30*	*100*	*300*	*3,000*
Agitation	0	1	1	1	0	0
Allodynia	1	1	1	1	0	0
Loss of corneal reflex	0	0	0	1	0	0
Loss of Pinna reflex	0	0	0	1	0	3
Flaccidity	0	0	1	3	6	5
Disturbance of righting reflex	1	0	2	6	6	5
Disturbance of placing and stepping response	0	0	1	4	6	5
Ambulation	0	0	0	0	0	2

The number of rats with each side effect is shown. The total number of animals studied at each dose was 8. Saline, 1, 3, 10, 30, and 100 μg in IT, and saline, 3, 30, 300, and 3000 μg in IP were tested. IT = intrathecal; IP = intraperitoneal.

response is caused by subsequent inflammation after formalin injection and central sensitization related to C-fibre activity.[6] It reflects activity in mechanically insensitive afferent fibres and activity of Aδ and C fibres.[7] The present results suggest that intraperitoneally administered midazolam has few effects at the level of the spinal cord while eliciting some antinociceptive effects in the periphery and/or the brain. In contrast, intrathecal administration of the drug provides spinally-mediated analgesia.

Yanez *et al.* reported that intrathecally administered midazolam produces dose-dependent antinociception on thermally induced pain.[2] Midazolam administered into the subarachnoid space of the rat could produce potent analgesia that was antagonized by flumazenil.[1] A segmental effect of intrathecal midazolam was demonstrated using transcutaneous electrical stimulation.[8] Enhancement of presynaptic inhibition might be a possible mechanism for the action of midazolam, because benzodiazepines are known to increase GABA transmission via their specific binding site co-located with the $GABA_A$ receptor in the spinal cord.[9] As shown in these previous studies and corroborated by the present results, it is clear that intrathecally administered midazolam has antinociceptive effects through the benzodiazepine/$GABA_A$ receptor complex in the spinal cord.

In a previous study using the rat tail flick test, 1–10 $mg \cdot kg^{-1}$ (about 300–3000 μg) of *ip* midazolam was shown to produce a hyperalgesic effect, which could be blocked by the benzodiazepine receptor antagonist flumazenil. In contrast, intrathecal midazolam in doses of 10–100 μg produces antinociception.[3] Tatsuo *et al.* also reported that *ip* injection of midazolam 10 $mg \cdot kg^{-1}$ induced a significant decrease of tail flick latency and produced a long-lasting nociceptive effect in the formalin test, characterizing a hyperalgesic effect.[10] However, in the present study, we did not observe a hyperalgesic response. The only important difference between studies was the species of the rat. We used Sprague Dawley rats, while Niv *et al.*[3] used albino rats and Tatsuo *et al.*[10] used Wistar rats. In addition, Niv *et al.*[3] observed sedation with the doses that achieved hyperalgesia. Therefore, variations in responses may be, in part, species-specific. Alternatively, sedation might be a confounding factor in interpretation of the tail flick response.

When midazolam was injected intracerebroventricularly, it reduced the antinociceptive effects of morphine as measured by the tail flick test.[11] Midazolam potentiates morphine action at the spinal level, while an antagonistic effect can be seen at the supraspinal level.[12] Benzodiazepines produce a clinically significant antagonism of opioid analgesia.[13] Systemically administered midazolam attenuates the antinociceptive effects of morphine, probably by inhibiting the descending inhibitory system.[14] The $GABA_A$ receptor is involved in modulating supraspinal actions of opioid receptor occupancy.[15] Therefore, benzodiazepines may modulate the antinociceptive effect of opioids in the brain by means of physiologic mechanisms that are distinct from the effects of benzodiazepines in the spinal cord. This mechanism is different from hyperalgesia.

It has been suggested that midazolam in conjunction with morphine will suppress both peripheral and central sensitization and enhance the effects of preemptive analgesia. Midazolam *iv* has been shown to decrease anesthetic requirements in enflurane-anesthetized dogs.[16] Therefore, midazolam might have antinociceptive effects in both the periphery and the brain.

Intravenous midazolam suppresses noxiously-evoked activity of spinal wide dynamic range neurons with maximum effect at a dose of 1 $mg \cdot kg^{-1}$ that is reversible by a benzodiazepine antagonist.[17] Systemically administered midazolam reduces Aδ fibre-evoked responses of the neurons of the dorsal horn of the spinal cord, and also reduces the C fibre-mediated activity in a spinal nerve ligation model of neuropathic pain, most likely acting at spinally located benzodiazepine receptors.[18] Therefore, systemically administered midazolam may have actions at the level of the spinal cord. The present formalin data support these findings.

Intraperitoneal midazolam had an ED50 which was five times greater in phase 2 compared to phase 1, while intrathecal midazolam was equally effective

for both phases 1 and 2. These results suggest that systemically administered midazolam was less effective for central sensitization than the nociceptive response in the periphery.

The present study used *ip* injection in rats. Therefore, these results cannot be extrapolated to clinical practice for patients receiving *iv* or *im* midazolam injections. Furthermore, although the formalin test was used to mimic postoperative pain, the nature and intensity of noxious stimulation with the formalin test may not be comparable to the varying sites and intensities of acute postoperative pain. Therefore, follow-up clinical trials are warranted to confirm the analgesic properties of systemically-administered midazolam.

In conclusion, systemically administered midazolam induced antinociception for inflammatory pain only, while intrathecal administration elicited antinociceptive effects on both acute thermal and inflammatory-induced pain. When given in relatively low and non-sedating doses, systemically administered midazolam might be a useful adjunct for postoperative pain management.

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