Ketorolac Potentiates Morphine Antinociception during Visceral Nociception in the Rat

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Background: Combinations of opioids and nonsteroidal antiinflammatory drugs (NSAIDs) are commonly used to control pain in the perioperative period, yet there are no quantitative evaluations of the interaction between opioids and nonsteroidal antiinflammatory drugs during visceral nociception. This study evaluated the interaction between morphine and ketorolac during visceral nociception in the rat.

Methods: The pressor response to noxious colorectal distention (80 mmHg, 20 s) was evaluated in 29 male Sprague-Dawley rats and dose–response curves were determined for intravenous morphine, ketorolac and the mixture of morphine and ketorolac. The data were interpreted using an isobolographic analysis to establish the nature of the interaction.

Results: Intravenous ketorolac alone (8–32 mg/kg) did not have a significant antinociceptive effect, whereas morphine alone (1–4 mg/kg) produced significant antinociception during noxious colorectal distention (dose yielding a 50% reduction in nociceptive response relative to baseline pressor response = 1.7 ± 0.6 mg/kg). Isobolographic analysis of the antinociceptive interaction demonstrated a highly significant, naloxone-reversible potentiation of intravenous morphine by ketorolac in the rat during visceral nociception (P < 0.001).

Conclusions: Ketorolac is a powerful potentiator of morphine antinociception during visceral nociception in the rat. However, intravenous ketorolac alone did not demonstrate antinociceptive properties during colorectal distention, a model of acute visceral nociception without a major inflammatory component. These data suggest that ketorolac may have a central modulatory effect on opioid pharmacology and the synergistic effect may be separate from its peripheral antiinflammatory properties. This study encourages further basic as well as clinical evaluations of the improved antinociception provided by combination therapy of opioids and nonsteroidal antiinflammatory drugs.


ADEQUATE control of perioperative pain has been a problem for many years. Improved knowledge about opioid pharmacology and delivery systems (e.g., patient-controlled analgesia) have provided better analgesia and greater patient satisfaction; however, the problems of adverse opioid effects remain. In response to this issue, ketorolac is commonly administered in the perioperative setting. Yet, while an occasional patient may only require nonsteroidal antiinflammatory drugs (NSAIDs) to maintain adequate analgesia, many patients receive a combination of NSAIDs and opioids. The potential advantage of using combination therapy is that analgesia can be maximized while minimizing the incidence of adverse effects. While clinical studies with NSAIDs and opioids suggest an additive or possibly synergistic interaction, few quantitative studies to establish the antinociceptive interaction have been conducted. Recently, Malmberg and Yaksh reported that intrathecal ketorolac potentiated intrathecal morphine analgesia during the formalin test in rats. In addition, Pirchio et al. had earlier demonstrated that the antinociceptive effect of butorphanol (an opioid agonist–antagonist) and acetaminophen was synergistic in the mouse writhing test.

There is evidence in humans that the analgesia provided by 30 mg parenteral ketorolac may be as effective as 12 mg parenteral morphine in the postoperative period. Nevertheless, if pain persists or breaks through the initial dose of NSAID, extended use of parenteral NSAIDs increases the risk of renal and hepatic impairment, bleeding complications, gastrointestinal erosions and ulcerations, as well as drowsiness and fluid retention. Administration of opioids in the perioperative setting may contribute to early or delayed respiratory depression, confusion, nausea and vomiting, decreased gastrointestinal motility, and pruritus. Many, if not all, of these adverse effects are dose-dependent. Therefore, using combinations of medications that offer analgesic synergy should allow a reduction in required dosage and decrease the incidence of adverse effects.

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Quantifying an antinociceptive synergistic effect presents practical and ethical limitations in human subjects; however, animal models of visceral nociception have been described. Cutting, crushing, pinching, and heat applied to human viscera do not produce pain. However, visceral pain is produced reliably in humans during distention of hollow organs. Ness and Gebhart characterized a model of visceral nociception produced by colorectal distention (CRD) in an awake, unrestrained rat. In this model, pressor responses to noxious intensities of CRD provide objective measures of visceral nociception. Because a major component of perioperative pain is visceral in origin, the CRD model provides a clinically relevant paradigm for the study of perioperative pain.

The current evaluation quantitatively examined the antinociceptive interaction between an opioid agonist (morphine) and an NSAID (ketorolac) during visceral nociception in the rat.

Materials and Methods

Subjects
The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Iowa. Twenty-nine unanesthetized male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 280–350 g were studied in five groups. One group (n = 6) received intravenous (iv) ketorolac; a second (n = 6) received morphine; a third (n = 6) and a fourth (n = 6) group received fixed ratio combinations of morphine and ketorolac; and a fifth group (n = 5) received a combination of morphine and ketorolac followed by naloxone.

Surgical Preparation
All surgical procedures were performed with the rats deeply anesthetized with sodium pentobarbital (40–50 mg/kg, intraperitoneally). Femoral arterial and venous catheters were implanted for measurement of arterial blood pressure and drug administration, respectively. Arterial and venous catheters were exteriorized and secured at the back of the head. After surgery, rats were housed individually with free access to food and water and allowed to recover for at least 1 week before use. Rats exhibiting any abnormalities after recovery were not included in this study.

Colorectal Distention
CRD was produced by air inflation of a 7–8-cm flexible latex balloon inserted intranally and connected to a pressure-controlled device described previously. Phasic CRD (20 s duration) was produced when a solenoid gate was opened to a constant pressure air reservoir at 80 mmHg, resulting in an instantaneous increase in the balloon pressure to 80 mmHg. Phasic CRD in awake rats results in a reliable, reproducible increase in mean arterial pressure (MAP). The pressor response to CRD has been well characterized as an objective measure of nociception and was defined as the change from baseline MAP (ΔMAP) during a 20 s phasic distention to 80 mmHg. ΔMAP was calculated as the mean of MAP during distention minus the MAP immediately before distention. Three distentions were performed in each unmedicated rat while distention pressure and MAP were monitored simultaneously to establish a baseline pressor response to CRD. Drugs were then administered via the femoral venous catheter and the pressor responses to CRD were recorded every 4 min.

Drugs and Drug Interactions
The NSAID used in this study was ketorolac (1–32 mg/kg iv; Toradol, Syntex, Palo Alto, CA), and the opioid was morphine sulfate (0.2–4 mg/kg iv; Merck, Rahway, NJ). The drug solutions were freshly prepared in 0.9% sterile saline. All drugs were administered iv. Five separate groups, 6 rats per group (except naloxone group, n = 5) were studied. One group received only iv morphine 1, 2, and 4 mg/kg with at least 2 days between successive experiments in any rat. A second group received only iv ketorolac 8, 16, and 32 mg/kg with at least 2 days between successive experiments in any rat. The third and fourth groups received combinations of morphine (0.2–0.9 mg/kg) and ketorolac (2–17 mg/kg) in a 1:10 or 1:20 ratio with at least 2 days between successive experiments in any rat. The combination doses were based on fixed weight ratios (e.g., each rat in the 1:10 group received 0.21 mg morphine in combination with 2.1 mg ketorolac, 0.42 mg morphine with 4.2 mg ketorolac, and 0.85 mg morphine with 8.5 mg ketorolac on separate days with at least 2 days between each dose trial). A fifth group received morphine 0.85 mg/kg and ketorolac 8.5 mg/kg iv followed by naloxone 1 mg/kg iv.

Trials using either ketorolac or morphine alone were performed as described above (see section Colorectal Distention). Tests using ketorolac–morphine combinations were conducted by measuring the pressor response to three baseline distentions followed by iv ketorolac administration and CRD every 4 min for the next 24 min. Morphine was then administered iv and

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CRD measurements were continued every 4 min for the next hour (except in the fifth group, which received iv naloxone 24 min after morphine administration). The interval used for data analysis included the four measurements at 8, 12, 16, and 20 min after morphine administration. This interval was chosen based on the time of maximal antinociceptive effect obtained from each drug alone (figs. 2 and 4 in Results), although ketorolac did not demonstrate a significant antinociceptive effect at any time.

Dose-response curves were derived for ketorolac alone, morphine alone, and ketorolac-morphine combinations in fixed ratios of 1:10 and 1:20 (morphine: ketorolac) on a weight basis.

Statistical Analysis

The effective dose 50 (ED_{50}) was defined as the dose that yielded a 50% reduction in nociceptive response (ΔMAP) relative to baseline (predrug) pressor responses to CRD. For example, if the MAP increased by 30 mmHg during baseline CRD measurements, the ED_{50} would be the dose that attenuated the pressor response to a 15 mmHg increase in MAP during CRD. ED_{50} and 95% confidence intervals (CIs) were calculated by a graded ED_{50} program. Data for ΔMAP are reported as the percentage of the maximal possible effect:

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\text{Maximal possible effect (\%)} = 100 \times \frac{\text{predrug } \Delta \text{MAP} - \text{postdrug } \Delta \text{MAP}}{\text{predrug } \Delta \text{MAP}}
\]

Isobolographic analysis for drug interaction was conducted according to the procedure of Tallarica et al.\(^2^{3}\) and a modified method described by Porreca et al.\(^2^{4}\) when one drug lacks efficacy. CIs for each point were calculated from the variances of each component alone. The CIs were evaluated for statistical significance with a Student's t test. A demonstration isobologram when one drug lacks efficacy is illustrated in figure 1. Data from individual and combination dose-response curves are used to generate the isobologram. The theoretical additive line in figure 1 is illustrated by the dashed horizontal line, which intersects the y-axis at the experimentally determined ED_{50} dose of drug Y. The theoretical additive line does not intersect the x-axis as it does in typical isobolographic illustrations because drug X lacks efficacy and an ED_{50} dose cannot be established. If the ED_{50} dose of the mixture of drugs X and Y falls on the theoretical additive line, the effect of the mixture of drug X and Y is additive (e.g., point A, fig. 1). Points that are statistically below the theoretical additive line would indicate that drug X has significantly potentiated the effect of drug Y (e.g., point B, fig. 1).

Results

Administration of iv morphine (1–4 mg/kg) produced a significant dose-dependent attenuation of responses to noxious CRD (fig. 2, P < 0.05). The antinociceptive effect of iv morphine was rapid in onset (by the first test 4 min after administration) and long-acting (greater than 1 h for the greatest dose tested). Data at the time of maximal morphine effect (8–20 min after administration) are presented in standard dose-response format in figure 3, where 100% maximal possible effect represents no pressor response to noxious CRD (i.e., ΔMAP = 0). Morphine iv produced a dose-dependent attenuation of the ΔMAP in response to phasic CRD with an ED_{50} ± 95% CI of 1.7 ± 0.6 mg/kg. Predistention (resting) MAP values were not statistically different before and after iv morphine administration for all doses tested (P < 0.05). Thus, the cardiovascular response to CRD represented an antinociceptive effect rather than a hemodynamic effect from morphine itself (see also\(^2^{2}\)).

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In contrast to morphine, however, there was no significant antinociceptive effect of ketorolac (8–32 mg/kg, figs. 3 and 4). In preliminary evaluations, doses of ketorolac less than this range (1–4 mg/kg) were also tested and did not produce an antinociceptive effect. In addition, four rats received 64 mg/kg of iv ketorolac and a significant decrease in the pressor response to CRD was observed; however, each rat rapidly developed severe hypotension and died during the experiment. Therefore, the greatest ketorolac dose tested in this experiment was 32 mg/kg. Although ketorolac 8–32 mg/kg tended to have a slight effect on pressor responses to CRD, the effect was not statistically different from baseline ΔMAP for any dose tested (fig. 3, P > 0.05). Therefore, ketorolac lacked efficacy and an ED50 dose of ketorolac could not be determined in this model of visceral nociception. As noted with morphine, iv ketorolac administration did not significantly change the predistention (resting) MAP values when compared to the MAP before ketorolac (P > 0.05).

Nevertheless, when “nonantinociceptive” doses of ketorolac (2–17 mg/kg) were coadministered with morphine, the antinociception produced by morphine was significantly potentiated (P < 0.001 by isobolographic analysis, below). The dose–response curves for the coadministration of iv morphine and ketorolac in a fixed ratio of 1:10 and 1:20 (morphine: ketorolac) are illustrated in figure 5. It is important to note that the dose of morphine present in the coadministration trials was significantly less than that used in establishing the dose–response curve for morphine alone. For example, whereas morphine 1, 2, and 4 mg/kg were used in the morphine only tests, the morphine dosage range in the coadministration tests was 0.2–0.9 mg/kg. Yet when these relatively small doses of morphine were combined with a nonantinociceptive dose of ketorolac (2–17 mg/kg), the result was a significant attenuation of responses to noxious CRD. This point is clearly illustrated by the finding that the ED50 ± 95% CI for morphine alone is 1.7 ± 0.6 mg/kg, whereas the morphine component of the ED50 ± 95% CI for the coadministration of morphine and ketorolac in 1:10 and 1:20 fixed ratios was significantly decreased, to 0.6 ± 0.3 mg/kg and 0.3 ± 0.2, respectively (P < 0.01, fig. 6). Thus, by adding a nonantinociceptive dose of ketorolac to morphine, the same magnitude of antinociception is maintained with a 65–82% reduction in the dose of morphine. In 5 additional rats, naloxone 1 mg/
Fig. 4. Time–effect curve for ketorolac in the colorectal distention (CRD) model of visceral nociception. The pressor response to noxious CRD (80 mmHg, 20 s) is reported as the change from baseline mean arterial pressure (ΔMAP) after intravenous ketorolac (8–32 mg/kg) administration. *P > 0.05 for all data relative to control.

Fig. 5. Dose–response curves for mixtures of morphine (mor) and ketorolac (ket) in a fixed ratio of 1:10 or 1:20 (mor:ket) in the colorectal distention (CRD) model of visceral nociception. The pressor response to noxious CRD (80 mmHg, 20 s) is reported as the percentage of the maximal possible effect (%MPE).

%MPE = 100 × \frac{\text{predrug ΔMAP} - \text{postdrug ΔMAP}}{\text{predrug ΔMAP}}

where MAP = mean arterial pressure and ΔMAP = mean of MAP during distention minus MAP immediately before distention. Dose (on the x-axis) is the total milligram dose of morphine plus ketorolac administered intravenously for each fixed ratio combination (e.g., in the 1:10 graph, the greatest dose reported was 9.35 mg/kg = 0.85 mg/kg morphine + 8.5 mg/kg ketorolac; *P < 0.05 compared to the lowest dose tested in each fixed ratio combination). Intravenous naloxone 1 mg/kg completely reversed the antinociceptive effect in an additional group of rats (n = 5) that received the greatest dose combination in the 1:20 group (open triangle).

Fig. 6. Summary of doses that yielded a 50% reduction in noxious response (change from baseline mean arterial pressure [ΔMAP]) relative to baseline (predrug) pressor response to colorectal distention (ED50) ± 95% confidence intervals, for morphine (mor) and mixtures of morphine and ketorolac (ket) in a fixed ratio of 1:10 or 1:20 (mor:ket). Bar graphs represent the dose of morphine alone or in combination with ketorolac that yielded a 50% reduction from the control ΔMAP during noxious colorectal distention. *P < 0.01 compared to morphine alone; 1:10 and 1:20 ED50 values are not statistically different from each other.

kg iv completely reversed the antinociceptive effect of the greatest combination dose in the 1:20 group (open triangle, fig. 5).

Isobolographic Analysis
The experimentally determined 1:10 (morphine:ketorolac) mixture ED50 ± 95% CI for the pressor response to noxious CRD was 0.6 ± 0.3 mg/kg for morphine and 5.6 ± 3.3 mg/kg for ketorolac. This point is plotted as 0.6, 5.6 on the 1:10 mixture isobologram (filled square, fig. 7). The theoretical additive ED50 ± 95% CI was calculated to be 1.7 ± 0.1 mg/kg for morphine and 17.2 ± 1.1 mg/kg for ketorolac (open square, fig. 7). The 95% CIs of these points do not overlap, and results of a Student’s t test for potency ratio were highly significant (P < 0.001). Similar results were obtained when a 1:20 fixed ratio of morphine to ketorolac was examined. The experimentally determined ED50 ± 95% CI for the 1:20 mixture group was 0.3 ± 0.2 mg/kg for morphine and 6.6 ± 3.8 mg/kg.
for ketorolac, while the theoretical additive ED$_{50}$ ± 95% CI was calculated to be 1.7 ± 0.1 mg/kg for morphine and 32.3 ± 2.0 mg/kg for ketorolac (filled and open triangles, respectively, fig. 7). The two points were compared by a Student's t test for relative potency and found to be significantly different ($P < 0.001$).

**Discussion**

This study has shown that iv ketorolac does not have a significant antinoceptive effect during noxious CRD in the rat; however, when coadministered with morphine, iv ketorolac produces a naloxone-reversible, marked potentiation of morphine antinoception.

The analgesic properties of various NSAIDs during somatic nociception (e.g., metastatic bone pain) are well described; however, the analgesic effects of NSAIDs during visceral nociception are not as well defined. In addition, several studies have suggested an opioid- or anesthetic-sparing effect after the perioperative administration of NSAIDs. Therefore, the focus of this study was to first examine the analgesic efficacy of iv ketorolac during visceral nociception relative to morphine, and second, to quantitatively evaluate the analgesic interaction between morphine and ketorolac during noxious CRD. The clinical implications of this study are important, given the desire to maximize analgesia while minimizing adverse effects in a variety of situations in which visceral pain is a major problem (e.g., cases of perioperative, cancer, or postpartum pain).

Several clinical studies have reported adequate analgesia with NSAID administration in the perioperative setting in which visceral nociception may have a contribution to the overall pain experience. Vangen et al. compared the analgesia and adverse effects of ketorolac and paracetamol/codeine in 107 patients after gynecological surgery, and found that both treatments were efficacious at relieving moderate pain with similar side effects. After gastric bypass surgery, Rubin et al. reported that ketorolac did not depress respiration or bowel function as much as morphine at equivalent analgesic levels. In addition, Oosterlinck et al. reported that a low dose of intramuscular ketorolac (10 mg) was an effective analgesic when compared to high dose intramuscular ketorolac (90 mg) or pethidine (100 mg) for the management of renal colic. Further, Oosterlinck et al. stated that the incidence of sedation and emesis was lower after ketorolac administration. These clinical studies support a role for ketorolac in the management of visceral pain.

In the current study, iv ketorolac by itself did not produce a significant antinoceptive effect during CRD. A similar result was reported by Molke Jensen et al. using iv acetaminophen during CRD in the rabbit. It is possible that in clinical studies, in which the analgesic effects of ketorolac on visceral pain are clear, the noxious input has a significant inflammatory component. The CRD model uses acute distention as the noxious stimulus with only a minor inflammatory component as a consequence of repeated distentions. Future studies using inflammatory visceral pain models may help to clarify this issue. In addition, most clinical studies use subjective pain scales to rate the degree of analgesia, whereas this study evaluated hemodynamic responses to assess antinoception. Thus the method of assessing antinoception is quite different and may
also play a role in the divergent results between the clinical and basic studies.

Nevertheless, it is clear from the current study that when an iv dose of ketorolac, which by itself lacks a significant antinociceptive effect, is combined with an iv dose of morphine, the result is a marked potentiation of morphine antinociception during CRD. These data are in agreement with a study by Pirchio et al. demonstrating a synergistic antinociceptive effect of butorphanol (an opioid agonist–antagonist) and acetaminophen in the mouse writhing test.13 These results have significant clinical importance and application. In addition, these studies substantiate several recent clinical observations of the opioid-sparing effects of NSAIDs. In a randomized, double-blind, placebo-controlled study, Gillies et al.9 reported that ketorolac significantly reduced the morphine requirements in the postoperative period after upper abdominal surgery. In addition, the patients who received ketorolac had significantly lower arterial carbon dioxide levels postoperatively, indicating less respiratory depression or splinting as compared with the morphine only control patients. Decreasing dose-dependent adverse effects associated with each analgesic is one of the obvious advantages of using drug combinations that produce analgesic synergy.

The mechanism by which NSAIDs and opioids may interact to produce antinociceptive synergy is unknown. In a review of the differences and similarities among the NSAIDs, Brooks and Dasy18 summarize the possible mechanisms of action, which include: cyclooxygenase inhibition, lipoxygenase inhibition, cell membrane processes, phospholipase C inhibition, and effects on transmembrane ion fluxes. Ketorolac appears to exert an antinociceptive response in situations in which inflammation or hyperalgesia or both are present, but exhibits little to no effect on pain thresholds in noninflamed or nonsensitized states.6,12 This is supported by the current results and by assays involving compression of yeast-inflamed paws in rats, which show that ketorolac increased the nociceptive threshold on the inflamed paw but not on the contralateral or noninflamed paw.32

Previously, it was believed that the primary mechanism of antinociception of NSAIDs was a peripheral antiinflammatory effect; however, several studies have since demonstrated a significant central action of NSAIDs.33,34 Malberg and Yaksh reported that intrathecal ketorolac attenuates the hyperalgesia produced by activation of spinal glutamate and substance P receptors.35 In addition, these investigators recently demonstrated that intrathecal ketorolac potentiated the antinociception produced by intrathecal morphine (a μ opioid receptor agonist) and intrathecal ST-91 (an α2 adrenoceptor agonist) in the formalin test, indicating a central mechanism of action.12 Whether NSAIDs may modulate opioid receptor function through prostaglandin synthesis or via excitatory amino acid, or neuropeptide actions is unknown. Nevertheless, these data as well as clinical observations have clearly demonstrated the potentiation of morphine antinociception by iv ketorolac.

In this study, the antinociception provided by the combination of ketorolac and morphine was completely reversed by naloxone, indicating that ketorolac did not change the nature of the opioid receptor interaction. In addition to possible pharmacodynamic modulation of the opioid antinociception, ketorolac may alter the pharmacokinetic properties of the opioid agonist. The authors are not aware of any data that answer this question; however, future studies will address this issue.

In summary, this study quantified the antinociceptive synergy between iv morphine and ketorolac during visceral nociception in the rat. From these data, we conclude that: (1) iv ketorolac, by itself, is not an efficacious antinociceptive drug during noxious CRD in the rat, (2) iv ketorolac significantly potentiates iv morphine antinociception during visceral nociception, and (3) the potentiated antinociception provided by morphine and ketorolac is completely reversed by naloxone. These data suggest that the mechanism by which ketorolac potentiates morphine analgesia may involve a central modulatory effect on the opioid receptor; however, a change in opioid pharmacokinetics cannot be ruled out and requires further evaluation. These results encourage the combined use of ketorolac and morphine in the treatment of patients suffering visceral pain (e.g., in certain perioperative, obstetric, and oncologic cases). In addition to improving the analgesia we may provide these patients, we may also decrease or avoid adverse drug effects by reducing the analgesic dose requirement.

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References

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