Spinal Prostaglandin Formation and Pain Perception Following Thoracotomy*
A Role for Cyclooxygenase-2

Connail McCrory, MD; and Desmond Fitzgerald, MD

Study objective: Prostaglandins (PGs) generated in the spinal cord may play a major role in pain perception. Consequently, the suppression of spinal cyclooxygenase (COX) and PG formation may contribute to the analgesic effect of nonsteroidal anti-inflammatory drugs (NSAIDs) in pain following surgery. Which isoform of COX is responsible for postsurgical pain and, consequently, should be targeted, is unclear.

Design: Prospective randomized blinded study.

Setting: University teaching hospital.

Patients: Thirty patients undergoing thoracotomy for lobectomy were recruited.

Interventions: Patients were randomized to receive the COX-2 selective inhibitor nimesulide, 100 mg orally twice daily, or ibuprofen (nonselective), 400 mg orally three times daily, in an open-label study. In addition, there was a randomized control group that received no NSAIDs. Cerebrospinal fluid (CSF) was analyzed for 6-keto-PGF1α, the principle metabolite of prostacyclin. COX-1 and COX-2 activity was determined by measuring serum thromboxane (TX) B2 and endotoxin-induced PGE2 generation in whole blood.

Measurements: Pain perception was measured by visual analog scores, and blinded assessment of opioid analgesic requirements and expiratory peak flow measurements were performed.

Results: At the doses used, nimesulide was selective for COX-2, while ibuprofen was nonselective based on serum TXB2 levels. The mean (± SEM) levels of 6-keto-PGF1α in CSF increased following surgery from 32 ± 4.9 to 127 ± 29 pg/mL (p < 0.001), and this was suppressed by nimesulide (49 ± 9.3 pg/mL; p = 0.0025) but not by ibuprofen (122 ± 35 pg/mL). Pain scores (p < 0.001), morphine requirement (p = 0.0175), and the fall in peak expiratory flow rate (p < 0.001) were significantly lower in the nimesulide group.

Conclusions: Increases in spinal PG synthesis after thoracotomy are repressed by a selective COX-2 inhibitor. This suggests that the inducible COX-2 mediates central PG synthesis, which may be important in the generation of pain, as the use of nimesulide also resulted in significant decreases in postoperative pain perception.

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Key words: cerebrospinal fluid; cyclooxygenase; cyclooxygenase-2 inhibition; nonsteroidal anti-inflammatory drugs; postoperative pain

Abbreviations: COX = cyclooxygenase; CSF = cerebrospinal fluid; EIA = enzyme immunoassay; LPS = lipopolysaccharide; NMDA = N-methyl-D-aspartate; NSAID = nonsteroidal anti-inflammatory drug; PG = prostaglandin; TX = thromboxane; VAS = visual analog scale

The effectiveness of nonsteroidal anti-inflammatory drugs (NSAIDs) in alleviating pain and reducing the requirement for therapy with opioids in the postoperative period has been demonstrated.1,2 The observations suggest that surgery in humans induces prostaglandin (PG) synthesis, the target for NSAIDs, which may contribute to nociception. PGs are generated from arachidonic acid by the enzyme cyclooxygenase (COX), of which there are two isoforms.3 COX-1 is present in most cells, while COX-2 is expressed to only a limited extent in normal tissues, including the brain.4 Animal models have

*From the Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin, Ireland.
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Correspondence to: Desmond Fitzgerald, MD, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin, Ireland; e-mail: dfitzgerald@rcsi.ie.
demonstrated that COX-2 is induced in the dorsal root neurones following peripheral injury.5,6 One product that may be important in pain perception is prostacyclin, as disruption of the prostacyclin receptor gene in mice attenuates the writhing response to peripheral tissue injury.7,8 Finally, several studies9,10 have reported an analgesic response to the intrathecal administration of a COX-2 inhibitor in experimental models. Thus, spinal COX-2 expression leading to prostacyclin generation may contribute to the perception of pain.

If spinal COX-2 also plays an important role in pain perception in humans, then therapy with selective COX-2 inhibitors may prove to be effective for pain control following surgery. Therapy with selective COX-2 inhibitors may be particularly attractive in the perioperative period as they are associated with a lower risk of acute gastric injury and do not inhibit platelets.11 However, Ballou and colleagues12 have reported that pain responses are attenuated in mice lacking the COX-1 gene, whereas disruption of COX-2 had no effect. To determine whether COX-2 plays a role in nociception in humans, we examined the effects of a selective COX-2 inhibitor, nimesulide,13 on pain control and on the concentrations of PG in cerebrospinal fluid (CSF) in patients undergoing thoracotomy.

**STUDY DESIGN**

**Subjects**

The study was approved by the Ethics Committee of St. James Hospital, Dublin, and all patients gave informed, written consent. Thirty patients undergoing elective lobectomy for bronchial carcinoma were recruited. Exclusion criteria included a history of peptic ulcer disease, renal and hepatic dysfunction, psychiatric illness, any chronic pain syndrome, consumption of NSAIDs, corticosteroids, or any other drug known to interfere with PG production for 14 days prior to surgery, and an inability to understand and use the visual analog scale (VAS) ruler.

Patients were randomly assigned in an open-label manner to one of three groups. One group (ie, the control group) received no NSAID, a second group received the COX-2 selective inhibitor nimesulide, 100 mg orally twice daily, and a third group received the nonselective COX inhibitor ibuprofen, 400 mg orally four times daily. The first dose of the COX inhibitor was administered just prior to the induction of general anesthesia. An intrathecal catheter (22G spinal catheter; B. Braun Medical; Melsungen, Germany) was placed through the lumbar 3–4 interspace prior to anesthesia and surgery. Two milliliters of CSF was withdrawn preoperatively and then once every 24 h postoperatively, with the first 500 µL CSF being discarded as the catheter dead space was 300 µL. At the same time, 6 mL peripheral venous blood was obtained. All patients received morphine, 1 mg, via the catheter prior to incision. This catheter was used for the delivery of morphine for postoperative analgesia and was removed at 48 h once the final CSF sample had been obtained.

Standardized anesthetic and surgical techniques were used. The incision was made above or below the sixth rib. No NSAIDs were administered intraoperatively, and the only opioid used was fentanyl, 1 µg/kg, as an intermittent bolus at the discretion of the anesthesiologist.

**Biochemical Analysis**

In preliminary studies, we demonstrated by mass spectrometry that the major PG product in human CSF was 6-keto-PGF$_{1α}$, the immediate hydrolysis product of prostacyclin, with only small amounts of PGE$_2$ detected (C McGrory, DJ Fitzgerald; unpublished observations). Thereafter, the CSF was analyzed for 6-keto-PGF$_{1α}$ by enzyme immunoassay (EIA) [R&D Systems; Minneapolis, MN].

COX-1 and COX-2 activities were analyzed in whole blood, as previously described. Briefly, non-anticoagulated whole blood was allowed to clot in a nonsiliconized glass tube at 37°C for 1 h. Serum was separated by centrifugation at 1,000 g for 10 min, was stored at −20°C, and was assayed for thromboxane (TX) B$_2$ by EIA. The assay measures TX formation by platelets, where the only isof orm is COX-1.14 In addition, a 1-mL aliquot of whole blood containing 10 IU sodium heparin was incubated in the presence of lipopolysaccharide (LPS) derived from *Escherichia coli* (Sigma; St. Louis, MO), 10 µg/mL at 37°C for 24 h. LPS induces the expression of COX-2 in blood monocytes over 24 h, while the contribution of platelet COX-1 is suppressed by the addition of 200 µmol/L aspirin. Plasma was separated by centrifugation at 1,000 g for 10 min and stored at −20°C. PGE$_2$ was measured by EIA as an index of COX-2 activity.15

**Patient Monitoring**

After surgery, the patients were returned to the High Dependency Unit, extubated, and with an intrathecal catheter in situ. Static pain was assessed by blinded observers using a 10-point VAS at rest every 2 to 4 h, and dynamic pain (immediately after coughing) was assessed every 6 to 8 h. The target VAS score was < 4 at rest, and intrathecal morphine was administered if the VAS exceeded this target. Pain score assessments commenced within 1 h of arrival in the High Dependency Unit with a VAS score of < 4 and were continued for 48 h. Intrathecal preservative-free morphine, 0.5 mg, was administered at intervals of not < 15 min (to avoid respiratory depression) according to pain scores. The maximum dose of intrathecal morphine was 1.5 mg in 45 min (three sequential doses), after which the duty anesthetist was called. The amount of morphine required throughout the study period was recorded. As a further assessment of the quality of analgesia following thoracotomy, pre- and postoperative peak flow measurements were compared. On the morning of the second postoperative day, 2 h after the administration of the study drug, the average of three peak flow readings was obtained using a portable, hand-held, peak flowmeter and was compared to preoperative values.

**Statistical Analysis**

The Box-Cox transformation diagnostic procedure was applied to all measured response variables. Where multiple responses were measured on study participants, a repeated-measures analysis of variance and associated Student t test of linear contrasts among the means were used. In cases in which a single response was measured, a one-way analysis of variance and associated Student t test were used. The p values quoted in the text are all single-tail values.
Results

Thirty patients were recruited, 17 men and 13 women, with a mean age of 63 years. Table 1 shows the demographic data.

PG Formation

Mean (± SEM) serum levels of TXB₂, a marker of COX-1 activity, was markedly suppressed by ibuprofen (day 2, 4.5 ± 1.1 ng/mL; before treatment, 180 ± 24 ng/mL; p < 0.001), whereas nimesulide had little effect (day 2, 171 ± 20 ng/mL; before treatment, 220 ± 19 ng/mL; p = 0.4). LPS-induced PGE₂ formation in whole blood, an index of COX-2 activity, was markedly inhibited by nimesulide (day 2, 1.2 ± 0.2 ng/mL; before treatment, 17.3 ± 2.5 ng/mL; p < 0.001), whereas ibuprofen had a modest inhibitory effect (day 2, 12.9 ± 3.3 ng/mL; before treatment, 16.4 ± 2.8 ng/mL; p = 0.01). Levels of serum TXB₂ and LPS-induced plasma PGE₂ were unaltered in the control group of patients who did not receive a COX inhibitor (Fig 1).

Figure 2 shows the concentration of 6-keto-PGF₁α in the CSF at the time of surgery and on the 2 days following surgery. There was a fourfold rise in the concentration in the control group following surgery (32 ± 4.9 to 127 ± 29 pg/mL; p < 0.001) that was blunted in patients treated with nimesulide (day 2 level, 49 ± 9.3 pg/mL; p = 0.0025 [vs control]). In contrast, ibuprofen had no effect (day 2 level, 122 ± 35 pg/mL).

Table 1—Demographic Data and Operative Procedures

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n = 10)</th>
<th>Ibuprofen (n = 10)</th>
<th>Nimesulide (n = 10)</th>
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Figure 1. Effect of ibuprofen and nimesulide on serum TXB₂ levels (COX-1 activity) and endotoxin-induced plasma PGE₂ levels (COX-2 activity) compared with no treatment (control subjects). Ibuprofen behaved largely as a COX-1 inhibitor, whereas nimesulide was selective for COX-2. The data are presented as the mean ± SEM for the preoperative (Pre-op) period and for postoperative days 1 (Day 1) and 2 (Day 2). ** = p < 0.01; *** = p < 0.001.
Analysis of Quality of Analgesia

Pain scores at rest and immediately after coughing were lower in the nimesulide group compared to the control group (p < 0.001), whereas ibuprofen at the dose used had no effect (Fig 3). The intrathecal morphine requirements were also lower in the nimesulide group compared to the control group (p = 0.0175), whereas again ibuprofen had no effect (Fig 4). Peak expiratory flow rate decreased following surgery in the control group. The reduction in peak expiratory flow rate was blunted by nimesulide (p < 0.001 vs control) but not by ibuprofen (Fig 5).

Discussion

Our studies show that thoracotomy is associated with a rise in CSF 6-keto-PGF$_{1\alpha}$ levels in the 48 h following surgery. The increase is likely to represent local generation as the levels far exceed those seen in plasma.\textsuperscript{16} The increase was in part mediated by COX-2, as it was suppressed by nimesulide to a greater degree than ibuprofen. Nimesulide, 100 mg given twice daily, is a selective COX-2 inhibitor, as selective as celecoxib.\textsuperscript{13,17} COX-2 selectivity was confirmed in this study, as nimesulide suppressed LPS-induced PGE$_2$ generation in plasma, a measure of COX-2 activity, while having no effect on serum TXB$_2$, a measure of COX-1 activity.\textsuperscript{14} Ibuprofen inhibits both COX isoforms,\textsuperscript{18} although with a 2 to 15-fold selectivity for COX-1.\textsuperscript{19} In contrast to nimesulide, at a dose that acted as a selective COX-1 inhibitor, ibuprofen had no effect on CSF 6-keto-PGF$_{1\alpha}$ levels. An alternative explanation is that ibuprofen failed to cross the blood-brain barrier. However, the concentration of free ibuprofen in the CSF at steady state exceeded that of plasma in humans.\textsuperscript{20} Notwithstanding the limitations that nimesulide and ibuprofen are selective and not specific inhibitors of COX isoforms, or that ibuprofen may not have reached a sufficient concentration in the CSF, the contrast between the two agents at doses exhibiting differential effects against COX isoforms adds credence to the hypothesis that the increase in CSF 6-keto-PGF$_{1\alpha}$ levels following thoracotomy is largely mediated by COX-2.
Figure 3. Assessment of pain relief based on VAS scores on day 1 and day 2 following surgery. The data are presented as box plots showing the median, and the 75% and 95% confidence intervals. The nimesulide group had the lowest pain scores at rest and the least increase in pain scores on coughing (gray shade) compared to control subjects. *** = p < 0.001 vs untreated patients.

Figure 4. Intrathecal morphine requirement over the 48 h following surgery. The data are presented as box plots showing the median and the 75% and 95% confidence intervals. Patients receiving nimesulide required a lower dose of morphine for pain control. * = p = 0.0175 vs untreated patients.
N-methyl-d-aspartate (NMDA) is a neurotransmitter that plays a key role in pain perception. PGs may exert their nociceptive effects by regulating NMDA-dependent neurotransmission, as PGs act as intermediaries in NMDA cell signaling. NMDA limits opioid receptor/G protein coupling, an effect that appears to be mediated by COX-2-dependent PG formation. Thus, the inhibition of PG formation may enhance the activity of opioid agonists. Here, we show that a COX-2 inhibitor suppresses CSF 6-keto-PGF1α levels following surgery and improves the pain relief achieved over that seen with an opioid analgesia alone. The improved analgesia was associated with a reduced requirement for intrathecal morphine and probably explains the improved respiratory function following surgery. The results suggest that the suppression of COX-2 is responsible for the observed enhancement of opioid analgesia by NSAIDs. While ibuprofen was less effective, higher doses may have exerted an equianalgesic effect by suppressing COX-2. Our findings in a relatively small number of patients should be confirmed in a larger study population.

There is now compelling experimental evidence to support a major role for COX in spinal nociceptive processing. In a rat model, the antihyperalgesic effects of NSAIDs is achieved through the inhibition of constitutive spinal COX-2, and central inhibition of COX-2 inhibits inflammatory pain hypersensitivity. Our study suggests that COX-2 is also involved in postoperative pain perception in humans. As COX-2 inhibitors are associated with less gastric injury (at least acutely), are considered safe to use in patients with asthma, and do not influence hemostasis, they may represent a safer alternative to nonselective NSAIDs in the management of postoperative pain.

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References


