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# *Cyclooxygenase-2 Induction after Oral Surgery Does Not Entirely Account for Analgesia after Selective Blockade of Cyclooxygenase 2 in the Preoperative Period*

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***Background:*** **The administration of selective cyclooxygenase-2 inhibitors before surgery is regarded as an innovative option to manage postoperative pain. This study was designed to (1) examine the efficacy of preoperative cyclooxygenase-2 blockade on postoperative oral pain and (2) compare pain intensity with prostaglandin $E_2$ ($PGE_2$) production and cyclooxygenase isoform (cyclooxygenase-1, cyclooxygenase-2) messenger RNA (mRNA) expression at the surgical site during the postoperative period.**

***Methods:*** **Sixty patients with impacted lower third molars were randomly allocated to three single-dose treatment groups—placebo, 50 mg rofecoxib, or 550 mg naproxen—1 h before extraction. Pain intensity was evaluated with categorical and visual analog scales every 30 min from 90 to 240 min after surgery. At these times, $PGE_2$ production in the alveolar socket was also evaluated. Cyclooxygenase-1 and cyclooxygenase-2 mRNA expression was examined by reverse-transcription polymerase chain reaction in gingival specimens collected during tooth removal and 240 min after surgery.**

***Results:*** **Pain intensity and $PGE_2$ production in the placebo group increased throughout the observation period. Naproxen prevented pain and decreased $PGE_2$ release at all time points. Rofecoxib reduced $PGE_2$ production *versus* placebo from 150 min onward, while inducing analgesia through the whole observation period. mRNA assay in gingival specimens collected at tooth extraction revealed cyclooxygenase-1 expression, whereas cyclooxygenase 2 was undetectable. At the end of observation, cyclooxygenase-1 mRNA expression was unchanged, whereas cyclooxygenase-2 mRNA was significantly induced.**

***Conclusions:*** **This study indicates that preoperative administration of a selective cyclooxygenase-2 inhibitor ensures effective control of postoperative pain. It is suggested that the selective blockade of inducible cyclooxygenase 2 at the surgical site does not entirely account for the analgesic action occurring in the postoperative period.**

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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NONSTEROIDAL antiinflammatory drugs (NSAIDs) are regarded as effective medications in the management of pain associated with oral surgery.[1] Their therapeutic action results mainly from the inhibition of cyclooxygenase with a subsequent decrease in the production of prostanoids, which act synergistically with other mediators to promote local inflammatory reactions and to determine hyperalgesia.[2] However, the same pharmacodynamic properties account for the occurrence of adverse effects, such as gastrointestinal injury and inhibition of platelet aggregation, with an increased risk of bleeding, which can limit the usefulness of these drugs in the perioperative period.[3]

After the identification of two cyclooxygenase isoforms, named COX-1 and COX-2, selective inhibitors of COX-2 (coxibs) were clinically developed as novel NSAIDs, based on the assumption that COX-2–derived prostanoids are responsible for inflammation and pain, whereas COX-1 would account for homeostatic functions.[4,5] A number of clinical studies has shown that coxibs can be as effective as conventional NSAIDs against postoperative oral pain.[6] However, with the exception of celecoxib and valdecoxib,[7,8] the possible benefits offered by a selective blockade of COX-2 isoform at the preoperative level deserve further investigation.

The administration of analgesic drugs before surgery is currently regarded as a strategic option to manage postoperative pain. This procedure is thought to counteract both peripheral hyperalgesia, resulting from sensitization of sensory neurons at their peripheral ends, and central hyperalgesia, related to changes in the excitability threshold of dorsal horn neurons in the spinal cord.[9] Cyclooxygenase inhibitors may act both at peripheral and central sites to lessen the pain threshold, and therefore, selective COX-2 blockers are being increasingly investigated as preoperative analgesic drugs.[10–12] However, the relative contribution of cyclooxygenase isoforms to pain control is debated, and the respective significance of peripheral and central sites in the analgesic actions of selective COX-2 inhibitors remains to be clarified.

The current study was designed to assess whether prostaglandin production at the surgical site accounts for analgesia associated with selective COX-2 blockade in the preoperative period. To achieve this goal, rofecoxib (a selective COX-2 inhibitor),[13,14] naproxen (a nonselective COX-1/COX-2 inhibitor), and placebo were

preoperatively administered to patients undergoing impacted third molar removal and compared in terms of postoperative analgesic efficacy. Therefore, postoperative pain was related with the dynamics of prostaglandin $E_2$ ($PGE_2$) release and messenger RNA (mRNA) expression of cyclooxygenase isoforms in the surgical area.

## Materials and Methods

*Selection of Subjects*

We aimed to recruit a total of at least 60 patients among new referrals to the oral surgery clinic of Pisa University Hospital, Pisa, Italy, from January to July 2004. Men and women aged at least 18 yr and scheduled to undergo removal of one lower impacted third molar were enrolled. A thorough medical history and radiographic examination were performed to confirm the need for tooth extraction and verify the absence of inflammation or infection at the extraction site. Patients had no history of drug allergy, and none of them had taken drugs within 2 weeks before the study. Patients were also excluded if they had infectious diseases, history of asthma, peptic ulceration, or other disorders of the upper gastrointestinal tract. Patients who had any concomitant major medical problem, known allergy or intolerance to study medications, or any ongoing pharmacologic treatment were also excluded, as were women who were pregnant or lactating or not using contraceptives. Informed consent was obtained from each patient before entering the trial, and the investigation was approved by the local University Hospital Ethics Committee.

*Study Design*

A double-blind, randomized design was used. After screening, patients were randomly assigned to receive one of the following treatments as a single dose by oral route 1 h before surgery: placebo, 50 mg rofecoxib (Dolostop®, Gentili, Pisa, Italy), or 550 mg naproxen (Synflex Forte®, Recordati, Milano, Italy). The random sequence was generated by computer. The subjects chose a sequentially numbered, opaque, sealed envelope, which enclosed the code for the treatment protocol they were to receive. The number of envelopes was equal to the number of subjects. Treatment groups were coded so that the operator, the examiner, and the patients remained blinded throughout the study.

*Surgical Procedures, Microdialysis, and Pain Evaluation*

Surgical extractions of one lower third molar per patient were performed by an experienced oral surgeon by a standardized technique and local anesthesia (3% lidocaine for inferior alveolar nerve block and 2% lidocaine with 1:100,000 epinephrine in the surgical area). Briefly, a mucoperiosteal flap was raised and retracted, bone tissue was removed with a bur, and the tooth was elevated. A specimen of mucosal tissue was then dissected from the surgical site, immediately snap-frozen in liquid nitrogen, and stored at −80°C for subsequent reverse-transcription polymerase chain reaction (RT-PCR) analysis of mRNAs coding for cyclooxygenase isoforms. After the extraction, a microdialysis probe (CMA/70 Microdialysis Bolt Catheter; CMA/Microdialysis, Solna, Sweden) was placed beneath the mucoperiosteal flap into the surgical site. The probe fiber consisted of a 10-mm, flexible, nonmetallic, semipermeable dialysis membrane with a molecular cutoff of approximatively 20,000 Da. Silk sutures were then used to secure the probe and to suture the surgical flap. Subsequently, a saline solution (Perfusion Fluid T1; CMA/Microdialysis) was pumped at a rate of 5 μl/min. The solution had the following composition: 147 mM $Na^+$, 4 mM $K^+$, 2.3 mM $Ca^{2+}$, and 156 mM $Cl^-$. The dialytic perfusate collected during the initial 60 min from probe implantation was discarded, and dialytic samples were then collected in vials placed on ice. Vials were changed every 30 min for the subsequent 180 min, and the collected samples were then stored at −80°C until $PGE_2$ assay.

Concomitantly with vial changes, pain intensity was assessed every 30 min by means of questionnaires, completed by every patient, using a categorical scale of none (0), mild (1), moderate (2), or severe (3) pain as well as a 100-mm visual analog scale (VAS). Patients were also requested to provide information about inferior alveolar nerve conductivity by rating feelings in their lips as "numb," "tingling," or "normal." If patients rated their pain as 40 or greater, based on the VAS scale, and requested additional analgesic medication, they were allowed to take codeine at the oral dose of 30–60 mg. At the end of the observation period, during local anesthesia, the restraining sutures were cut, and the probe was removed. At this time, an additional specimen of oral mucosa was dissected from the surgical area and stored at −80°C for subsequent RT-PCR assay.

*$PGE_2$ Assay*

Levels of $PGE_2$ in samples of dialytic perfusate were determined by competitive enzyme-linked immunoassay (Cayman, Ann Arbor, MI) in accordance with manufacturer instructions. Each sample was assayed in duplicate. $PGE_2$ levels were then expressed as concentrations at various time points (pg/ml).

*RT-PCR Analysis*

Specimens of oral mucosa were disrupted with cold glass pestles, and total RNA was isolated by Trizol® (Life Technologies, Carlsbad, CA) and chloroform. RT-PCR was performed by specific primers based on cloned COX-1 and COX-2 human genes[15] and carried out as previously reported.[16] PCR consisted of 30 cycles of

denaturation at 94°C (1 min), annealing at 50°C (1.5 min), extension at 72°C (2 min), and final extension at 72°C for 10 min. Amplified products were separated by 1.5% agarose gel electrophoresis and stained with ethidium bromide. Complementary DNA bands were visualized by ultraviolet light, quantitated by densitometric analysis with NIH Image program (Scion Corporation, Frederick, MD), and normalized to $\beta$-actin.

*Statistical Analysis*

Results are given as mean ± SD. The significance of differences was evaluated by one-way analysis of variance for unpaired data followed by *post hoc* analysis with Bonferroni test (pain intensity and $PGE_2$ assay), or the Student *t* test for paired data (RT-PCR analysis). *P* values lower than 0.05 were considered significant.

## Results

*Characteristics of Patients*

A total of 63 patients (28 men and 35 women; age ranging from 20 to 32 yr; mean age, 24.5 ± 4.7 yr) were included in the study. However, 60 patients completed the trial, because 3 patients were withdrawn because of probe failure. Therefore, the study groups consisted of 20 patients each (placebo: 8 men and 12 women; mean age, 25.3 ± 5.1 yr; naproxen: 11 men and 9 women; mean age, 24.7 ± 4.1 yr; rofecoxib: 7 men and 13 women; mean age, 23.8 ± 5.3 yr).

*Clinical Observations*

No adverse events were recorded in any treatment group throughout the study period. No alterations of the inferior alveolar nerve conductivity were noted, because all patients returned to their normal lip sensitivity within 180 min from the intervention.

*Assessment of Pain Intensity*

Pain intensity in the placebo group, as assessed by VAS scale, increased progressively throughout the postoperative period, reaching a peak level of 44 ± 5.9 at 180 min from surgery (fig. 1A). Pain intensity values recorded for patients treated with rofecoxib or naproxen were significantly lower than those included in the placebo group, whereas no significant differences were observed when comparing the rofecoxib group with the naproxen group (fig. 1A). Reports of pain intensity evaluated by means of categorical rating scale yielded data similar to those obtained with the VAS scale. Figure 1B displays pain intensity values recorded with the categorical scale at three different times of the study period (120, 180, and 240 min). Three patients in the placebo group required additional analgesic medication because they approached a VAS value of 40 at 180 min.

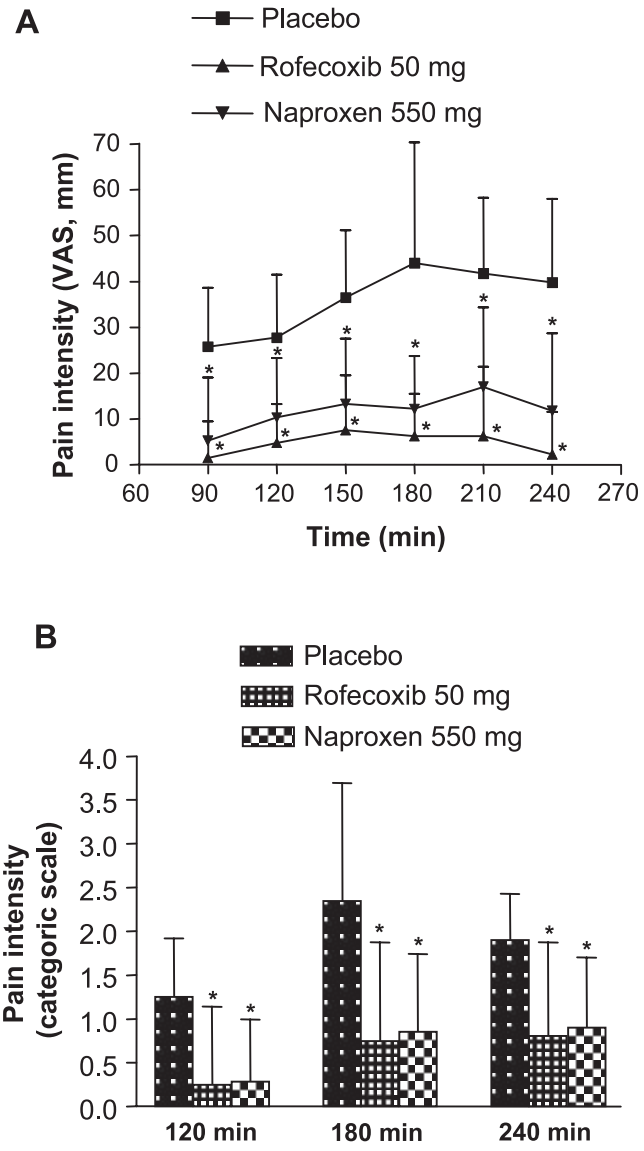


**Fig. 1. Pain intensity, evaluated by visual analog scale (VAS; *A*) or categorical scale (*B*), in patients treated preoperatively with single oral doses of placebo, 50 mg rofecoxib, or 550 mg naproxen. Patients underwent impacted lower third molar removal 1 h after drug administration, and they were then monitored from 60 to 240 min during the postoperative period. Each *point* or *column* indicates the mean value ± SD (*vertical bars*) obtained from 20 patients. * $P < 0.05$, significant difference *versus* values from patients treated with placebo.**

*$PGE_2$ Assay*

The collection of dialytic samples, 60 min after probe implantation into the surgical site, allowed measurement of a $PGE_2$ concentration of 426.8 ± 61.1 pg/ml at 90 min in patients treated with placebo. Such a value increased progressively throughout the postoperative period, reaching a level of 631.5 ± 36.2 pg/ml at 240 min (fig. 2). $PGE_2$ concentrations in the naproxen group were significantly lower than those recorded in placebo patients at all time points examined. After naproxen administration, $PGE_2$ levels accounted for 113.8 ± 41.1 pg/ml at 90 min, and this value did not change signifi-

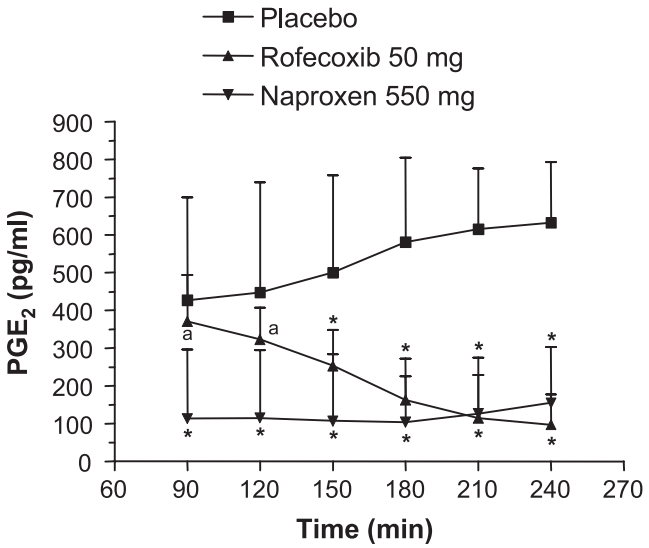


**Fig. 2. Prostaglandin $E_2$ ($PGE_2$) concentration in samples of dialytic perfusate collected from the surgical site of patients treated preoperatively with single oral doses of placebo, 50 mg rofecoxib, or 550 mg naproxen. Patients underwent impacted lower third molar removal and microdialysis probe implantation 1 h after drug administration, and dialytic samples were then collected from 60 to 240 min during the postoperative period. Each *point* or *column* indicates the mean value ± SD (*vertical bars*) obtained from 20 patients. * $P < 0.05$, significant difference *versus* placebo. [a] $P < 0.05$, significant difference *versus* naproxen.**

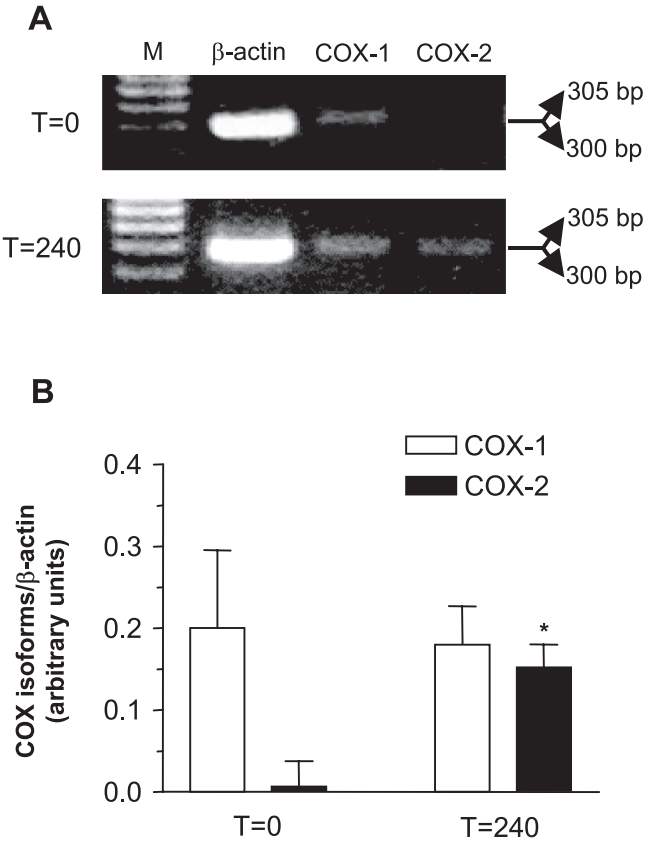


**Fig. 3. Reverse-transcription polymerase chain reaction analysis of cyclooxygenase (COX)-1, COX-2, and β-actin messenger RNA in samples of gingival tissue collected from the surgical site of patients treated preoperatively with placebo. Patients underwent impacted lower third molar removal 1 h after placebo administration. Specimens of gingiva were collected immediately after tooth extraction (T = 0) as well as at the end of the postoperative observation period (T = 240). (*A*) Two representative agarose gels referring to the amplification of β-actin, COX-1, and COX-2 complementary DNAs (cDNAs) at T = 0 and T = 240. (*B*) Column graph referring to the densitometric analysis of COX-1 and COX-2 cDNA bands normalized to the expression of β-actin. bp = base pairs; M = size markers. Each *column* represents the mean value ± SD (*vertical bars*) obtained from 10 patients. * $P < 0.05$, significant difference *versus* values obtained at T = 0. COX-isoform/β-actin ratios estimated in rofecoxib- and naproxen-treated patients did not differ significantly from those obtained in the placebo group.**

cantly throughout the evaluation period (fig. 2). $PGE_2$ concentrations in dialytic samples collected from patients of rofecoxib group displayed a different trend in comparison with naproxen group. After rofecoxib administration, $PGE_2$ levels were 370.6 ± 27.5 pg/ml at 90 min of the postoperative period, and this value declined progressively up to the end of the experimental period. When compared with placebo, $PGE_2$ values in rofecoxib-treated patients were significantly lower from 150 min onward, whereas significant differences between naproxen and rofecoxib groups were noted at both 90 and 120 min (fig. 2).

*RT-PCR Analysis*

The mRNA expression of cyclooxygenase isoforms was assessed by RT-PCR analysis of gingival specimens obtained from patients immediately before probe implantation (T = 0) as well as at the end of dialytic perfusion and probe removal (T = 240 min). When examining tissue samples retrieved from placebo-treated patients at T = 0, RT-PCR revealed the presence of COX-1 mRNA, whereas COX-2 expression was undetectable (or very faint in few cases). At the end of the observation period, COX-1 mRNA expression was barely unchanged, according to semiquantitative densitometric analysis, whereas a significant induction of gene expression could be detected for COX-2 mRNA (fig. 3). Similar expression patterns were found for COX-1 and COX-2 mRNA, at both T = 0 and T = 240, when performing RT-PCR analysis on gingival samples obtained from patients subjected to treatment with rofecoxib or naproxen (data not shown).

## Discussion

A number of studies suggest that preoperative treatments with coxibs ensure good postoperative analgesia.[10,12,17] However, the anatomical locations and molecular mechanisms underlying coxib-induced preoperative analgesia remain undetermined. In the current investigation, patients undergoing third molar extraction were preoperatively treated with a selective COX-2 inhibitor or a conventional NSAID and, by a combined assessment

of pain intensity with local $PGE_2$ production and mRNA COX isoform expression in the postoperative period, consistent evidence was obtained that both drugs exerted significant analgesic effects while displaying different inhibitory profiles on prostaglandin release.

The evaluation of postsurgical pain in patients treated preoperatively with placebo showed that pain intensity increased progressively after surgery and that maximal levels could be recorded by 180 min. Concomitantly, the analysis of dialytic perfusate revealed an active release of $PGE_2$ at the surgical site by 90 min after tooth extraction, with progressive increments in $PGE_2$ concentrations throughout the observation period. The mRNA coding for cyclooxygenase isoforms was also measured by RT-PCR in tissue samples and, at variance with the stable expression of COX-1, COX-2 mRNA was undetectable in perialveolar gingiva at the time of tooth removal, and a significant increment of its expression could be observed 4 h later. Considering that the increase in COX-2 mRNA could be due to its induction in resident cells or to recruitment of COX-2 expressing cells triggered by surgical injury, our RT-PCR results support the view that COX-2 induction in the operative site may contribute to the increase in $PGE_2$ production at a late phase of postsurgical period. Of note, the above lines of evidence, in keeping with previous reports based on similar methodologies,[8,18] might be taken to suggest that postoperative pain is driven mainly by an enhancement of cyclooxygenase activity and prostaglandin production in oral tissues subjected to surgical injury. However, our data from patients treated with cyclooxygenase inhibitors argue against this hypothesis.

A major finding of the current study was that rofecoxib and naproxen were equieffective against postoperative pain, while acting with different time patterns to modulate $PGE_2$ release at the surgical site. In particular, naproxen exerted a significant analgesic action that was closely paralleled by a marked inhibition of tissue $PGE_2$ production, whereas rofecoxib ensured a full prevention of postoperative pain that was associated with a significant reduction of local $PGE_2$ release only in a late phase of the observation period. In the light of data provided by RT-PCR analysis of COX-1 and COX-2 mRNA expression, the results obtained with naproxen and rofecoxib suggest that (1) COX-1 is mainly responsible for $PGE_2$ production at the surgical site in the early postoperative phase, (2) a local induction of COX-2 can account for the enhanced $PGE_2$ release in the late observation period, and (3) the analgesic effect observed after preoperative rofecoxib administration is likely to result from a pharmacologic modulation of COX-2 constitutively active at distinct locations from the surgical area. Most importantly, our findings in the setting of drug treatment do not support the hypothesis that local $PGE_2$ production plays a significant role in driving postoperative pain, as one might infer by measurements performed in the placebo group.

Cyclooxygenase inhibitors are thought to act both on peripheral tissues and central nervous pathways to counteract pain sensitization.[3,19] It is also acknowledged that, after acute noxious stimuli, the production of prostanoids responsible for peripheral hyperalgesia requires COX-2 induction.[2] However, whereas this mechanism has been assumed as the main rationale for clinical development of coxibs, it can hardly account for the analgesic effects of selective COX-2 inhibitors in the early stage of postoperative period, because few hours are required to achieve maximal levels of COX-2 induction.[20] In this respect, our results emphasize from a clinical perspective the importance of a constitutive COX-2 isoform in preemptive analgesia achievable with cyclooxygenase inhibitors.

Studies on preclinical models have shown that cyclooxygenase isoforms are constitutively expressed in dorsal horns of spinal cord and that central hyperalgesic responses to peripheral stimuli are highly sensitive to COX-2 blockade, indicating that spinal nerves implicated in pain transmission are under the control of COX-2–derived prostaglandins.[21] Other authors have suggested that spinal COX-1 might be important also in sensitization to postoperative pain.[22,23] In addition, spinal COX-2 expression can be further enhanced by peripheral inflammation to subserve a late phase of central hyperalgesia, which requires some hours to develop fully.[20] Therefore, current preclinical evidence supports the notion that the primary site of NSAID action is in the spinal cord, where acute stimuli can activate $PGE_2$ production by constitutive COX-2, to initiate facilitated release of primary afferent nociceptive transmitters and block intrinsic dorsal horn inhibition, and where acutely induced hyperalgesia can be blocked by systemic or spinally administered COX-2 inhibitors.[24–26] Consistent with these preclinical findings, our data, showing that preoperative rofecoxib administration ensured analgesia in the early stage of postsurgical period, without a concomitant reduction of local $PGE_2$ production, can be interpreted as resulting from the inhibition of a constitutive COX-2 responsible for the activation of a rapid central hyperalgesia elicited by peripheral surgical injury. In support of this proposal, it has been recently reported that, after oral administration to healthy volunteers, at a single dose of 50 mg, rofecoxib penetrates rapidly into the central nervous system and reaches cerebral spinal fluid concentrations sufficient to inhibit COX-2 activity.[27]

In conclusion, the current study provides clinical evidence that the preoperative administration of a selective COX-2 inhibitor determines an effective control of postoperative oral pain. It is also suggested that the selective blockade of inducible COX-2 at the peripheral level does not entirely account for the analgesic action occurring in the postoperative period.

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