The Journal of Pain, Vol 8, No 4 (April), 2007: pp 349-354
Available online at www.sciencedirect.com

ELSEVIER

Expression of COX-1 and COX-2 in a Clinical Model of Acute Inflammation

Asma A. Khan,* Michael Iadarola,* Hsiu-Ying T. Yang,* and Raymond A. Dionne†

*National Institute of Dental and Craniofacial Research and
†National Institute of Nursing Research, National Institutes of Health, Bethesda, Maryland.

Abstract: **Cyclooxygenase (COX) plays an important role in the induction of pain and inflammation as well as the analgesic actions of NSAIDs and coxibs. This study evaluates the expression of the two isoforms COX-1 and COX-2 in a clinical model in which the surgical removal of impacted third molars is used to evaluate the analgesic activity of anti-inflammatory drugs. A 3-mm punch biopsy was performed on the oral mucosa overlying 1 impacted third molar immediately before extraction of 2 impacted lower third molars. After the second tooth was extracted, a second biopsy was performed adjacent to the surgical site either immediately after surgery or 30, 60, or 120 minutes after surgery. RNA was extracted from the biopsy specimens, and RT-PCR was performed to assess mRNA levels of COX-1, COX-2, and glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The RT-PCR products in the biopsy specimens were normalized to G3PDH and compared with baseline. COX-2 mRNA was progressively increased at 30, 60, and 120 minutes after surgery ($P < .05$); COX-1 mRNA was transiently decreased at 60 minutes during the postsurgical period ($P < .05$). The results demonstrate peripheral elevation of COX-2 after tissue injury, which may contribute to increased prostaglandin E_2 at the site of injury, pain onset, and the analgesic activity of both nonselective NSAIDs and selective COX-2 inhibitors.**

Perspective: ***This clinical study uses a physiologically relevant model to determine the time course of expression of COX-1 and COX-2 in acute inflammation of the human oral mucosa. This study furthers our understanding of the contribution of the COX isoforms to acute pain.***

Key words: *COX-1, COX-2, cyclooxygenase, inflammation, lipopolysaccharides, NSAID, prostaglandin, polymorphonuclear.*

Cyclooxygenase (COX), also known as prostaglandin H synthase, is the key enzyme in the synthesis of prostaglandins (PGs). Elucidation of the 2 COX isoforms gave rise to the concept that the constitutive enzyme COX-1 was responsible for the production of the PGs with homeostatic functions in tissues such as the stomach, kidney, and platelets, whereas COX-2, the inducible enzyme, was responsible for the production of the proinflammatory PGs.[15,26,32]

There is extensive evidence based on animal as well as human studies supporting the role of COX-2 in the development of inflammation.[26,31,33] Animal models of inflammation have demonstrated that COX-2 mRNA and protein as well as PGs increase in a time-dependent manner that parallels the inflammatory process.[1] Inflammatory cytokines and endotoxins can induce a 10- to 80-fold increase in the level of COX-2 expression in monocytes, macrophages, chondrocytes, fibroblasts, and endothelial cells.[1,2] The contribution of COX-2 to inflammation is further supported by demonstration that the expression of COX-2 and production of PGs can be inhibited by anti-inflammatory cytokines and glucocorticoids.[6,24]

The concept that COX-2 is the only COX isoform involved in inflammation has been challenged by a number of studies.[8,10,22,34] It is now believed that COX-1 is responsible for the initial prostanoid response to inflammatory stimuli, whereas COX-2 becomes the major contributor to prostanoid synthesis as inflammation progresses.[9,15,23,30] PGE_2 can be produced by PGE synthase from many different cell types including neurons,

Received June 13, 2006; Revised September 18, 2006; Accepted October 24, 2006.
Supported by The National Institutes of Health.
Address reprint requests to Raymond A. Dionne, DDS, PhD, National Institutes of Health, 10 Center Drive, Room 2-NE-1339, Bethesda, MD 20892. E-mail: dionner@mail.nih.gov
1526-5900/$32.00

doi:10.1016/j.jpain.2006.10.004

endothelial cells, and neutrophils. PGE_2 released in inflamed tissue sensitizes the terminals of afferent nerve fibers, thereby enhancing nociceptive processing within the spinal cord and brain to evoke hyperalgesia.[28] COX-1 mRNA has a half-life of about 12 to 15 hours, whereas COX-2 has a shorter half-life of less than 3.5 hours,[18] suggesting a close temporal link between tissue injury, COX-2 expression, and elevated PGE_2 in comparison to constitutively expressed COX-1.

A previous study in the oral surgery model demonstrated differential production of products of COX-1 (thromboxane B_2, the stable metabolite of thromboxane A_2) and PGE_2 production mediated by both COX-1 and COX-2. The selective COX-2 inhibitor celecoxib did not have any detectable effect on thromboxane B_2 levels and only suppressed PGE_2 levels from 120 to 240 minutes after oral surgery.[14] A similar time course of action was also demonstrated for rofecoxib in the oral surgery model, with the effects of the selective COX-2 inhibitor being seen at 60 to 240 minutes after surgery (based on unpublished observations). These data suggest that the expression of COX-2 after tissue injury takes 1 to 2 hours to produce increased prostanoid levels that contribute to pain and the acute inflammatory process. We conducted a study to examine the in vivo expression of COX-1 and COX-2 in the human oral mucosa before and after postsurgical trauma and the onset of inflammation. Our results demonstrate that COX-2 mRNA rapidly increases in a time-dependent manner after surgery, whereas the level of COX-1 mRNA transiently decreases but otherwise shows no sustained alteration during the postsurgical period.

Materials and Methods

Subjects and Study Design

The study was approved by the Institutional Review Board, National Institute of Dental and Craniofacial Research, National Institutes of Health. Informed consent was obtained from all subjects. Subjects, 16 years or older, were enrolled as outpatients and underwent surgical removal of impacted mandibular third molars. Inclusion criteria were the presence of 2 mandibular third molars, classified as partial or full bony impaction by clinical and radiographic examination. Exclusion criteria included the presence of infection or inflammation at either of the 2 extraction sites as determined by clinical examination. Subjects who were pregnant or nursing were excluded from the study, as were those taking antidepressants, diuretics, aspirin, coumadin, or any other anticoagulants and any drugs such as steroids, which might influence pain report or the synthesis and activity of COX.

Preoperative and postoperative punch biopsies were obtained from each subject. The preoperative biopsy was performed from the oral mucosa overlying 1 impacted third molar, immediately before the surgical extraction. After this, both mandibular third molars were removed and the postoperative biopsy was obtained from the other extraction site. Subjects ($n > 10$ per group) were randomly allocated with respect to the second biopsy into 1 of 4 groups: (1) time 0, immediately after surgery ($n = 10$); (2) 30 minutes after surgery ($n = 13$); (3) 60 minutes after surgery ($n = 10$); or (4) 120 minutes after surgery ($n = 10$).

RNA Extraction and RT-PCR Analysis

The samples were immediately frozen and maintained at −80°C. Total RNA was extracted by using the RNeasy RNA extraction kit (Qiagen; Valencia, CA). RNA yield was quantified by using the RiboGreen RNA Quantitation Kit (Molecular Probes; Eugene, OR). The RNA yield was 246 ± 42 ng/mg tissue weight. RT-PCR was carried out by using the Access RT-PCR system (Promega; Madison, WI). Primer sequences and assay parameters are given in Table 1.

The program used for RT-PCR on a thermocycler (Robocycler Gradient 96; Stratagene, La Jolla, CA) consisted of 48°C for 45 minutes and 94°C for 2 minutes for the synthesis of first-strand cDNA by reverse transcriptase, followed by 40 cycles of a denaturation step at 94°C for 30 seconds, an annealing step at 55°C for 1 minute, and an extension step at 68°C for 2 minutes for amplification of cDNA reverse transcribed from COX-2 mRNA. The final extension was done at 72°C for 7 minutes. The program for COX-1 was similar except that the extension was done at 72°C. For G3DPH, the extension and the final extension were done at 68°C. The amount of RNA template and number of cycles that would yield relative values in the linear range of amplification for each target transcript in the RT-PCR were determined by preliminary experiments. Each group of RT-PCR experiments included a negative control in which the extracted RNA was replaced by RNase-free water.

The PCR products were detected by electrophoresis in 2% agarose gels visualized with ethidium bromide stain-

Table 1. Sequence of Primers Used and Their Product Length

Gene	Sequence of Primers (5′ to 3′)	Product Length (BP)	RNA Template (ng)	Number of Cycles for PCR
COX-1	Forward-CAGACGACCCGCCTCATCCTCATAG Reverse-GCCTCAACCCCATAGTCCACCAACA	275	4	40
COX-2	Forward-TGGGAAGCCTTCTCTAACCTCTCCT Reverse-CTTTGACTGTGGGAGGATACATCTC	388	8	40
G3DPH	Forward-GACCCCTTCATTGACCTCAACTAC Reverse-CATCGCCCCACTTGATTTTG	167	2	26

Table 2. Demographic and Surgical Features of the Patient Sample

Time of Biopsy From End of Surgery (min)	N	Age (Years)	Sex M/F	Extraction Difficulty*	Midazolam Dose (mg)	Lidocaine Dose (mg)
0	10	19.0 ± 3.9	6/4	6.4 ± 1.6	4.5 ± 0.7	155.6 ± 13
30	13	18.9 ± 3.5	7/6	6.5 ± 1.1	4.3 ± 0.7	144.8 ± 30
60	10	23.1 ± 5.9	3/7	7.0 ± 1.2	4.7 ± 0.5	164.0 ± 25
120	10	19.0 ± 3.3	4/6	7.0 ± 1.2	4.6 ± 0.6	162.0 ± 26

*Extraction difficulty is the sum calculated by assigning a score of (2) for soft tissue impactions, (3) for partial bony impactions, and (4) for full bony impactions.

ing. A fluorescence imaging system (AlphaImager; Alpha Innotech Corp., San Leandro, CA) was used to acquire the image of the fluorescent bands. Analysis of band intensity was performed on a Macintosh computer, using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image). The RT-PCR products were normalized to G3PDH, and the results of postsurgical biopsies were compared with those of presurgical biopsies.

Statistical Analysis

Changes in the ratio of COX-1/G3PDH and COX-2/G3PDH over time were compared with ratios in the presurgical biopsies. A permutation test for 2 related samples tested for change over time in levels of each COX isoforms was performed with post hoc testing to determine if any time points differed from baseline. Statistical analyses were performed with SPSS 6.1 (SPSS; Chicago, IL).

Results

Subjects

The study sample consisted of 43 usable subjects distributed among the treatment groups (Table 2). The mean age, 20 years, is characteristic of the young adult population undergoing the removal of impacted third molars. Subjects in the 4 groups did not differ in terms of demographic and surgical factors such as extraction difficulty, the dose of midazolam, or the amount of lidocaine administered, which could affect the outcome of the study.

COX-2 mRNA

Under the conditions used, COX-2 expression in the preoperative biopsy specimens was either not detectable or was seen as a faint band (Fig 1). Low levels of COX-2 message were detected in 51% of the presurgical biopsy specimens. The ratio of COX-2 mRNA/G3PDH mRNA in biopsy specimens obtained at time 0 immediately after surgery did not differ from that in the presurgical biopsies. Biopsy specimens collected at 30, 60, and 120 minutes after surgery demonstrated a progressive significant increases ($P < .01$) in the level of COX-2 message over time as compared with preoperative levels (Figs 1 and 2). The presence of additional bands in Fig 1 probably is due to the large number of PCR cycles needed to detect COX-2.

COX-1 mRNA

The COX-1 transcript was detected in all the presurgical and postsurgical biopsy specimens (Figs 1 and 3). The ratio of COX-1 mRNA/G3PDH mRNA in the biopsy specimens collected immediately after surgery did not differ significantly from that of the preoperative biopsy specimens (Fig 3). We detected a significant decrease in the ratio of COX-1 mRNA/G3PDH mRNA in samples collected at 60 minutes after surgery, as compared with baseline

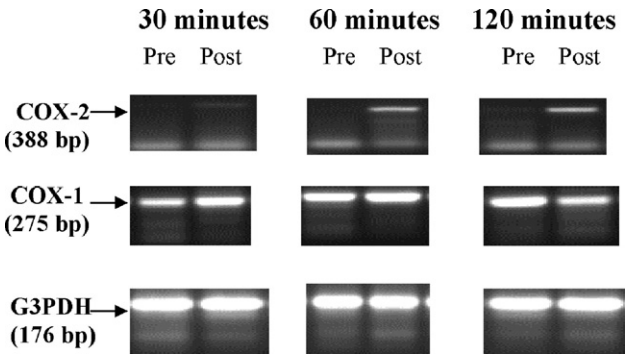

Figure 1. Representative products of the RT-PCR for COX-2, COX-1, and G3PDH. Notice that COX-2 is either not detectable in the presurgical biopsy specimens or is barely detected and is induced at 30, 60, and 120 minutes after surgery in these samples. In contrast, COX-1 was detected at all time points examined.

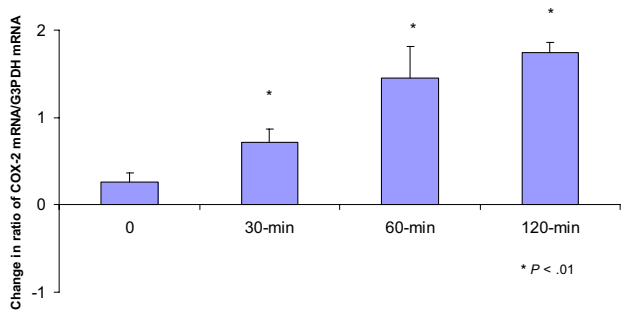

Figure 2. Change in the ratio of COX-2 mRNA/G3PDHmRNA in the postoperative biopsy specimens as compared with the preoperative biopsy specimens. "0" represents biopsy specimens that were obtained immediately after surgery. A significant increase was detected in the biopsy specimens obtained at 30, 60, and 120 minutes after surgery. *$P < .05$.

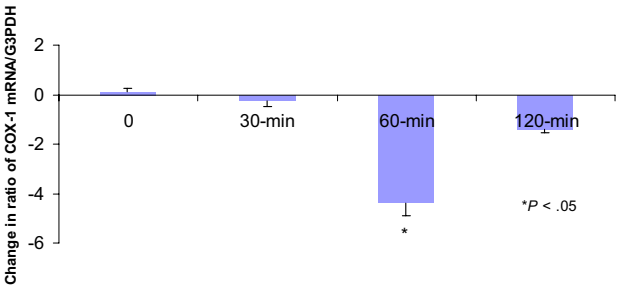

Figure 3. Change in the ratio of COX-1 mRNA/G3PDH mRNA in the postsurgical biopsy specimens as compared with the presurgical ones. "0" represents biopsy specimens that were obtained immediately after surgery. A significant decrease in COX-1 mRNA was detected at 60 minutes after surgery. *$P < .05$.

($P < .05$). The level of COX-1 mRNA was numerically lower at 30 and 120 minutes after surgery, as compared with baseline, but was not statistically significant. No consistent change was observed with G3PDH.

Discussion

It is widely accepted that COX-1 is responsible for the immediate prostanoid response to inflammatory stimuli, whereas COX-2 becomes the primary contributor to prostanoid synthesis as inflammation progresses. The results of this study are supportive of this concept as we demonstrate COX-1 message at baseline and throughout the postoperative period in comparison to negligible COX-2 message before surgery with an increase in the level of COX-2 message during the postsurgical period.

This study demonstrates a significant decrease in the level of COX-1 mRNA in biopsy specimens obtained 60 minutes after surgery ($P < .05$), as compared with the level of COX-1 in preoperative biopsy specimens. Prior studies have reported that the level of COX-1 mRNA and protein in the peripheral tissues do not change during acute inflammation.[26,31] It is conceivable that the discrepancy between these reports and our data is due to differences in the types of stimuli used and in the tissue examined. Liu et al[17] report a 2- to 5-fold decrease in the level of COX-1 mRNA in the myocardial and pleural tissues of rats after systemic administration of lipopolysaccharides (LPS). A similar study reported a decrease in COX-1 mRNA in the rodent renal medulla 1 hour after injection of LPS.[13] Although these data support our observations, the systemic administration of LPS is a model of sepsis and may not be reflective of the physiological changes of postsurgical trauma and acute inflammation. A subsequent study in the oral surgery model[16] using quantitative real-time PCR in a larger sample replicated the observed decrease in COX-1 mRNA in the immediate postoperative period, suggesting that acute tissue injury and inflammation in humans not only stimulates increased COX-2 mRNA but also transiently inhibits COX-1 mRNA transcription similar to immune stimuli such as LPS.[13,17]

The results from this study clearly demonstrate increased expression of COX-2 after the induction of inflammation. Previous studies examining the levels of PGE_2 in the extraction sites after oral surgery demonstrate a decrease in PGE_2 levels in the immediate postoperative period[10,14] followed by an increase, which is coincident with report of moderate to severe pain.[10,14,25] Although the relative contributions of COX-1 and COX-2 to PGE_2 production are not known, it is likely that COX-2 is primarily responsible for the increased levels of PGE_2, but with continued production of PGE_2 by COX-1.

COX-2 is induced in cultured gingival fibroblasts after application of proinflamatory agents such as interleukin-1β (IL-1β), LPS, and bradykinin.[19,21,22,37] The increased expression of COX-2 in fibroblasts results in enhanced synthesis of PGE_2.[22,35-37] The same phenomenon can be observed in endothelial cells on induction with IL-1α.[11] Polymorphonuclear cells also upregulate COX-2 expression in experimental systems after LPS stimulation.[19] Taken together with our results, it appears that after tissue trauma, mainly 3 types of cells contribute to COX-2 induction and subsequently to PG synthesis: the resident fibroblasts and endothelial cells as well as the invading PMNs. The increased levels of PGs may in turn influence the maintenance of nociceptive processes in postoperative pain.

The oral surgery model is a useful and reproducible model of acute pain, widely used in analgesic research.[3,4] Adapting microdialysis to the oral surgery model has facilitated examination of the relations among mediators of inflammation, pain report, and analgesic activity in humans.[5,12,25,29] The results of previous studies using microdialysis and the oral surgery model demonstrated increased PGE_2 production at later time points during the postoperative period (120 to 240 minutes) suggestive of increased COX activity. The present study supports these observations by demonstrating increased COX-2 expression with negligible changes in COX-1 expression at the same time points. This increase in COX-2 expression also suggests a role of this isoform in the inflammatory response and subsequent resolution of tissue injury and repair, although the exact role needs clarification.[7,27] Taken together with previous demonstrations of a PGE_2 time course after surgery,[10] differential effects of coxibs on biomarkers for COX-1 and COX-2[14] (and based on unpublished observations) and the ability to simultaneously measure pain and analgesia in humans support the utility and clinical relevance of the oral surgery model for mechanistic studies of inflammation.

To conclude, this study demonstrated the induction of COX-2 mRNA in acute inflammation in humans. The use of a physiologically relevant model of acute inflammation further enhances the generalizability of these findings, which are relevant to other types of acute inflammatory pain.

References

1. Anderson GD, Hauser SD, McGarity KL, Bremer ME, Isakson PC, Gregory SA: Selective inhibition of cyclooxygenase: (COX)-2 reverses inflammation and expression of interleukin-6 in rat adjuvant arthritis. J Clin Invest 97:2672-2679, 1996

2. Buttar NS, Wang KK: The 'aspirin' of the new millennium: Cyclooxygenase inhibitors. Mayo Clin Proc 75:1027-1038, 2000

3. Cooper SA, Beaver WT: A model to evaluate mild analgesic in oral surgery outpatients. Clin Pharmacol Ther 20:241-250, 1976

4. Dionne RA, Berthold CW: Therapeutic uses of non-steroidal anti-inflammatory drugs in dentistry. Crit Rev Oral Biol Med 12:315-330, 2001

5. Dionne RA: Evaluation of analgesic mechanisms and NSAIDs in the oral surgery model. In, Rainsford KD, Powanda MD (eds), Safety and Efficacy of Non-Prescription (OTC) Analgesics and NSAIDs. London: Kluwer Academic Publishers, 1994, pp 105-117

6. Fernandez-Morata JC, Mullol J, Fuentes M, Pujols L, Roca-Ferrer J, Xaubet A, Picado C: Regulation of cyclooxygenase-1 and cyclooxygenase-2 in human nasal mucosa: Effects of cytokines and dexamethasone. Clin Exp Allergy 30:1275-1284, 2004

7. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA: Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med 5:698-701, 1999

8. Gilroy DW, Tomlinson A, Willoughby DA: Differential effects of inhibition of isoforms of cyclooxygenase (COX-1, COX-2) in chronic inflammation. Inflamm Res 47:79-85, 1998

9. Gilroy DW, Tomlinson A, Willoughby DA: Differential effects of inhibitors of cyclooxygenase (cyclooxygenase 1 and cyclooxygenase 2) in acute inflammation. Eur J Pharmacol 355:211-217, 1998

10. Gordon SM, Brahim JS, Rowan J, Kent A, Dionne RA: Peripheral prostanoid levels and nonsteroidal anti-inflammatory drug analgesia: Replicate clinical trials in a tissue injury model. Clin Pharmacol Ther 72:175-183, 2002

11. Habib A, Creminon C, Frobert Y, Grassi J, Pradelles P, Maclouf J: Demonstration of an inducible cyclooxygenase in human endothelial cells using antibodies raised against carboxyl-terminal region of cyclooxygenase-2. J Biol Chem 268: 23448-23454, 1993

12. Hargreaves KM, Costello A: Glucocorticoids suppress levels of immunoreactive bradykinin in inflamed tissue as evaluated by microdialysis probes. Clin Pharmacol Ther 48: 168-178, 1990

13. Ichitani Y, Holmberg K, Maunsbach AB, Haeggstrom JZ, Samuelsson B, De Witt D, Hokfelt T: Cyclooxygenase-1 and cyclooxygenase-2 expression in rat kidney and adrenal gland after stimulation with systemic lipopolysaccharide: In situ hybridization and immunocytochemical studies. Cell Tissue Res 303:235-252, 2001

14. Khan AA, Brahim JS, Rowan JS, Dionne RA: In vivo selectivity of a selective cyclooxygenase 2 inhibitor in the oral surgery model. Clin Pharmacol Ther 72:44-49, 2002

15. Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD, Kim HS, Smithies O: Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 83:483-492, 1995

16. Lee Y, Kim H, Wu T, Wang X, Dionne RA: Genetically mediated inter-individual variation in analgesic responses to COX inhibitory drug. Clin Pharmacol Ther 79:407-418, 2006

17. Liu SF, Newton R, Evans TW, Barnes PJ: Differential regulation of cyclo-oxygenase-1 and cyclo-oxygenase-2 gene expression by lipopolysaccharide treatment in vivo in the rat. Clin Sci Lond 90:301-306, 1996

18. Lukiw WJ, Bazan NG: Cyclooxygenase 2 RNA message abundance, stability, and hypervariability in sporadic Alzheimer neocortex. J Neurosci Res 50:937-945, 1997

19. Maloney CG, Kutchera WA, Albertine KH, McIntyre TM, Prescott SM, Zimmerman GA: Inflammatory agonists induce cyclooxygenase type 2 expression by human neutrophils. J Immunol 160:1402-1410, 1998

20. McAdam BF, Mardinin IA, Habib A, Burke A, Lawson A, Kapoor A, Fitzgerald GA: Effect of regulated expression of human cyclooxygenase isoforms on eicosanoid and isoeicosanoid production in inflammation. J Clin Invest 105:1473-1482, 2000

21. Nakao S, Ogata Y, Modeer T, Segawa M, Furuyama S, Sugiya H: Bradykinin induces a rapid cyclooxygenase-2 mRNA expression via Ca2+ mobilization in human gingival fibroblasts primed with interleukin-1 beta. Cell Calcium 29: 446-452, 2001

22. Noguchi K, Shitashige M, Endo H, Kondo H, Yotsumoto Y, Izumi Y, Nitta H, Ishikawa I: Involvement of cyclooxygenase-2 in serum-induced prostaglandin production by human oral gingival epithelial cells. J Periodontal Res 36:124-130, 2001.

23. Noguchi K, Shitashige M, Yanai M, Morita I, Nishihara T, Murota S, Ishikawa I: Prostaglandin production via induction of cyclooxygenase-2 by human gingival fibroblasts stimulated with lipopolysaccharides. Inflammation 20:555-568, 1996

24. Ristimaki A, Narko K, Hla T: Down-regulation of cytokine-induced cyclo-oxygenase-2 transcript isoforms by dexamethasone: Evidence for post-transcriptional regulation. Biochem J 318(Pt 1):325-331, 1996

25. Roszkowski MT, Swift JQ, Hargreaves KM: Effect of NSAID administration on tissue levels of immunoreactive prostaglandin E2, leukotriene B4, and (S)-flurbiprofen following extraction of impacted third molars. Pain 73:339-345, 1997

26. Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Isakson P: Distribution of COX-1 and COX-2 in normal and inflamed tissues. Adv Exp Med Biol 400A:167-170, 1997

27. Simon AM, Manigrasso MB, O'Connor JP: Cyclo-oxygenase 2 function is essential for bone fracture healing. J Bone Miner Res 17:963-976, 2002

28. Svensson CI, Yaksh TL: The spinal phospholipase-cyclooxygenase-prostanoid cascade in nociceptive processing. Annu Rev Pharmacol Toxicol 42:553-583, 2002

29. Swift JQ, Garry MG, Roszkowski MT, Hargreaves KM: Effect of flurbiprofen on tissue levels of immunoreactive bradykinin and acute postoperative pain. J Oral Maxillofac Surg 51:112-116; discussion 116-117, 1993

30. Tilley SL, Coffman TM, Koller BH: Mixed messages: Modulation of inflammation and immune responses by prostaglandins and thromboxanes. J Clin Invest 108:15-23, 2001

31. Tomlinson A, Appleton I, Moore AR, Gilroy DW, Willis D, Mitchell JA, Willoboughy DA: Cyclo-oxygenase and nitric oxide synthase isoforms in rat carrageenin-induced pleurisy. Br J Pharmacol 113:693-698, 1994

32. Vane JR, Botting RM: Mechanism of action of non-steroidal anti-inflammatory drugs. Am J Med 104:2S-8S, 1998

33. Vane JR, Mitchell JA, Appleton I, Tomlinson A, Bishop-Bailey D, Croxtall J, Willoboughy DA: Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. Proc Natl Acad Sci U S A 91:2046-2050, 1994

34. Wallace JL, Bak A, McKnight W, Asfaha S, Sharkey KA, MacNaughton WK: Cyclooxygenase-1 contributes to inflammatory responses in rat and mice: Implications for GI toxicity. Gastroenterology 115:101-109, 1998

35. Yucel-Lindberg T, Brunius G, Wondimu B, Anduren I, Modeer T: Enhanced cyclooxygenase-2 mRNA expression in human gingival fibroblasts induced by cell contact with human lymphocytes. Eur J Oral Sci 109:187-192, 2001

36. Yucel-Lindberg T, Ahola H, Carlstedt-Duke J, Modeer T: Involvement of tyrosine kinases on cyclooxygenase expression and prostaglandin E2 production in human gingival fibroblasts stimulated with interleukin-1beta and epidermal growth factor. Biochem Biophys Res Commun 257:528-532, 1999

37. Yucel-Lindberg T, Ahola H, Nilsson S, Carlstedt-Duke J, Modeer T: Interleukin-1 beta induces expression of cyclooxygenase-2 mRNA in human gingival fibroblasts. Inflammation 19:549-560, 1995