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Acetaminophen selectively suppresses peripheral prostaglandin E_2 release and increases COX-2 gene expression in a clinical model of acute inflammation

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Abstract

Acetaminophen is widely used for pain management as an alternative to NSAIDs and selective COX-2 inhibitors, but its action at a molecular level is still unclear. We evaluated acetaminophen's effect on PG release and the expression patterns of genes related to PG production in a clinical model of tissue injury and acute inflammation. Subjects (119 outpatients) received either 1000 mg acetaminophen, 50 mg rofecoxib (a selective COX-2 inhibitor), 30 mg ketorolac (a dual COX-1/COX-2 inhibitor), or placebo before the surgical removal of two impacted mandibular third molars. Microdialysis was used to collect inflammatory transudate from the surgical site for measurement of PGE_2 and TXB_2 levels at the site of injury. Biopsies were collected to investigate the expression patterns of genes related to PG production at baseline prior to surgery and at 3 or 24 h following surgery. PGE_2 release was suppressed by ketorolac, rofecoxib and acetaminophen compared to placebo at 3 h coincident with increased COX-2 gene expression in biopsies collected from the surgical site. TXB_2 release was suppressed only by ketorolac. COX-2 gene expression remained elevated at 24 h with continued ketorolac and acetaminophen treatment. COX-1 gene expression was significantly down-regulated at 24 h by ketorolac, rofecoxib and acetaminophen. Acetaminophen suppression of PGE_2 without inhibiting TXB_2 release, when COX-2 gene expression is up-regulated, suggests that acetaminophen is a selective COX-2 inhibitor *in vivo*. The up-regulation of COX-2 gene and down-regulation of COX-1 gene expression suggests that acetaminophen may result in changes in COX-derived prostanoids with repeated doses.

Keywords: Gene expression; NSAIDs; Acetaminophen; Selective COX-2 inhibitor

1. Introduction

The analgesic ability of nonsterioid anti-inflammatory drugs (NSAIDs) is due to inhibition of the cyclooxygenase (COX) enzyme, which converts arachidonic acid to the PG precursor, PGH_2. There are two isoforms of the COX enzyme. COX-1 is expressed constitutively and generally produces PGs to modulate physiological processes while COX-2 is inducible and typically produces proinflammatory PGs in response to physiological stresses such as infection and inflammation. These findings provided a rationale for developing selective COX-2 inhibitors, with anti-inflammatory efficacy comparable to traditional NSAIDs and reduced adverse effects,

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particularly on the gastrointestinal (GI) tract. However, two selective COX-2 inhibitors were subsequently withdrawn due to increased risk of adverse thromboembolic events.

Acetaminophen is widely used for pain management and antipyresis as an alternative to aspirin, NSAIDs and selective COX-2 inhibitors, but its action at the molecular level is still poorly defined. Although it does not inhibit COX enzymes at therapeutic concentrations *in vitro*, acetaminophen has been demonstrated to inhibit a variant of COX enzymes *in vivo*. Simmons et al. demonstrated a COX-2 variant which is especially sensitive to acetaminophen (Simmons et al., 1999). Each COX isoform has been reported to be differentially activated according to arachidonic acid concentration: COX-2 is 2- to 4-fold more active than COX-1 at arachidonic acid concentrations below 0.5 μM; COX-1 is more active than COX-2 when arachidonic acid concentration is above 2.5 μM (Swinney et al., 1997; Chen et al., 1999). Acetaminophen is a potent inhibitor of PG synthesis in intact cells at low concentrations of added arachidonic acid, but this effect decreases with increasing concentration, suggesting that it may inhibit COX-2 function (Graham and Scott, 2003). It has also been suggested that a splice variant of COX-1, named as COX-3, is related to the mechanism of action of acetaminophen (Chandrasekharan et al., 2002), but its low expression level with genomic and kinetic analysis indicates that this selective interaction is unlikely to be clinically relevant (Graham and Scott, 2005; Kis et al., 2005).

The surgical removal of impacted third molars is one of the most frequently used methods in clinical trials for evaluating analgesics for management of inflammatory pain of moderate to severe intensity. It is also well suited for the measurement of endogenous markers of inflammation (Hargreaves and Dionne, 1991), evaluating peripheral mechanisms of drug action *in vivo* (Dionne et al., 2001) and has been shown to be sensitive to the effects of NSAIDs (Cooper, 1984; Bjornsson et al., 2003).

In this study, we hypothesized that acetaminophen inhibits peripheral COX enzymes, resulting in decreased PG release. To examine this hypothesis, we evaluated acetaminophen's effect on PG release and the expression patterns of the genes encoding enzymes related to PG production, and compared to the effects of a dual COX-1/-2 inhibitor (ketorolac), a selective COX-2 inhibitor (rofecoxib) and placebo in the oral surgery model.

2. Methods

2.1. Subjects

The study was approved by the Institutional Review Board of the National Institute of Dental and Craniofacial Research and informed consent was obtained from all subjects. A total of 119 healthy subjects aged 16–35 (18.9 ± 3.3) undergoing the surgical removal of two impacted mandibular third molars were evaluated in this study (Table 1). Inclusion and exclusion criteria were similar to those in a previous study (Khan et al., 2002).

Subjects were randomly allocated to one of four treatment groups and administered a dose of blinded medication or placebo: 1000 mg acetaminophen, 50 mg rofecoxib or placebo administered orally 1 h prior to oral surgery; 30 mg ketorolac or placebo was administered intravenously 30 min prior to surgery (Table 2). All drugs and matching placebos were randomized by the Pharmaceutical Development Service of the Clinical Center (National Institutes of Health, Bethesda, MD, USA). The number of subjects in the rofecoxib treatment group is lower than the other groups as no subjects were assigned to rofecoxib after it was withdrawn from the market.

After receiving premedication with intravenous midazolam (4.7 ± 0.9 mg) and local anesthesia with 2% lidocaine (173.8 ± 20.1 mg) with epinephrine 1:100,000, a mucoperiosteal flap was raised and retracted, bone removed, and the teeth were sectioned as needed to facilitate extraction of the impacted lower third molars. Fig. 1 summarizes the study timeline schematically.

Oral mucosal punch biopsies (4 mm, Acu-punch, Acuderm, Inc., Fort Lauderdale, FL, USA) were taken from the planned surgical site prior to surgical extraction for the evaluation of baseline gene expression. For the evaluation of gene expression induced by the surgical injury and medication, subjects within each treatment group were further randomized into two groups for a second biopsy collection done under local anesthesia from another surgical site at an area immediately adjacent to the mucosal incision, either at 3 or at 24 h following surgery (Table 2). The biopsies were immediately frozen in liquid nitrogen and stored at a temperature of −70 °C.

Table 1
Summary of demographic characteristics of the sample population

Treatment group	No. of subjects	Age (year)	Sex (M/F)	Mean time to re-medication (minutes)	No. of subjects medicated	Successful No. of microdialysis collections
Placebo	31	19.5 ± 3.7	15/16	134.6 ± 47.5/18	18/22	17
Acetaminophen	33	18.5 ± 2.6	16/17	139.0 ± 42.3/19	19/21	22
Ketorolac	35	18.5 ± 2.5	13/22	163.6 ± 29.2/10	10/18	22
Rofecoxib	20	19.2 ± 4.5	13/7	142.3 ± 50.2/10	10/15	18
Total	119	18.9 ± 3.3	57/62	145.7 ± 43.0/57	57/76	79

Table 2
Drugs allocated to the treatment groups

Treatment group	Postoperative biopsy time		
	3 hour biopsy group ←→		
	24 hour biopsy group ←→		
	Preoperative drug PO (1 h prior)	Preoperative drug IV (30 min prior)	Postoperative drug PO qid (first 24 h)
Placebo	Placebo	Placebo	Placebo
Acetaminophen	1 gm acetaminophen	Placebo	1 gm acetaminophen
Ketorolac	Placebo	30 mg ketorolac	20 mg ketorolac
Rofecoxib	50 mg rofecoxib	Placebo	Placebo

Subjects randomized to have the post-operative biopsy at 3 h received a standard post-operative analgesic after the biopsy and received follow-up care as needed. If subjects requested pain medication prior to the 3 h biopsy, they were given one dose of 100 mg tramadol, which has no known effect on COX in peripheral tissues. Those subjects randomized to have the post-operative biopsy at 24 h continued to receive the drug that was randomly allocated prior to surgery: ketorolac 20 mg PO Q6H or acetaminophen 1000 mg Q6H (Table 2). The rofecoxib group did not receive any additional doses of this drug as the recommended dosing is once daily (QD), but continued to take matching placebo medication Q6H. The placebo group also received placebo medication Q6H. The final doses of testing drugs were received approximately 2 h prior to the second 24 h biopsy. All subjects in the 24 h biopsy group received an additional supply of tramadol 50 mg to be taken at 4–6 h intervals if needed for pain.

2.2. Microdialysis

Immediately after extraction of two lower third molars, microdialysis probes (CMA/20 Microdialysis Probes, CMA/Microdialysis, North Chelmsford, MA) were placed into both surgical sites for collection of inflammatory transudate. The probe fiber consists of a 10 mm flexible, semipermeable dialysis membrane with a 20,000 Da molecular cutoff. Sterile Ringer's lactate solution is pumped at 10 μL/min and samples collected in opaque vials placed extraorally for 3 h. The vials are changed every 20 min and each sample is assayed in duplicate by enzyme immunoassay for immunoreactive PGE_2 and thromboxane B_2 (TXB_2) (Cayman Chemical Company, Ann Arbor, Mich). Detailed procedures were similar to those in a previous study (Khan et al., 2002). PGE_2 was interpreted as indicative of both COX-1 and COX-2 activity, whereas TXB_2, the stable metabolite of TXA_2, was used as a measure of COX-1 activity. We measured TXB_2 instead of TXA_2 as TXA_2 is rapidly hydrolyzed to the inactive compound TXB_2.

In a successfully collected microdialysis sample set, a total of 9 vials of transudate samples are serially collected from one surgical site and each vial contains approximately 200 μL transudate sample: 120 μL of the sample is assayed to measure the level of PGE_2; 60 μL of sample is assayed to measure the level of TXB_2 as it is usually detected at higher concentrations than PGE_2 and requires less sample volume to stay within the recommended assay concentration range. Therefore, all data used for PGE_2 and TXB_2 analysis originated from the same samples.

2.3. Analysis of gene expression

Total RNA was isolated from frozen tissues using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) protocol. The real-time PCR assays were performed in triplicate for each sample in 96 subjects with eight different sequence-specific primer and TaqMan 6-FAM dye-labeled MGB probe sets (Assay-on-Demand, Applied Biosystems, Foster City, CA, USA). Seven primer and probe sets for genes encoding enzymes related to PG production: COX-1 gene (*PTGS1*), COX-2 gene (*PTGS2*), interleukin-1β gene (*IL1B*), cytosolic PGE synthase gene (*TEBP*, also known as *P*23), microsomal

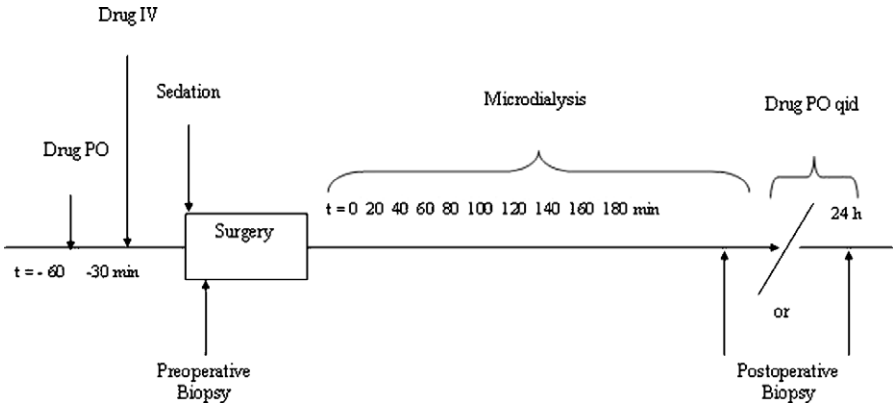

Fig. 1. Schematic of study timeline.

PGE synthase-1 gene (*PTGES*), prostaglandin I_2 (prostacyclin) synthase (*PTGIS*) and thromboxane A synthase 1 (*TBXAS1*) and one set for 18S rRNA as an endogenous control. Relative quantitation of gene expression was determined by standard $2^{(-\Delta\Delta Ct)}$ calculations (Livak and Schmittgen, 2001).

There are two transcriptional variants of thromboxane A synthase: isoform I (TXS-I) and isoform II (TXS-II). TXS-I is the full-length transcript; its encoded protein has thromboxane A synthase activity. However, TXS-II lacks 163 nucleotides between exon 12 and exon 13 of *TBXAS1* which encodes the heme binding site, and its protein product has a distinct C-terminus due to a change in reading frame and does not have thromboxane A synthase activity. Therefore, primers for *TBXAS1* were designed between exon 12 and exon 13 to avoid nonspecific amplification of TXS-II.

2.4. Statistical analysis

Data were analyzed with SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) and statistical significance was set at $p < .05$. Statistical differences between treatment groups for PGE_2 and TXB_2 levels were analyzed by use of repeated-measures ANOVA followed by a Tukey post hoc comparison. To compare levels of gene expression at baseline prior to surgery and either at 3 or at 24 h following surgery, a paired-samples *t*-test was used. A sample size of 20 subjects per group for analysis of PG release and gene expression was calculated on the basis of previous studies that used the oral surgery model (Gordon et al., 2002; Khan et al., 2002).

3. Results

3.1. PG measurements in microdialysis

We could not compare the PGE_2 and TXB_2 level change to the baseline values since it was not possible to collect microdialysis samples before tooth removal. Hence, the first evaluation of PGE_2 and TXB_2 levels was at 20 min post-surgery when comparatively high levels of both PGE_2 and TXB_2 levels were seen in placebo, acetaminophen, and rofecoxib groups compared to placebo control (Figs. 2A and B). Ketorolac suppressed both PGE_2 and TXB_2 levels to nearly unmeasurable levels through the 3 h microdialysis period.

In the placebo, acetaminophen, and rofecoxib groups, PGE_2 release at the site of injury in the placebo group gradually decreased during the first hour, and then increased over time (Fig. 2A). PGE_2 levels after ketorolac, rofecoxib and acetaminophen administration were significantly suppressed compared to placebo ($p < .05$). Ketorolac suppressed PGE_2 levels through the post-operative period; rofecoxib and acetaminophen had no effects on PGE_2 levels at the earlier time points (<60 min) but suppressed PGE_2 levels at the later time points (80–180 min; $p < .05$). PGE_2 suppression by acetaminophen was less than ketorolac ($p < .05$) but comparable to rofecoxib.

TXB_2 levels, used as a measure of COX-1 activity, were maximal at 20 min following surgery in the placebo, acetaminophen, and rofecoxib groups, then gradually decreased during the 180 min after surgery (Fig. 2B). Compared to placebo, TXB_2 levels were significantly suppressed only by ketorolac ($p < .01$). The

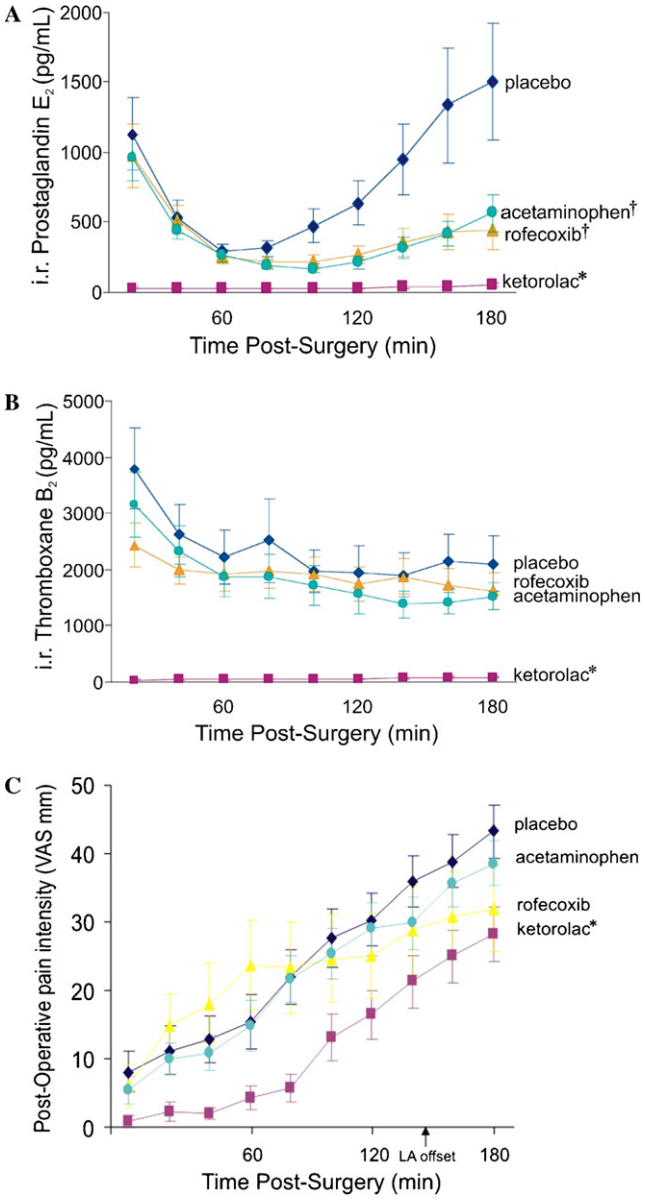

Fig. 2. Effects of pretreatment with acetaminophen, ketorolac, rofecoxib or placebo on levels of immunoreactive (i.r.) PGE_2 (A), TXB_2 (B) and pain intensity (C). (A) PGE_2 levels; PGE_2 suppression by acetaminophen was less than ketorolac but comparable to rofecoxib. *Ketorolac suppressed PGE_2 levels through the post-operative period; †rofecoxib and †acetaminophen had no effects on PGE_2 levels at the earlier time points (<60 min) but suppressed PGE_2 levels at the later time points (80–180 min). (B) TXB_2 levels; TXB_2 was suppressed only by *ketorolac. (C) Pain ratings. *Ketorolac group showed lower pain intensity compared to placebo group after the local anesthetics offset while rofecoxib and acetaminophen groups rated the pain intensity in between. LA: local anesthetics Error bars: standard error of means.

effects of rofecoxib and acetaminophen on TXB_2 levels did not differ from that of placebo.

3.2. Clinical pain ratings

During the 3 h post-operative period, pain intensity gradually increased in all four groups (Fig. 2C). Remaining local anesthetic effect started to wear off with the change of lip numbness to a tingling sensation after the surgery (mean 133.5 min; 95% CI, 126–141) and patients requested medication (mean 146 min; 95% CI, 138–154). At 3 h following surgery, significant difference was found in pain intensity among four groups (ANOVA, $p = .047$). Pain intensity at 3 h was higher in placebo group (mean 43.6; 95% CI, 35.8–51.5) compared to ketorolac group (mean 28.6; 95% CI, 20.3–36.8). The acetaminophen group (mean 38.9; 95% CI, 32.2–40.2) and the rofecoxib group (mean 32.3, 95% CI, 19.1–45.4) rated their pain intensity in between. However, the percentage of patients who took rescue medicine was inversely related to pain at 3 h: placebo (58.1%), acetaminophen (57.5%), rofecoxib (50%) and ketorolac (28.6%) group.

3.3. Expression of genes encoding enzymes related to PG production

No subjects were assigned to rofecoxib after it was withdrawn, resulting in only nine biopsy samples at 3 h and 11 samples at 24 h. We first analyzed gene expression changes compared to baseline levels in the pre-operative samples. Overall, changes in gene expression from baseline appear to represent the inflammatory response and its modulation by drug administration. At 3 h following surgery, *PTGS1* expression was slightly decreased ($p < .01$) compared to pre-surgical levels in all groups (non-significantly for rofecoxib) while *PTGS2* markedly increased ($p < .01$) in all groups (Fig. 3A). *PTGS2* expression remained elevated in the ketorolac and acetaminophen groups at 24 h (Fig. 3B) but decreased towards baseline expression in the rofecoxib and placebo groups. *PTGS1* expression remained

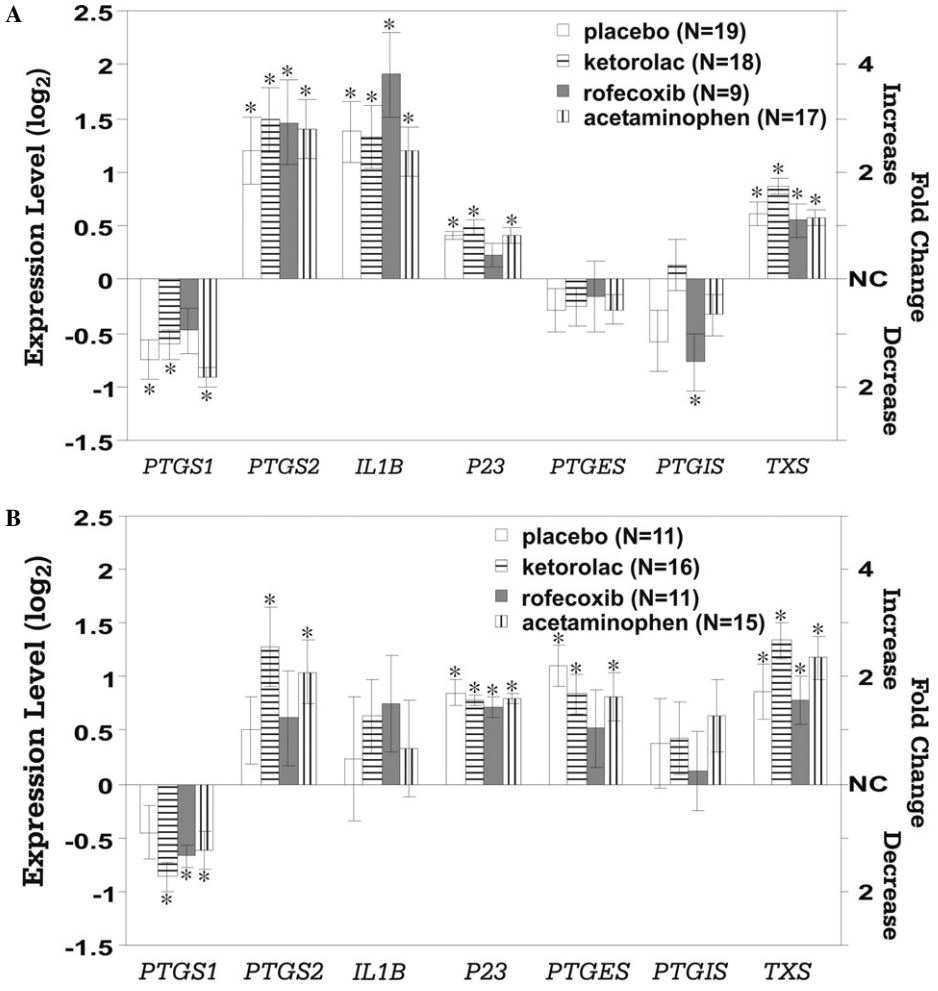

Fig. 3. Effects of treatment with 1000 mg acetaminophen, 30 mg ketorolac or placebo on gene expression levels at 3 h (A) and at 24 h (B) following surgery. $^*p < .01$ compared to pre-surgical levels of each gene. Error bars: standard error of means.

slightly below baseline at 24 h in the three drug groups (Fig. 3B). *IL1B* expression was significantly elevated in all groups at 3 h ($p < .01$) but returned to pre-surgical levels by 24 h. *P*23 and *TBXAS1* expression increased at 3 and 24 h ($p < .01$), but there was no difference in the gene expression pattern among the treatment groups. *PTGES* and *PTGIS* did not change appreciably by 3 h, but *PTGES* was significantly increased at 24 h in all groups except for the rofecoxib group ($p < .01$). Rofecoxib did not have a detectable effect on *PTGES* or *PTGIS* expression other than a significant decrease in *PTGIS* at 3 h.

Comparison of gene expression changes among treatment groups revealed that ketorolac induced significantly higher expression of *P*23 and *TBXAS1* than rofecoxib at 3 h post-operatively ($p < .05$). The down-regulation in PTGIS gene expression at 3 h by rofecoxib was also significantly different ($p < .05$) from the ketorolac effect. Gene expression among the drug treatments and placebo did not differ for the other genes at 3 h or for any of the genes at 24 h.

Total amount of rescue drug (tramadol) medication in placebo group was significantly ($p < .05$) greater (mean 175.0 mg, 95% CI, 123.7–226.3) than in the ketorolac group (mean 108.8 mg, 95% CI, 66.9–150.7). Tramadol use was similar for the rofecoxib group (mean 113.6 mg, 95% CI, 97.9–129.3) and the acetaminophen group (mean 115.6 mg, 95% CI, 67.3–164.0).

4. Discussion

The time course of PG production in this clinical model of acute inflammation is consistent with early synthesis by constitutive COX-1 followed by induction of COX-2 leading to enhanced PG production (Gordon et al., 2002; Khan et al., 2002). These observations are supported by the time course of PG production in the placebo group, reduced PGE_2 and TXB_2 levels by the non-selective NSAID ketorolac and selective suppression of PGE_2 but not TXB_2 at later time points by rofecoxib. Acetaminophen suppressed PGE_2 release at time points associated with COX-2 activation similar to rofecoxib without affecting TXB_2 release, used as a measure of COX-1 activity, or thromboxane A synthase expression. This suggests that acetaminophen inhibits COX-2 function *in vivo* and that its analgesic effect, at least in part, may be attributed to decreased peripheral PGE_2 release in addition to centrally mediated analgesic effects of acetaminophen (Bjornsson et al., 2003). It is also suggested that the analgesic effect of acetaminophen is due to activation of descending serotonergic pathways (Pelissier et al., 1996; Alloui et al., 2002; Chen and Bazan, 2003), but that its primary site of action may still be inhibition of PG synthesis (Muth-Selbach et al., 1999; Botting and Ayoub, 2005). The action of acetaminophen could be related to the production of reactive metabolites by the peroxidase function of COX-2, which could deplete glutathione, a cofactor of enzymes such as PGE synthase (Bonnefont et al., 2003; Graham and Scott, 2005). Considering the similar behavior of acetaminophen and rofecoxib on PGE_2 and TXB_2 levels following surgery, these data indicate that acetaminophen influences peripheral COX-2 function during acute inflammation similar to rofecoxib.

The effects of the dual COX-1/-2 inhibitor ketorolac on gene expression of *PTGS1* and *PTGS2* are similar to a previous report (Khan et al. submitted) with elevated expression in biopsies collected in the immediate post-operation and at 24 h. Similarly, acetaminophen slightly decreased *PTGS1* expression ($p < .01$) and markedly increased *PTGS2* ($p < .01$) at both 3 and 24 h. Rofecoxib induced similar changes in gene expression for *PTGS1* at both 3 and 24 h, and for *PTGS2* at 3 h. However, rofecoxib's effect on *PTGS2* expression was similar to placebo at 24 h in this study. A previous study (Lee et al., 2006) showed that repeated rofecoxib administration increased *PTGS2* expression at 48 h compared to placebo. The non-significant effect in the present study may have been due to the small sample size following rofecoxib's withdrawal and 24 h that elapsed from the pre-operative dose and biopsy without any additional rofecoxib administration, only indicated for one dose per 24 h. In general, these data suggest that continued administration of COX inhibitory drugs increases *PTGS2* expression, possibly altering the analgesic and adverse effects of these drugs over time. While it is generally accepted that a single or repeated doses of acetaminophen have no effect on the cardiovascular or respiratory system, platelet function or coagulation (Brunton et al., 2006), the similarity of acetaminophen's actions on *PTGS1* and *PTGS2* to the effects of rofecoxib during acute inflammation is suggestive of the need for further investigation.

An inducible form of PGE synthase, microsomal PGE synthase-1 (mPGES-1), has been demonstrated *in vitro* to be functionally coupled with COX-2 (Jakobsson et al., 1999) and this coupling is crucial for the delayed synthesis of PGE_2 in response to proinflammatory stimuli (Dieter et al., 2000; Murakami et al., 2000). In contrast to mPGES-1, cytosolic PGE synthase (cPGES) is functionally coupled with COX-1 (Tanioka et al., 2000) and is responsible for basal synthesis of PGE_2 and the immediate release of PGE_2. However, expression level of mPGES-1 gene (*PTGES*) in this study was not changed while *PTGS2* expression was significantly increased at early time point, but was significantly increased at later time point except for the rofecoxib group. Conversely, expression level of cPGES gene (*P*23) was significantly increased at early time point and even more at later time point except for the rofecoxib group. This finding suggests that the COX-1-cPGES coupling may be crucial for the immediate early synthesis

of PG while the COX-2-mPGES-1 coupling may account for the delayed synthesis of PG in response to inflammatory stimuli.

COX-1 and COX-2 have a common function, at least in part (Breder et al., 1995; Wilborn et al., 1995; Diaz et al., 1998), to produce PGE_2. The increase in COX-2 activity and *PTGS2* expression after the surgical injury was paralleled by a decrease of *PTGS1* expression. In general, increased gene expression in response to pharmacologic inhibition of the encoding enzymes is consistent with an inverse relationship between the product of enzymatic activity and the expression level of the encoding genes. While there are other mechanisms interacting molecularly with the COX-2 pathway such as iNOS (Kim et al., 2005), the reciprocal relationship between COX-1 and COX-2 gene expression may be modulated by inhibition of their activity by administration of COX inhibitory drugs.

Surgical injury increases the expression of the inducer of COX-2, *IL1B*. At both early and later time points, *IL1B* expression showed a significant positive correlation with *PTGS2* expression (data not shown), suggesting that IL-1β is the inducer of COX-2 upregulation not only at central sites (Samad et al., 2001) but also in the periphery.

Although TXB_2 levels gradually decreased in the placebo group during the 3 h after surgery, expression level of thromboxane A synthase 1 gene (*TBXAS1*) was significantly increased at 3 h and even more in the 24 h biopsy. The arachidonic acid endogenously released via phospholipase catalyses is usually less than 1 μM (Shitashige et al., 1998; Chen et al., 1999) and its intracellular concentration is limited. Therefore, lower expression of *TBXAS1* may be due to the lack of arachidonic acid as inflammatory cascade progresses in addition to the decreased expression of *COX-1* as demonstrated in the 3 h biopsies.

Pain intensity induced by the surgery during the first 3 h period increased gradually in all four groups as the effects of the local anesthetics dissipated. The pain rating at 3 h after surgery was minimally confounded by the residual local anesthetic effects and demonstrated difference between ketorolac and placebo groups in pain intensity. Ketorolac suppressed both TXB_2, a product of COX-1, and PGE_2, produced by both COX-1 and COX-2 coincident with lower levels of pain as the local anesthetic wore off and the underlying inflammatory pain was being reflected in the pain reported by subjects. Considering the mean of the onset time of the post-operative pain was 146 min, it is not surprising that the effects of medication on pain intensity during most of the 3 h period are not consistent with the level of PGE_2 at the surgery site. Over the last two observations taken after the anesthetics offset, acetaminophen reduced PGE_2 level as much as rofecoxib and had a similar effect on pain, although both were well differentiated from placebo.

Because we used standard $2^{(-\Delta\Delta Ct)}$ calculations for the relative quantitation compared to 18S rRNA, our result suggests that gene expression at the surgery site changes regardless of increasing cell numbers or infiltration of specific cell types. As gene expression prior to surgery was compared to that after surgery, infiltration of inflammatory cells could contribute to the increased expression of *PTGS2*. However, some patients showing decreased expression of *PTGS2* following surgery which indicated there must be other factors contributing to *PTGS2* expression. Here, we can only say the expression pattern changed following surgery and medication but we cannot say how much infiltrated inflammatory cells contribute to this change.

Peripheral PG release and related changes in gene expressions cannot fully explain diversity of clinical responses to analgesic medications as acetaminophen (Bonnefont et al., 2003; Altman, 2004), NSAIDs (Bjorkman, 1995) and selective COX-2 inhibitors (Ibuki et al., 2003; Muller et al., 2004) which also have central effects. Conversely, peripherally mediated effects by NSAIDs and coxibs on both COX-dependent and COX-independent pathways may provide an alternative explanation for adverse effects associated with these drug classes. Tissue inhibitor of metalloproteinase (TIMP) level, for example, is significantly associated with myocardial infarction (Cavusoglu et al., 2006) and rofecoxib alters the expression of matrix metalloproteinase pathway genes including TIMP (Wang et al., 2006). Further studies are necessary to investigate the effect of long-term administration of acetaminophen on PG release and *PTGS2* expression and determine whether inhibition of COX-2 function by acetaminophen results in similar effects that are attributed to COX-2 inhibition by NSAIDs and coxibs (Kearney et al., 2006).

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References

Alloui A, Chassaing C, Schmidt J, Ardid D, Dubray C, Cloarec A, et al. Paracetamol exerts a spinal, tropisetron-reversible, antinociceptive effect in an inflammatory pain model in rats. Eur J Pharmacol 2002;443:71–7.

Altman RD. A rationale for combining acetaminophen and NSAIDs for mild-to-moderate pain. Clin Exp Rheumatol 2004;22:110–7.

Bjorkman R. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Experimental studies in the rat. Acta Anaesthesiol Scand Suppl 1995;103:1–44.

Bjornsson GA, Haanaes HR, Skoglund LA. A randomized, double-blind crossover trial of paracetamol 1000 mg four times daily vs

ibuprofen 600 mg: effect on swelling and other postoperative events after third molar surgery. Br J Clin Pharmacol 2003;55:405–12.

Bonnefont J, Courade JP, Alloui A, Eschalier A. Antinociceptive mechanism of action of paracetamol. Drugs 2003;63(Spec. No. 2):1–4.

Botting R, Ayoub SS. COX-3 and the mechanism of action of paracetamol/acetaminophen. Prostaglandins Leukot Essent Fatty Acids 2005;72:85–7.

Breder CD, Dewitt D, Kraig RP. Characterization of inducible cyclooxygenase in rat brain. J Comp Neurol 1995;355:296–315.

Brunton L, Lazo J, Parker K. Goodman & Gilman's Teh pharmacological basis of therapeutics. New York: McGraw-Hill Professional; 2006.

Cavusoglu E, Ruwende C, Chopra V, Yanamadala S, Eng C, Clark LT, et al. Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction. Am Heart J 2006;151:1101e1–8e1.

Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci USA 2002;99:13926–31.

Chen C, Bazan NG. Acetaminophen modifies hippocampal synaptic plasticity via a presynaptic 5-HT2 receptor. Neuroreport 2003;14:743–7.

Chen W, Pawelek TR, Kulmacz RJ. Hydroperoxide dependence and cooperative cyclooxygenase kinetics in prostaglandin H synthase-1 and -2. J Biol Chem 1999;274:20301–6.

Cooper SA. Five studies on ibuprofen for postsurgical dental pain. Am J Med 1984;77:70–7.

Diaz A, Chepenik KP, Korn JH, Reginato AM, Jimenez SA. Differential regulation of cyclooxygenases 1 and 2 by interleukin-1 beta, tumor necrosis factor-alpha, and transforming growth factor-beta 1 in human lung fibroblasts. Exp Cell Res 1998;241:222–9.

Dieter P, Scheibe R, Jakobsson PJ, Watanabe K, Kolada A, Kamionka S. Functional coupling of cyclooxygenase 1 and 2 to discrete prostanoid synthases in liver macrophages. Biochem Biophys Res Commun 2000;276:488–92.

Dionne RA, Lepinski AM, Gordon SM, Jaber L, Brahim JS, Hargreaves KM. Analgesic effects of peripherally administered opioids in clinical models of acute and chronic inflammation. Clin Pharmacol Ther 2001;70:66–73.

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 2002;72:175–83.

Graham GG, Scott KF. Mechanisms of action of paracetamol and related analgesics. Inflammopharmacology 2003;11:401–13.

Graham GG, Scott KF. Mechanism of action of paracetamol. Am J Ther 2005;12:46–55.

Hargreaves KM, Dionne RA. Evaluating endogenous mediators of pain and analgesia in clinical studies. Adv Pain Res Ther 1991;18:579–97.

Ibuki T, Matsumura K, Yamazaki Y, Nozaki T, Tanaka Y, Kobayashi S. Cyclooxygenase-2 is induced in the endothelial cells throughout the central nervous system during carrageenan-induced hind paw inflammation; its possible role in hyperalgesia. J Neurochem 2003;86:318–28.

Jakobsson PJ, Thoren S, Morgenstern R, Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci USA 1999;96:7220–5.

Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. BMJ 2006;332:1302–8.

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 2002;72:44–9.

Kim SF, Huri DA, Snyder SH. Inducible nitric oxide synthase binds, *S*-nitrosylates, and activates cyclooxygenase-2. Science 2005;310:1966–70.

Kis B, Snipes JA, Busija DW. Acetaminophen and the cyclooxygenase-3 puzzle: sorting out facts, fictions, and uncertainties. J Pharmacol Exp Ther 2005;315:1–7.

Lee Y, Kim H, Wu T, Wang X, Dionne RA. Genetically mediated inter-individual variation in analgesic responses to COX inhibitory drugs. Clin Pharmacol Ther 2006;79:407–18.

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402–8.

Muller N, Riedel M, Schwarz MJ. Psychotropic effects of COX-2 inhibitors—a possible new approach for the treatment of psychiatric disorders. Pharmacopsychiatry 2004;37:266–9.

Murakami M, Naraba H, Tanioka T, Semmyo N, Nakatani Y, Kojima F, et al. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. J Biol Chem 2000;275:32783–92.

Muth-Selbach US, Tegeder I, Brune K, Geisslinger G. Acetaminophen inhibits spinal prostaglandin E2 release after peripheral noxious stimulation. Anesthesiology 1999;91:231–9.

Pelissier T, Alloui A, Caussade F, Dubray C, Cloarec A, Lavarenne J, et al. Paracetamol exerts a spinal antinociceptive effect involving an indirect interaction with 5-hydroxytryptamine3 receptors: in vivo and in vitro evidence. J Pharmacol Exp Ther 1996;278:8–14.

Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, et al. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 2001;410:471–5.

Shitashige M, Morita I, Murota S. Different substrate utilization between prostaglandin endoperoxide H synthase-1 and -2 in NIH3T3 fibroblasts. Biochim Biophys Acta 1998;1389:57–66.

Simmons DL, Botting RM, Robertson PM, Madsen ML, Vane JR. Induction of an acetaminophen-sensitive cyclooxygenase with reduced sensitivity to nonsteroid antiinflammatory drugs. Proc Natl Acad Sci USA 1999;96:3275–80.

Swinney DC, Mak AY, Barnett J, Ramesha CS. Differential allosteric regulation of prostaglandin H synthase 1 and 2 by arachidonic acid. J Biol Chem 1997;272:12393–8.

Tanioka T, Nakatani Y, Semmyo N, Murakami M, Kudo I. Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. J Biol Chem 2000;275:32775–82.

Wang XM, Wu TX, Lee YS, Dionne RA. Rofecoxib regulates the expression of genes related to the matrix metalloproteinase pathway in humans: implication for the adverse effects of cyclooxygenase-2 inhibitors. Clin Pharmacol Ther 2006;79:303–15.

Wilborn J, DeWitt DL, Peters-Golden M. Expression and role of cyclooxygenase isoforms in alveolar and peritoneal macrophages. Am J Physiol 1995;268:L294–301.