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Effects of Bupivacaine versus Lidocaine on Inflammatory Regulation

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Abstract

Background and Objectives: Postoperative pain is common following surgery. Perioperative administration of long-acting local anesthetics like bupivacaine can inhibit central sensitization and decrease pain but may cause inflammation that prolongs both pain and recovery. This study evaluated the effects of the local anesthetics bupivacaine versus lidocaine on inflammatory gene and protein expression and postoperative pain.

Methods: We stimulated cultured whole blood with saline, anesthetic (bupivacaine or lidocaine), or lipopolysaccharide to determine the effect of anesthetics on inflammation *ex vivo*. We also conducted an exploratory, prospective, randomized, double-blind clinical trial in which subjects undergoing root-end endodontic surgery received 2% lidocaine or 0.5% bupivacaine, both with 1:200,000 epinephrine. Biopsies were obtained before as well as immediately following and 48 h after surgery for evaluation of gene expression. Subjects rated pain intensity using a visual analog scale up to 48 h after surgery.

Results: Compared to controls, bupivacaine but not lidocaine elevated protein levels of prostaglandin E2 but no other inflammatory mediators *ex vivo*. In the exploratory clinical study, analyses revealed differential gene expression between treatment groups. Significant differences in baseline-normalized gene expression levels between groups occurred for tyrosinase-related protein 1 and sphingosine kinase 1 immediately following surgery ($p<0.001$) and matrix metalloproteinase 1 at 48 h after surgery ($p<0.0001$). There was a trend for higher subject-reported pain in the bupivacaine group at 48 h ($p=0.080$).

Conclusions: Our results confirm that bupivacaine increased certain inflammatory mediators, which may increase postoperative pain after surgery. Further studies assessing alternate bupivacaine formulations or different pain conditions are needed.

Keywords: Bupivacaine; Inflammation; Local Anesthetics; Pain; Peripheral Sensitization

Introduction

Postoperative pain is common following dental surgery. Pain from surgical incisions and tissue manipulation begins immediately but gives way to pain caused by cell recruitment to the injury over several hours, which typically peaks on the day of the operation after dissipation of the local anesthetic. Tissue injury instigated by surgery activates the inflammatory cascade by increasing expression of proinflammatory cytokines and cyclooxygenase 2 (COX-2), thereby leading to central and peripheral sensitization [1,2]. Therefore, a primary strategy for reducing postoperative pain involves proactively inhibiting postoperative pain through administration of long-lasting anesthetics during the perioperative period [3-6].

Bupivacaine is a long-lasting anesthetic recommended following surgical procedures projected to cause prolonged pain [7] and is associated with a decreased need for opioids to manage breakthrough pain [1,3,4]. However, several lines of evidence

have prompted concern regarding the use of bupivacaine. First, there are conflicting reports regarding bupivacaine's influence on analgesic use in clinical practice [4,8]. Second, animal and human studies have demonstrated that bupivacaine can cause local tissue reactions and proinflammatory effects that prolong healing [9-14] and result in rebound hyperalgesia [15]. Finally, bupivacaine has a narrower safety margin than lidocaine and has been implicated in several FDA complaints [16].

Therefore, the purpose of this study was to evaluate the effects of the long-acting local anesthetic bupivacaine as compared with lidocaine on inflammation and pain. We hypothesized that bupivacaine promotes local inflammation, leading to increased postoperative pain.

Methods

Study Conduct

This study was approved by the Institutional Review Board of the University of Maryland, Baltimore, and written informed consent was obtained from all subjects prior to participation in the study. The trial was registered in Clinicaltrials.gov (NCT01060774) on 31 January 2010.

Ex-Vivo Study

Because cell culture allows evaluation of human cytokine production in a complex environment without the variability introduced by surgery, we used a whole blood stimulation assay to determine the effect of the 2 anesthetics on inflammatory cascade protein levels *ex vivo*. We stimulated whole blood with physiologically relevant concentrations of saline (negative control), anesthetic bupivacaine (Hospira Inc, Lake Forest, Illinois) or lidocaine (Astra Pharmaceuticals Inc, Worcester, MA), or lipopolysaccharide (LPS; *InvivoGen,* San Diego, CA) as a positive control. Anesthetics tested included commercially available, medical-grade multi-use vials (2% lidocaine/1:200,000 epinephrine or 0.5% bupivacaine/1:200,000 epinephrine) and dental cartridges (2% lidocaine/1:100,000 epinephrine or 0.5% bupivacaine/1:200,000 epinephrine).

Whole blood cell culture: Blood was collected from healthy adult volunteers free of systemic disease and who had not taken medications during the preceding 2 weeks that could alter immune response. Whole blood cell cultures were performed using a commercially available assay (K_2 EDTA tubes; Vacutainer; BD, Franklin Lakes, NJ). Briefly, venous blood was collected by venipuncture and cultured at 37°C for 24 h under one of 4 stimulation conditions: physiologic saline, LPS (10 µg/mL), bupivacaine (9 mg/mL) with epinephrine 1:200,000, and lidocaine (18 mg/mL) with epinephrine 1:200,000. Physiologically relevant concentrations of the local anesthetics were derived from published maximum blood and tissue levels of lidocaine after applications in dentistry [17], and optimal LPS concentrations were determined empirically. For experiments evaluating the contribution of stimulant pH to inflammation, the pH of the stimulants was adjusted to equivalence using concentrated hydrochloric acid. Following culturing, blood samples were centrifuged and plasma decanted and stored at -70°C for analysis.

Protein Quantification: Total protein concentrations were determined via the Bradford Assay (BCA Protein Assay Kit, Pierce Biotechnology, Rockford, IL) according to manufacturer's instructions and read by spectrophotometry ($Spectra_{Max}$ M5, $V_{max}^{®}$ microplate spectrophotometer, Molecular Devices, Sunnyvale, CA). Sample concentrations were adjusted for total protein content.

Prostaglandin E2 (PGE2) levels were measured in plasma using a commercially available Enzyme-Linked Immune Sorbent Assay (ELISA) kit (PGE2 EIA Kit-Monoclonal, ACE, Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. ELISA plates were read using spectrophotometry ($V_{max}^{®}$ microplate spectrophotometer, Molecular Devices, Sunnyvale, CA). Data acquisition was done using User Bulletin #2 software (v1.6, Applied Biosystems).

Levels of COX-2, interleukin (IL)-1ß, IL-6, interferon (IFN)-γ, and tumor necrosis factor (TNF) α were quantified in duplicate using a multiplex enzyme immunoassay according to manufacturer's instructions (Searchlight Inflammatory Cytokine Array 2, Pierce Biotechnology, Rockford, IL). Samples were imaged by chemiluminescence (FluoroChem 8900, Alpha Innotech Corp, San Leandro, CA), and image analysis was performed using Array Vision Evaluation 8.0 software (GE Healthcare Life Science, Buckinghamshire, United Kingdom).

Exploratory Clinical Study

Study Design and Subject Selection: The exploratory clinical study was a prospective, randomized, double-blinded clinical trial of bupivacaine versus lidocaine anesthesia following endodontic surgery. Male and female subjects with the clinical indication for endodontic surgery who met eligibility criteria were invited to participate in the study (see Supplemental Information). To ensure consistency in surgical difficulty, treatment was limited to root-end resection surgeries. All subjects received the same preoperative local anesthesia (2% lidocaine/1:50,000 or 1:100,000 epinephrine) but were assigned to one of 2 treatment groups for intra- and postoperative anesthesia: 2% lidocaine/1:200,000 epinephrine or 0.5% bupivacaine/1:200,000 epinephrine. The study's primary endpoint was the difference in inflammatory gene expression between treatment groups, and the secondary endpoint was subject-reported pain measured at 48 h after surgery.

Study Procedures: Study procedures are summarized (Figure 1). Preoperative local anesthetics (2% lidocaine/1:50,000 and 1:100,000 epinephrine) were used per standard clinical practice, and subjects were tested for positive lip sign for mandibular anesthesia and for no response to a sharp explorer on soft tissue for

maxillary anesthesia. Following satisfactory local anesthesia, a baseline preoperative tissue biopsy was taken and surgery performed. Surgical treatments were performed by endodontic residents under double-blind conditions. For the purpose of consistency and to ensure adequate anesthetization for biopsy and suturing, blinded intra-operative local anesthetic (1/2 of a 1.7 cc carpule) was administered to all patients for reinforcement of anesthesia to complete the surgical procedure. Prior to suturing, a second biopsy was obtained to assess inflammation resulting from the surgical procedure. The location of the punch biopsy was not specified, because the location was dependent upon the location of the tooth undergoing the procedure.

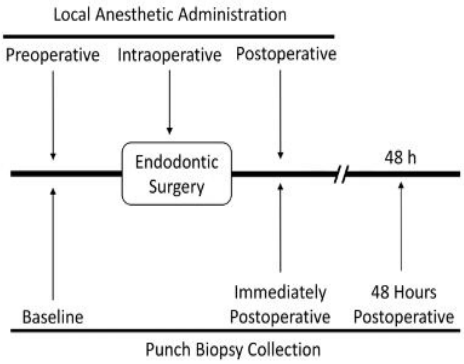


Figure 1: Study procedures. Prior to endodontic surgery, all subjects in the study underwent identical preoperative local anesthesia and baseline tissue biopsy. Blinded intra- and postoperative local anesthetics were given based on the subject's randomly assigned treatment group; additional tissue biopsies were taken immediately following surgery and 48 h later.

The location could have been on either side of the operated tooth. After suturing, the quadrant was injected via local infiltration with the blinded anesthetic (i.e., lidocaine or bupivacaine). Subjects were observed for 30 minutes after surgery and then discharged home with postoperative instructions, acetaminophen, and a prescription for oxycodone for breakthrough pain. Subjects recorded analgesic use and pain intensity in diaries at the initial onset of pain and at 24 and 48 h after surgery. At 48 h, they returned for a third tissue biopsy (performed under anesthesia with 2% lidocaine/1:100,000 epinephrine) and to return completed diaries. The third tissue biopsy was taken to measure the local anesthetic effect on tissue inflammation. All biopsies were 3 mm in diameter.

Tissue Analyses: Total RNA was extracted from homogenized biopsies using TRIzol reagent (Roche, Switzerland) per the manufacturer's instructions, and RNA precipitation and purification was done using an RNeasy kit (Qiagen, Valencia, CA). RNA concentrations were determined using Experion chips (Bio-Rad Laboratories, Hercules, CA), and isolated RNA was stored at -80°C for analysis.

Microarray analysis was performed by the UMB Genomics/Proteomics core facility. The Gene Chip Human Gene 1.0 ST array (Affymetrix, Santa Clara, CA) was used according to the manufacturer's instructions to assess changes in gene expression in tissue biopsies. Microarrays were scanned on a Gene Pix 4000B scanner (Axon Instruments). Acquired images were recorded as paired 16-bit TIFFs, and data extraction was performed with Axon Gene Pix Pro 4 software. Each array was normalized using an intensity-dependent locally weighted scatter plot smoothing regression analysis implemented in the TIGR Microarray Data Analysis System software package; gene expression data were normalized by the robust multichip average method.

To validate microarray data, real-time RT-PCR was performed on an ABI Prism 7700 Sequence Detection System. PCR primers were selected for specificity by NCBI BLAST of the human genome, and amplicon specificity was verified by first derivative melting curve analysis (Perkin-Elmer/Applied Biosystems software). Quantization and normalization of relative gene expression were done using the comparative threshold cycle method.

Analyses of Clinical End points: Pain intensity was assessed using a 100-mm visual analog scale (anchors of "No Pain" and "Worst Pain Imaginable") and a 4-point category scale (none, mild, moderate, and severe) at baseline, at pain onset, and at 24 and 48 h after surgery. A pill count at the 48 h return visit was done to assess analgesic intake, and reported and observed adverse effect frequencies were recorded.

Statistical Analysis

SPSS (version 16.0; Chicago, IL) was used for data analysis. For the *ex vivo* study, significant differences among treatment groups were tested via one-way Analysis of Variance (ANOVA) and effects of pH via two-way ANOVA. For the exploratory clinical study, sample size was estimated based on the projected COX-2 gene expression difference between the groups (1:1 allocation ratio and equal group sizes) and prior data [9]. Sixty subjects were planned for the study to permit statistical significance assessment at a 5% alpha level with 80% power. Population, demographic, baseline, and safety data were analyzed descriptively by treatment group. Although no between-group differences in baseline characteristics were expected, t- and chi square tests were performed as appropriate to confirm treatment group comparability. The primary endpoint was assessed using the significance analysis of microarrays method with a false discovery rate of ≤5%, followed by EDGE or Ingenuity (Ingenuity Systems, Redwood City, CA) analysis. Secondary endpoint analysis of the visual analog pain scale used the Summed Pain Intensity Difference (SPID) scores, calculated by subtracting the baseline pain rating from each pain rating and summing the values, were summarized descriptively. Differences between treatment groups were tested using a t-test, and p-values were calculated along with 95% confidence intervals. Postoperative pain ratings generally exhibited normality and equality of variance. For all statistical tests, a p-value of <0.05 was considered statistically significant; where applicable, use of more stringent statistical criteria to minimize the risk of error from mul-

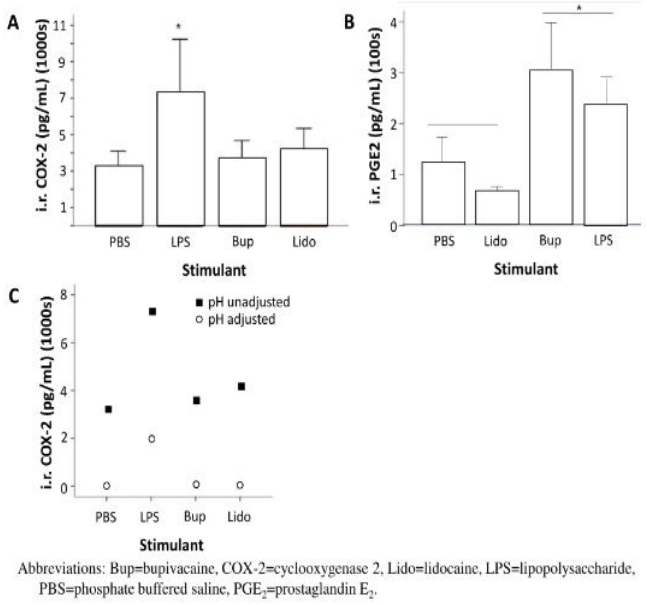
tiple comparisons is indicated in the text.

Results

Ex-Vivo Study

As expected, stimulation of whole blood with the positive control LPS increased protein concentrations of COX-2 ($p<0.001$; Figure 2A), PGE2 ($p<0.001$; Figure 2B), and other inflammatory cytokines (data not shown) compared to saline. Bupivacaine, but not lidocaine, elevated PGE2 protein levels (Figure 2B); there were no significant differences between the anesthetics with respect to other inflammatory mediators.

Because pH may affect the extent of an inflammatory response, we next examined the pH of the local anesthetics and found them to be within the manufacturer's reported pH range (Table 1). We then adjusted the pH of all stimulants to be equivalent and repeated the experiments described above for COX-2. Although pH-adjusted stimulants induced significantly less COX-2 expression than did non-adjusted stimulants ($p<0.05$), the overall pattern of stimulant-induced changes persisted after pH adjustment (Figure 2C); thus, small differences in pH were unlikely to be a primary contributor to bupivacaine's effects on PGE2 protein levels.



Abbreviations: Bup=bupivacaine, COX-2=cyclooxygenase 2, Lido=lidocaine, LPS=lipopolysaccharide, PBS=phosphate buffered saline, PGE_2=prostaglandin E_2.

Figure 2A-C: Effect of anesthetics on inflammatory cascade protein levels in *ex vivo* whole blood cultures. **(A)** Stimulation of whole blood cultures with pH-unadjusted LPS, but not saline, bupivacaine, or lidocaine, significantly elevated COX-2 protein levels ($p<0.001$). **(B)** Stimulation of whole blood cultures with pH-unadjusted LPS and bupivacaine, but not saline or lidocaine, significantly elevated PGE2 protein levels ($p<0.001$). **(C)** pH-adjusted stimulants induced significantly less COX-2 expression than did non-adjusted stimulants ($p<0.05$). However, the effects of stimulants on COX-2 levels remained proportionate with significant LPS-induced COX-2 production. Data are plotted as mean ± standard deviation.

	Treatment Group		
Parameter	**Lidocaine**	**Bupivacaine**	**P-value**
Demographic Characteristics			
Age, years			0.139
Median (range)	43.5 (33-51)	50 (40-58)	
Mean (SD)	43.3 (6.5)	49.8 (6.6)	
Sex, n (%)			0.376
Female	2 (33.3)	3 (60.0)	
Male	4 (66.7)	2 (40.0)	
Race, n (%)			0.355
White	2 (33.3)	4 (80.0)	
Black	2 (33.3)	0 (0)	
Hispanic	1 (16.7)	1 (20.0)	
Unknown	1 (16.7)	0 (0)	
Weight, lbs	194.3 (52.2)	164 (22.3)	0.26
Height, in	68.75 (4.5)	66.2 (3.3)	0.319
Surgical Variables			
Measured Anesthetic pH[a]			
Medical vial	4.26±0.064	3.63±0.047	
Dental cartridge	3.57±0.14	3.91±0.062	
Total clinical lidocaine volume, mL	10.34 (3.01)	9.52 (2.58)	0.643
Lidocaine volume 1:50,000 epi, mL	2.67 (1.25)	2.6 (0.89)	0.703
Lidocaine volume 1:100,000 epi, mL	3.41 (0.66)	3.00 (0.71)	0.67
Study drug volume, mL	7.37 (1.28)	6.30 (2.21)	0.342
Total dosage of local anesthetics, mg	354.7 (64.9)	221.8 (62.7)	0.169
Surgical Difficulty[b]	19.0 (1.0)	19.0 (0.4)	0.355
Total osteotomy time, min	14.1 (6.5)	11.0 (4.5)	0.395
Periodontal graft, n (%)	3 (50.0)	1 (20.0)	>0.05
Excessive hemorrhage, n (%)	1 (16.7)	1 (20.0)	>0.05

Abbreviations: epi=epinephrine, SD=Standard Deviation. Unless otherwise noted, values are presented as mean (SD). **(a)** Per the anesthetic package inserts, the pH of each anesthetic was expected to fall within the range of 3.3 to 5.5. **(b)** Sum of difficulty scores per surgery, where 1=simple, 2=mild complexity, 3=moderate complexity, and 4=difficult.

Table 1: Demographic Characteristics and Surgical Variables.

Exploratory Clinical Study

Subjects: To evaluate the clinical relevance of the *ex vivo* results, we conducted an exploratory study in patients undergoing root-end

endodontic surgery. Eleven subjects were enrolled, and 6 subjects provided biopsies with sufficient RNA for gene expression analysis (Figure 3). Treatment group characteristics were comparable at baseline (Table 1); there were no significant differences in age, race, gender, weight, height, surgical difficulty, osteotomy time, anesthetic volume, number of periodontal grafts, or cases of excessive bleeding between groups (all p-values >0.05).

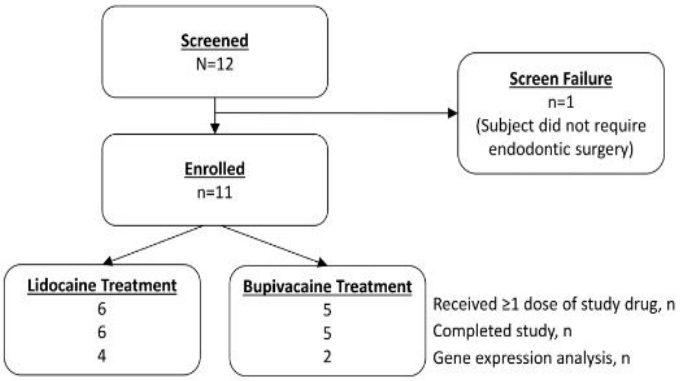


Figure 3: CONSORT diagram. A total of 11 subjects were enrolled in the study into the lidocaine (n=6 subjects) and bupivacaine (n=5 subjects) treatment groups. Of these, 4 lidocaine- and 2 bupivacaine-treated subjects had sufficient RNA recovery for analysis of gene expression. All enrolled subjects completed the study.

Exploratory Gene Expression Analysis: For subjects with evaluable biopsies, gene expression profiles were measured via microarray at 3 time points: prior to surgery (baseline), immediately after surgery (to measure surgical inflammation), and 48 h after surgery (to assess inflammation due to study drug).

Analysis of the microarray data using EDGE with a false discovery rate of 1% revealed differential expression of 112 genes over time, and 9 genes had significant differences in expression between groups ($p<0.001$, Table 2). To minimize error from multiple comparisons, we used a fold change of 2 or a p-value of $p<0.0001$ as more stringent criteria for statistical significance. Two of the 9 identified genes met these criteria: chemokine (C C motif) ligand 20 (CCL20) had a fold change of 2.273 immediately after surgery, and matrix metalloproteinase-1 (MMP-1) had a fold change of 2.270 at 48 h after surgery.

Gene Symbol(s)	Location	Type	Fold Change	Significant Time Point
EDGE Analysis[a]				
GHRL	Extracellular space	Growth factor	1.185[b]	Baseline
KIAA1161	Unknown	Other	0.84[b]	Baseline
ACAA1	Cytoplasm	Enzyme	1.08[b]	Immediately postoperative
CCL20[b]	Extracellular space	Chemokine	2.273b,[c]	Immediately postoperative
EHD1	Cytoplasm	Other	0.705[b]	Immediately postoperative
GLI3	Nucleus	Transcription regulator	-0.593[b]	Immediately postoperative
SNF8	Nucleus	Enzyme	1.502[b]	Immediately postoperative
MMP-1b	Extracellular space	Peptidase	2.270b,[c]	48 hours postoperative
PRTN3	Extracellular space	Peptidase	0.815[b]	48 hours postoperative
Ingenuity Analysis				
PIK3C2G, SIX3, GDF 15, MOXD1, CXCL10, CA14, LCN8, SPRYD5	Various	various	NA	Baseline
SPHK1	Cytoplasm	enzyme	NA[b]	Immediately postoperative
TYRP1	Cytoplasm	enzyme	NA[b]	Immediately postoperative
MMP-1	Extracellular space	peptidase	NA[c]	48 hours postoperative

Abbreviations: ACAA1=Acetyl-Coenzyme A Acyltransferase 1, CA14=Carbonic Anhydrase XIV, CCL20=Chemokine (C-C motif) Ligand 20, CXCL10=Chemokine (C-X-C motif) Ligand 10, EHD1=EH domain-containing protein 1, GDF 15=Growth Differentiation Factor 15, GHRL=ghrelin/obestatin prepropeptide, GLI3=GLI family zinc finger 3, LCN8=Lipocalin 8, MMP-1=Matrix Metalloproteinase 1, MOXD1=Monooxygenase, DBH-like 1, NA=Not Applicable, PIK3C2G=Phosphoinositide 3-Kinase Class 2 Gamma, PRTN3= Proteinase 3, SIX3=homeobox protein SIX3, SNF8=ESCRT-II complex subunit, homolog, SPHK1=Sphingosine Kinase 1, SPRYD5=SPRY Domain containing 5, TYRP1=Tyrosinase-Related Protein 1. (a) All subject samples analyzed had a minimum of 3 replicates. (b) Met predefined significance criterion of $p<0.001$. (c) Met additional significance criterion of $p<0.0001$ or fold change >2.

Table 2: Differentially Expressed Genes.

Because the treatment groups showed no significant differences at baseline, we normalized the microarray data using baseline expression levels. Ingenuity analysis (Ingenuity Systems, Redwood City, CA) of the normalized data revealed differential expression of 11 genes (Table 2). Of these, differences between groups were significant for 3 genes: tyrosinase-related protein 1 (TYRP1) and sphingosine kinase 1 (SPHK1) immediately following surgery ($p<0.001$) and MMP-1 at 48 h after surgery ($p<0.0001$). RT-PCR analysis of all 3 genes correlated significantly with the microarray results, thereby validating the microarray findings

($p<0.05$, data not shown).

Postoperative Pain: Postoperative pain was assessed at baseline, pain onset, and 24 and 48 h after surgery. Overall, pain was rated as moderate at onset and diminished over 48 h (Figure 4). There were no significant differences in postoperative pain between treatment groups at baseline, at pain onset, or 24 h after surgery. At 48 h after surgery, pain intensity tended to be higher for the bupivacaine than lidocaine treatment group ($p=0.08$). However, this difference did not reach significance due to the small sample size; a post-hoc power analysis using the 48 h pain scores revealed study power (0.421) to be below the preplanned level of 0.80. Nevertheless, subjects treated with bupivacaine also used more acetaminophen tablets (15±7.8 vs 12.7±7.2 tablets; $p=0.619$) and reported more adverse events (3 [60%] vs 0 subjects; $p=0.061$) than those treated with lidocaine during the study. All adverse events were reported under the preferred term of nausea/vomiting and considered related to the opioid analgesic prescribed for breakthrough pain.

Discussion

In the context of literature illustrating tissue injury via inflammatory mechanisms and FDA complaints questioning bupivacaine's safety, we conducted this study to evaluate the effect of locally administered bupivacaine on inflammation and pain. In this study, we demonstrate that local bupivacaine anesthesia activates the inflammatory cascade and leads to greater postoperative pain as compared with lidocaine anesthesia.

Consistent with bupivacaine-induced tissue injury and inflammatory cell recruitment, we found that bupivacaine anesthesia increased expression of several proteins and genes related to inflammation (Figure 2, Table 2). These proteins and genes included PGE2, a prostaglandin that increases sensitivity to pain; CCL20, a pro-inflammatory chemotactic factor; SPHK1, a lipid messenger that regulates cellular pathways involved in extracellular matrix remodeling [18]; TYRP1, a melanosomal enzyme that plays an important role in the melanin biosynthetic pathway; and MMP-1, a protease product of the inflammatory cascade that is involved in the breakdown of the extracellular matrix. It is notable that increased MMP-1 expression was detected by both EDGE and Ingenuity analysis in our exploratory clinical study. Although our *ex vivo* and clinical studies assessed gene expression at different times after anesthetic administration, their complementary results clearly indicate a pattern of bupivacaine-induced inflammatory up-regulation. Together, these results lay the groundwork for larger-scale studies investigating bupivacaine's effects on inflammatory mediators.

Bupivacaine-induced changes in protein and gene expression occurred without concomitant changes in COX-2 expression in both our *ex vivo* and patient-derived tissue analyses. Indeed, that PGE2 protein was up regulated in the absence of a COX-2 elevation *ex vivo* suggests the existence of an alternative, COX-2-independent contribution to PGE2 synthesis. Potential sources for this contribution include constitutively expressed COX-1, proteinase-activated receptor [19-21], IL-1β or TNF-α [22], or gene activation due to calcium influx and mobilization [23]. Combined with the lack of COX-2 gene up regulation in our clinical study, our findings thus indicate that tissue injury can activate multiple signaling pathways that culminate in remodeling and repair.

For example, bupivacaine altered the expression of SPHK1 in this study. SPHK1 is well poised to regulate the COX-2-independent biologic responses observed here. SPHK1 controls cytokine-stimulated pathways through 2 distinct lipid mediators: dhS1P and S1P [18]. S1P (but not dhS1P) induces proinflammatory mediator COX-2, whereas dhS1P (but not S1P) induces MMP-1 degradation. Thus, selective function of SPHK1-driven dhS1P could drive the COX-2-independent MMP-1 production observed in this study.

The clinical endpoint of our exploratory study was postoperative pain as an indicator of inflammation. Similar to prior work [9], subject-reported pain in this study was highest for both treatment groups at pain onset (ie, at the time of cessation of local anesthetic action when analgesics had not yet reached therapeutic levels) and diminished over 48 h (Figure 4).

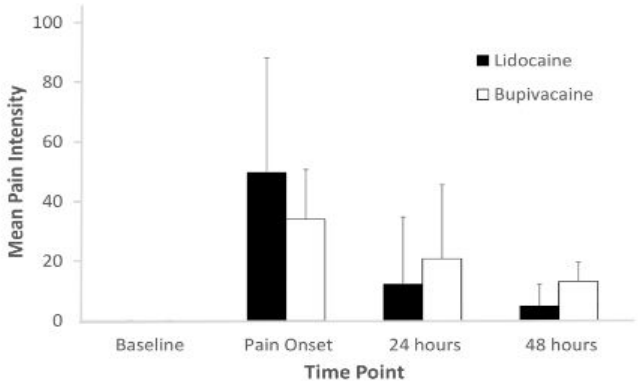


Figure 4: Subject-reported pain by treatment group and time point. Subject-reported pain, assessed on a 100-mm visual analog scale (0=no pain to 100=worst pain imaginable) before administration of any study medication, was highest for both groups at the pain onset and decreased over 48 h. Pain onset was reported by subjects at the time of actual pain onset and varied from subject to subject. Despite a trend for higher pain in the bupivacaine-treated subjects at 48 h ($p=0.08$), there were no significant differences in reported pain between treatment groups at any time point. Data are plotted as mean ± standard deviation.

Although statistical significance was not achieved due to the exploratory nature of the clinical study, several reports suggest a latent effect of local anesthetic treatment group on postoperative pain. First, bupivacaine-treated subjects generally report higher average pain scores 48 h after surgery than their lidocaine-treated counterparts. Further, analysis of analgesic intake as a measure of postoperative pain revealed that bupivacaine-treated subjects used numerically more acetaminophen tablets and experienced

a greater number of adverse events attributed to opioid use than did lidocaine-treated subjects. Although the exploratory study was not sufficiently powered to detect significant differences in these variables, taken together, these results suggest that bupivacaine-treated subjects required greater use of analgesics (ie, acetaminophen and opioids) to control their pain after dental surgery than did lidocaine-treated subjects.

In summary, the results presented here demonstrate that bupivacaine increased certain inflammatory mediators, which may increase postoperative pain after surgery. Our results are consistent with previous reports demonstrating an association between bupivacaine and inflammation, leading to increased postoperative pain [9-15]. It will be interesting for future work to evaluate whether the use of liposomal bupivacaine can circumvent these effects [24]. The inflammation observed in this study occurred in the absence of significant COX-2 elevation, suggesting the existence of multiple pathways to prostanoid expression following tissue injury that culminate in signaling for tissue remodeling and repair. Further studies examining the relationship between bupivacaine and postoperative pain, as well as the effect of bupivacaine formulation on inflammatory gene expression and pain, are needed.

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