Int. J. Oral Maxillofac. Surg. 2017; xxx: xxx–xxx
http://dx.doi.org/10.1016/j.ijom.2017.05.007, available online at http://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Clinical Paper
Oral Surgery

Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study

A. F. M. Albuquerque[1,2], C. S. R. Fonteles[3], D. R. do Val[4], H. V. Chaves[5], M. M. Bezerra[5], K. M. A. Pereira[3], P. G. de Barros Silva[6], B. B. de Lima[7], E. C. S. Soares[3], T. R. Ribeiro[3], F. W. G. Costa[1]

[1]Post-graduate Program in Dentistry, School of Dentistry, Federal University of Ceará, Ceará, Fortaleza, Brazil; [2]Division of Oral Surgery, School of Dentistry, Fortaleza University (UNIFOR), Ceará, Fortaleza, Brazil; [3]Post-graduate Program in Dentistry, School of Dentistry, Federal University of Ceará, Ceará, Fortaleza, Brazil; [4]Renorbio Post-graduate Program, Federal University of Pernambuco, Recife, Pernambuco, Brazil; [5]Post-graduate Program in Health Science, Medical School, Federal University of Ceará, Sobral, Ceará, Brazil; [6]Division of Oral Surgery, School of Dentistry, Fortaleza University (UNIFOR), Ceará, Fortaleza, Brazil; [7]Division of Oral Surgery, Walter Cantídio University Hospital, Ceará, Fortaleza, Brazil

A. F. M. Albuquerque, C. S. R. Fonteles, D. R. do Val, H. V. Chaves, M. M. Bezerra, K. M. A. Pereira, P. G. de Barros Silva, B. B. de Lima, E. C. S. Soares, T. R. Ribeiro, F. W. G. Costa: Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study. Int. J. Oral Maxillofac. Surg. 2017; xxx: xxx–xxx. © 2017 International Association of Oral and Maxillofacial Surgeons. Published by Elsevier Ltd. All rights reserved.

Abstract. This study aimed to evaluate whether pre-emptive analgesia modifies the tissue expression of tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β), and whether there is an association with postoperative surgical outcomes. A triple-blind, randomized, placebo-controlled study of patients undergoing mandibular third molar removal was performed. Volunteers were allocated randomly to receive etoricoxib 120 mg, ibuprofen 400 mg, or placebo 1 h before surgery. Twenty-four surgical sites per group were required (95% confidence level and 80% statistical power). Pain scores differed significantly between groups ($P < 0.001$). Etoricoxib and ibuprofen reduced pain scores compared to placebo ($P < 0.05$). Pain scores peaked at 4 h postoperative in the experimental groups, but at 2 h postoperative in the placebo group ($P < 0.05$). A significant reduction in TNF-α concentration from time 0′ to time 30′ was seen for ibuprofen ($P = 0.001$) and etoricoxib ($P = 0.016$). The ibuprofen group showed a significant reduction in IL-1β levels from time 0′ to time 30′ ($P = 0.038$). In conclusion, TNF-α and IL-1β levels and the inflammatory events in third molar surgery were inversely associated with the degree of cyclooxygenase 2 selectivity of the non-steroidal anti-inflammatory drugs used pre-emptively. Patients given pre-emptive analgesia showed significant reductions in the clinical parameters pain, trismus, and oedema when compared to the placebo group.

Key words: third molar; inflammatory events; TNF-α; IL-1β; non-steroidal anti-inflammatory drugs.

Accepted for publication

0901-5027/000001+011 © 2017 International Association of Oral and Maxillofacial Surgeons. Published by Elsevier Ltd. All rights reserved.

Please cite this article in press as: Albuquerque AFM, et al. Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study, *Int J Oral Maxillofac Surg* (2017), http://dx.doi.org/10.1016/j.ijom.2017.05.007

The surgical removal of mandibular third molars is one of the most commonly performed dental procedures in the outpatient setting. The level of dental impaction may be explained by the lack of physical space for eruption. This is usually the result of the size of the maxillary bone being insufficient to properly accommodate all teeth present within the arch. Thus, third molar extraction is a procedure commonly related to local tissue injury, associated with varying degrees of postoperative pain[1–3].

From a clinical perspective, the removal of such teeth can affect the patient's quality of life postoperatively, particularly during the first 3 days, due to the intensity of pain and inflammation arising from the surgical procedure. Approximately 40–60% of these patients experience moderate to severe pain, requiring the use of rescue analgesics[4–6]. In this context, pre-emptive analgesia is used as a pharmacological strategy for the management, reduction, or even prevention of postoperative pain related to dental procedures. This strategy of pain control has been studied widely in recent decades[7].

In addition to pain, oedema and limited mouth opening (trismus) are the postoperative complications most commonly associated with the removal of mandibular third molars. These clinical signs and symptoms result from a local inflammatory process generated by the activation of the cyclooxygenase (COX) pathway and the subsequent increase in levels of prostaglandins within the injured site. Both of these play important roles in the release of proinflammatory cytokines (tumour necrosis factor alpha (TNF-α), interleukin 1 (IL-1), and interleukin 6 (IL-6)) related to the pathophysiology of pain and inflammation[8,9].

Clinical and experimental studies evaluating the pathogenesis of inflammation resulting from third molar surgery, as well as the role of the inflammatory mediators implicated in the subsequent processes of pain and inflammation, are extremely relevant and currently warranted. Certain mechanisms have been proposed to explain the inflammation that occurs as a result of surgical procedures, but a complete understanding of how these events are fully triggered remains unclear. Proinflammatory cytokines such as TNF-α and interleukin 1 beta (IL-1β) have often been described as important mediators in this process[10–13]. Experimental studies involving acute pain models suggest that IL-1β sensitizes nociceptors and causes hyperalgesia, therefore working actively in the pathophysiology of this type of pain[14–16]. On the other hand, it is recognized that TNF-α exerts remarkable effects, including activating lymphocytes, stimulating the synthesis of other proinflammatory cytokines such as IL-1β and IL-6, and triggering the production of prostaglandins[17–21].

Although clinical studies have investigated the effectiveness of pre-emptive analgesia in third molar surgery[22–26], it is currently highly relevant to publish translational studies aimed at assessing the influence of the preoperative administration of non-steroidal anti-inflammatory drugs (NSAIDs) on the pathophysiology of inflammatory events established within these clinical situations. Another question that has yet to be answered is whether or not the selectivity of NSAIDs to COX isoforms may render different effects in the expression of proinflammatory cytokines such as TNF-α and IL-1β in these surgical situations.

Therefore, this study aimed to test the hypothesis that pre−emptive analgesia, through the administration of NSAIDs prior to the removal of mandibular third molars, can quantitatively change the tissue expression of TNF−α and IL−1β, and that these changes may be related to clinical effects (pain, oedema, and trismus) postoperatively.

Materials and methods

Study design and sample

This study was approved by the Ethics Committee of Walter Cantídio University Hospital and was performed in accordance with the Helsinki statements. A triple−blind, randomized, crossover, placebo−controlled study of patients undergoing mandibular third molar extractions was performed. These patients were recruited from the Division of Oral and Maxillofacial Surgery of Walter Cantídio University Hospital at the Federal University of Ceará (Brazil). The volunteers were recruited between March 2014 and December 2015 according to the CONSORT protocol[27].

The sample unit used in this study was the surgical site. Healthy individuals (American Society of Anesthesiologists, ASA 1) of both sexes, aged between 18 and 35 years, requiring the removal of both mandibular third molars, were invited to participate in this study.

The following inclusion criteria were adopted to standardize the level of traumatic injury generated by surgery: (1) patients with third molars requiring ostectomy, with or without associated tooth sectioning; (2) patients with third molars that showed similar patterns of root formation, position, and degree of impaction. Patients were excluded if they met any of the following criteria: smokers, pregnant or breast feeding, users of medications that could interact with the drugs used in this study, patients with orthodontic bands on the mandibular second molars, confirmed history of allergy to NSAIDs, signs of any preoperative inflammatory or infectious condition, systemic chronic disease, use of NSAIDs within the past 21 days, or the presence of periodontal disease, swelling, fever, or trismus prior to surgery. Patients who did not follow the recommendations prescribed, who underwent surgical procedures that exceeded 2 h, who had an intolerance to the pharmacological regimen, who presented a postoperative infection, and those who did not return for postoperative assessment consultations were removed from the study.

All recruited individuals were informed about the objectives and study design, and those who consented to participate signed a written informed consent agreement.

Sample size calculation

The sample size calculation was based on that described in the study conducted by Al-Shukun et al.[26]. These authors observed a negative response to the pharmacological treatment instituted of 58% in the control group and 18% in the experimental groups. Thus, 24 samples of gingival tissue per group were required to conduct this clinical trial and statistically reject the null hypothesis with 80% power and a 95% confidence interval. For this sample calculation, the type 1 error associated with the test was 0.05; the χ^2 test without correction was used to evaluate the null hypothesis.

Interventions

The following data were collected prior to surgery: sex, age, general health status, periodontal condition, and intraoral and extraoral aspects of the dental impaction. A panoramic radiograph was acquired and the tooth position according to the classifications of Pell and Gregory[28] and Winter[29], degree of tooth development, and level of impaction were recorded.

The surgical procedures were performed in two distinct clinical sessions (one side at a time). Each patient underwent removal of only one third molar per surgical session (i.e., one surgical site). Patients were instructed to call and schedule their second surgical appointment after a period of 21 days, and surgeries were

Please cite this article in press as: Albuquerque AFM, et al. Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study, *Int J Oral Maxillofac Surg* (2017), http://dx.doi.org/10.1016/j.ijom.2017.05.007

scheduled 1 week later. Hence, the second surgery was performed 28 days after the first (wash-out period).

In order to exclude a possible confounding factor, it was established that both surgical sites in the same patient could not be allocated to a single experimental group. Each treatment was coded as a different group, and groups were identified with the letters A, B, or C. Based on the nomenclature of the blinded groups previously provided to the statistician, six blocks of combinations were created (AB, BA, BC, CB, AC, and CA). Each block represented the treatment that would be administered prior to the removal of the right and left mandibular third molars, respectively. They were randomized through a computer-generated randomization code in Microsoft Excel, confirming that each patient received two different medications. The drugs used in this study were etoricoxib 120 mg, ibuprofen 400 mg, and placebo (without active drug). The medications were administered 1 h before surgery. No antibiotic prophylaxis was administered to the volunteers.

All patients underwent a standardized surgical technique, performed in an outpatient setting, following a strict biosafety protocol. A surgeon with experience in oral and maxillofacial surgery performed all of the surgical procedures. The same surgical protocol was adopted for both sides of the mouth, with the aim of reducing differences in the level of intraoperative trauma. The third molar removal was performed under local anaesthesia with mepivacaine 2% and epinephrine 1:100,000 (DFL, Rio de Janeiro, Brazil), using a maximum of three 1.8-ml cartridges.

A triangular full-thickness flap was raised, followed by peripheral ostectomy using a high-speed hand piece under irrigation with cooled double-distilled water. One sample of soft tissue was collected from the region distal to the third molar before the surgical flap was raised (time 0′) and a second soft tissue sample was obtained 30 min after the surgical procedure (time 30′) for the laboratory analysis of cytokines. The surgical wound was closed with a 4–0 silk suture.

After surgery, ibuprofen 300 mg was prescribed as rescue analgesic, to be taken at intervals of 8 h. Postoperative instructions were also carefully read and explained to the patients. They were instructed to maintain a liquid and soft diet and to avoid hot liquids and/or foods during the first 24 h, and to perform careful oral hygiene without vigorous mouthwashes in order to prevent postsurgical bleeding. The patients were instructed to contact the surgeon by telephone in the case of persistent bleeding, or if they deemed it necessary. In addition, the patients were also asked to report any physical symptoms experienced during the postoperative period of the study, such as nausea, vomiting, dizziness, headache, insomnia, and signs of infection.

Outcome measures

The primary outcome of the study was the occurrence of postoperative inflammatory events (pain, facial oedema, and trismus). The intensity of postoperative pain was measured using a visual analogue scale (VAS) of 10 cm, with 0 representing the absence of pain or discomfort and 10 representing the maximum pain or discomfort. After the surgical procedure, each patient received a standardized form with the VAS to report postoperative pain values. Study participants were asked to mark the intensity of their pain at 0, 2, 4, 6, 8, 10, 12, 24, 48, and 72 h, as well as on days 5 and 7 after surgery. In addition, data were collected on the use of rescue medication, including: (1) the time elapsed between the end of the surgical procedure and the ingestion of the medicine by the patient; (2) number of rescue analgesic consumed.

Postoperative oedema was measured using lines drawn between facial points (Fig. 1). These were the distances from the mandibular angle (MA) to: (1) tragus (MA–Tr distance), (2) external corner of the eye (MA–ECE distance), (3) nasal border (MA–NB distance), (4) labial commissure (MA–LC distance), and (5) soft pogonion (MA–SP distance). The preoperative values and those obtained at 24 h, 72 h, and 7 days after surgery were analyzed.

Furthermore, to estimate the degree of mouth opening, the maximum mouth opening was measured in the pre- and postoperative periods (after 24 h, 72 h, and 7 days) by measuring the distance in millimetres between the upper and lower central incisors using a calibrated ruler.

The secondary outcome of this study was the occurrence of changes in the tissue levels of TNF-α and IL-1β in each study group. The gingival tissue samples were stored at −80 °C in Eppendorf tubes containing Radio-Immunoprecipitation Assay solution (Santa Cruz Biotechnology, Santa Cruz, CA, USA) until required for each assay. The collected tissue was homogenized and followed by centrifugation (10,000 rpm/10 min/4 °C). The supernatant was used to determine the expression levels of TNF-α and IL-1β by ELISA

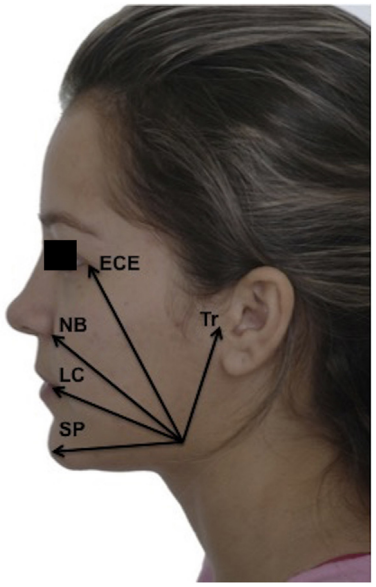

Fig. 1. Linear measurements for the assessment of postoperative oedema: mandibular angle (MA) to: (1) tragus (MA–Tr distance), (2) external corner of the eye (MA–ECE distance), (3) nasal border (MA–NB distance), (4) labial commissure (MA–LC distance), and (5) soft pogonion (MA–SP distance).

method, using commercial kits (Quantikine Human TNF-α Kit (catalogue DY210) and Quantikine Human IL-1β/IL-1f2 Kit (catalogue DY201); R&D system, Minneapolis, MN, USA). The assays were performed in accordance with the manufacturer's instructions. The levels of TNF-α and IL-1β were recorded in picograms per millilitre (pg/ml).

Randomization

The method used to generate the random allocation sequence was the function 'randbetween' in Microsoft Excel, 2010 version. The randomization was based on simple type, without any restriction. The mechanism used to implement the random allocation sequence was envelopes that stated the numbers of randomization on the outside. These envelopes contained information specifying the group to which the patient would belong. A collaborating researcher who did not participate in the surgical procedures was responsible for generating the random allocation sequence, as well as for organizing and distributing the participants in each group.

Blinding

Through the blinding protocol used in this study, the patient, researcher, and statistician did not know to which group each patient belonged. Before the surgical

Please cite this article in press as: Albuquerque AFM, et al. Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study, *Int J Oral Maxillofac Surg* (2017), http://dx.doi.org/10.1016/j.ijom.2017.05.007

procedures, a list containing the random distribution of all surgical sites and respective medicines to be administered was kept in a sealed envelope by an external collaborator who remained unaware of the study protocol until the data analysis. The statistical analysis was initially performed with groups encoded with the letters 'A', 'B', and 'C', which represented the different groups studied. These codes were revealed at the end of the study.

Statistical analysis

The data were expressed as the mean and standard deviation and submitted to the Kolmogorov–Smirnov normality test prior to further analysis by Kruskal–Wallis test followed by the Dunn or Wilcoxon post-hoc test (non-parametric data), or one- or two-way analysis of variance (ANOVA) for repeated (or not) measures followed by the Bonferroni post-hoc test (parametric data). Pearson's correlation analysis was used to assess the correlation between the sum of the pain scores and the total consumption of rescue medication (parametric data), and Spearman's correlation analysis was used to assess the correlation with the levels of cytokines (non-parametric data). Categorical data were analyzed by χ^2 test.

All analyses were performed in GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) adopting a 95% confidence interval; $P < 0.05$ was considered statistically significant.

Results

Sample characterization

A total of 540 patients were screened for study eligibility (Fig. 2). From this total, 504 individuals were excluded because they did not meet the study criteria. The final sample was composed of 36 volunteers (16 male, 44.4%; 20 female, 55.6%),

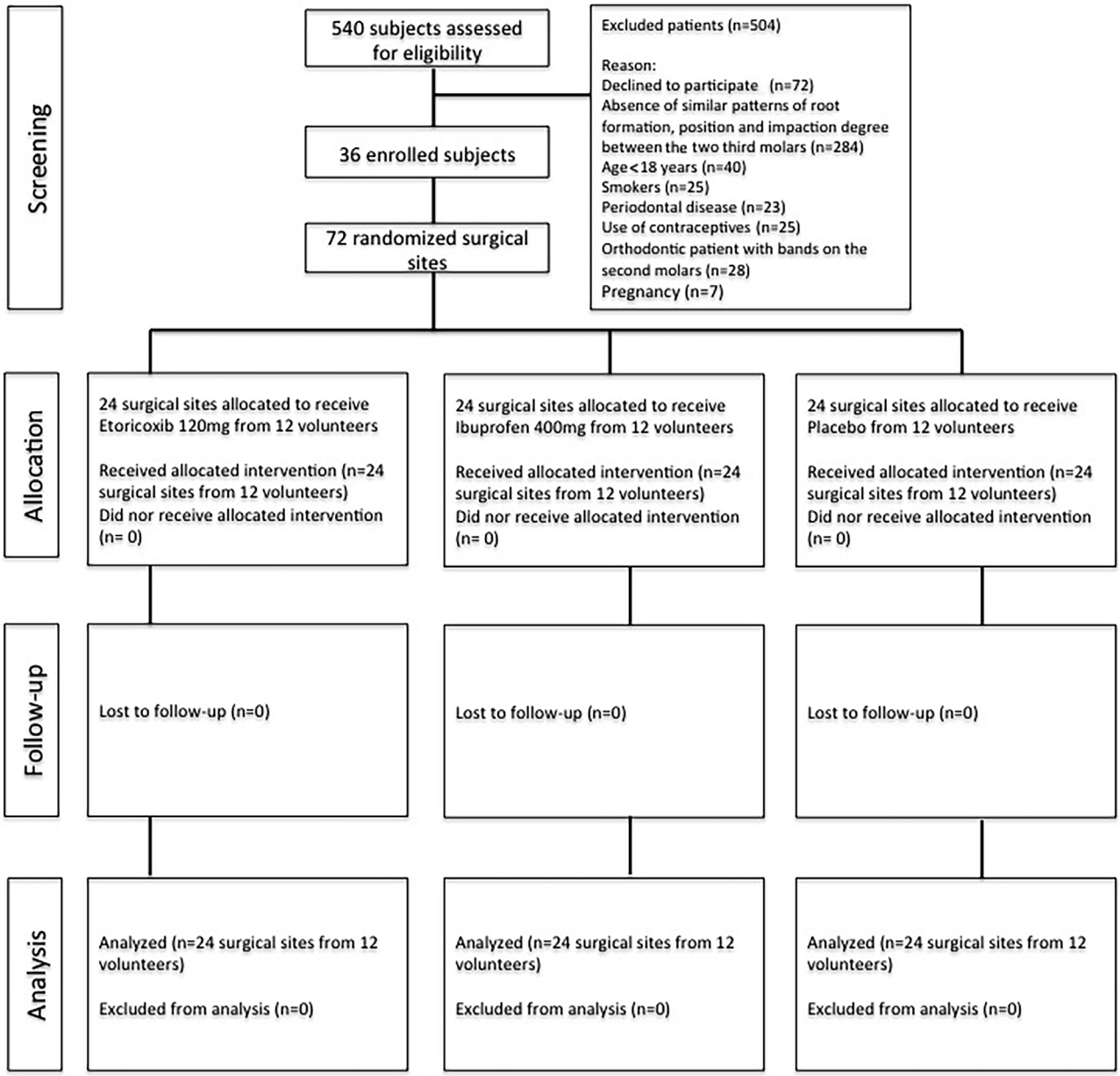

Fig. 2. Flowchart of patient recruitment according to the CONSORT protocol.

for a total of 72 surgical sites, divided into 24 procedures per group.

There were no statistically significant differences in clinical, radiographic, or surgical characteristics between the groups ($P > 0.05$); the level of operative difficulty was similar across the surgical procedures. The distribution of surgical sites did not differ between males (9 placebo, 11 ibuprofen, 12 etoricoxib) and females (15 placebo, 13 ibuprofen, 12 etoricoxib) ($P = 0.590$). There was no statistically significant difference in age group distribution between the groups ($P = 0.436$): <20 years (4 placebo, 1 ibuprofen, 5 etoricoxib), 20–30 years (19 placebo, 21 ibuprofen, 16 etoricoxib), >30 years (1 placebo, 2 ibuprofen, 3 etoricoxib). There was also no statistically significant difference between teeth with total bone inclusion (5 placebo, 7 ibuprofen, 8 etoricoxib), partial bone inclusion (11 placebo, 6 ibuprofen, 7 etoricoxib), and semi inclusion (8 placebo, 11 ibuprofen, 9 etoricoxib) ($P = 0.490$).

When considering the Pell and Gregory classification regarding the amount of tooth covered by the anterior border of the ramus, no differences were identified between the groups ($P = 0.817$): placebo (12 class I, 11 class II, 1 class III), ibuprofen (15 class I, 9 class II, 0 class III), and etoricoxib (14 class I, 9 class II, 1 class III). In addition, there were no differences between the groups regarding the impaction depth relative to the adjacent tooth: placebo (11 class A, 12 class B, 1 class C), ibuprofen (15 class A, 8 class B, 1 class C), and etoricoxib (10 class A, 13 class B, 1 class C) ($P = 0.787$). Tooth position according to the Winter classification did not differ between the placebo (11 vertical, 8 mesioangular, 1 distoangular, 4 horizontal), ibuprofen (13 vertical, 5 mesioangular, 1 distoangular, 5 horizontal), and etoricoxib (9 vertical, 8 mesioangular, 0 distoangular, 7 horizontal) groups ($P = 0.614$).

The need for tooth sectioning during surgery did not differ between the groups: placebo (12 presence, 12 absence), ibuprofen (11 presence, 13 absence), and etoricoxib (12 presence, 12 absence) ($P = 0.829$). The total number of anaesthetic cartridges used ranged from 1.5 to 2, with no difference between the groups ($P > 0.05$). Furthermore, the time required for third molar removal (range 10–40 min) did not differ between the groups ($P = 0.875$).

Analysis of pain

The mean time to the need for rescue medication was significantly reduced in the etoricoxib (2.0 ± 1.8 h) and ibuprofen (2.6 ± 1.1 h) groups, when compared to the placebo group (4.5 ± 1.7 h) ($P < 0.001$), but the range of mouth opening did not differ between the groups ($P = 0.682$).

The placebo group presented a significantly elevated pain peak after 2 h (VAS 6.3 ± 2.9), and the groups treated with ibuprofen and etoricoxib showed significantly elevated pain peaks at 4 h after the surgical procedure (3.9 ± 2.4 and 3.0 ± 2.3, respectively) (Fig. 3). The pain peak reduced significantly in both groups at 6 h after the end of surgery (3.6 ± 1.9 in the placebo group, and 2.6 ± 1.8 and 1.9 ± 1.5 for the ibuprofen and etoricoxib groups, respectively) ($P < 0.001$). The group treated with ibuprofen showed significantly less pain than the placebo group at 2 h and 4 h after surgery, and the group treated with etoricoxib showed significantly less pain than the placebo group at 2 h, 4 h, 6 h, 8 h, and 10 h following surgery ($P < 0.001$). The cumulative effect of all scores up to 6 h and 12 h postoperatively was significantly lower in the ibuprofen group (2.3 ± 2.3 and 1.5 ± 1.8, respectively) and etoricoxib group (1.6 ± 1.8 and 1.0 ± 1.4, respectively), when compared to the placebo group (3.8 ± 3.2 and 2.5 ± 2.8, respectively) ($P < 0.001$). The group treated with etoricoxib showed a lower mean accumulated pain during 12 h when compared to the group treated with ibuprofen ($P < 0.001$).

The total consumption of rescue analgesics showed a statistically significant correlation with the sum of the pain scores in all groups (placebo: $P = 0.019$, $r = 0.495$; ibuprofen: $P = 0.027$, $r = 0.492$; etoricoxib: $P = 0.011$, $r = 0.509$).

Analysis of oedema

Measurement of MA–Tr showed a significant increase in the placebo (65.7 ± 9.4 mm), ibuprofen (61.6 ± 7.3 mm), and etoricoxib groups (58.3 ± 5.1 mm) at 24 h after surgery, followed by a significant reduction in the placebo group after 7 days (60.0 ± 65.0 mm), while the ibuprofen and etoricoxib groups showed a significant reduction after 72 h (59.5 ± 5.0 mm and 57.2 ± 4.5 mm, respectively) ($P < 0.001$). The mean MA–Tr distance was significantly lower in the group treated with etoricoxib when compared to the placebo at 24 h and 72 h postoperatively ($P < 0.001$). The cumulative effect of all the mean MA–Tr distances within the different postoperative times in the placebo group (61.9 ± 8.0 mm) showed a higher value than the ibuprofen group (59.6 ± 5.7 mm) and etoricoxib group (57.2 ± 4.7 mm), while the etoricoxib group had a lower value than the ibuprofen group ($P < 0.001$).

The MA–ECE peak measurement was significantly increased at 24 h after the surgical procedure in the placebo (107.8 ± 8.2 mm), ibuprofen (105.7 ± 6.9 mm), and etoricoxib groups (105.4 ± 7.0 mm), and demonstrated a significant reduction in the ibuprofen group after 7 days (103.2 ± 6.2 mm) and in the placebo

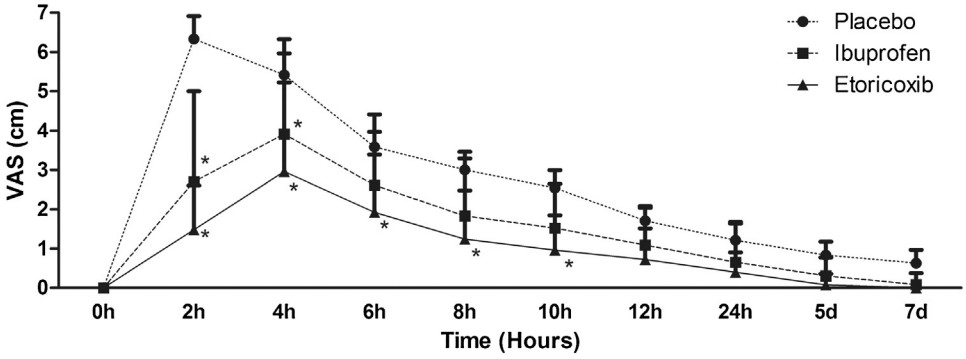

Fig. 3. Pain intensity measured on a visual analogue scale (VAS, cm) according to the study groups and specific postoperative time intervals. Data expressed as the mean ± standard deviation (*$P < 0.05$ when vs. placebo; repeated measures two-way ANOVA/Bonferroni test).

Please cite this article in press as: Albuquerque AFM, et al. Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study, *Int J Oral Maxillofac Surg* (2017), http://dx.doi.org/10.1016/j.ijom.2017.05.007

and etoricoxib groups after 72 h (105.6 ± 6.0 mm and 104.2 ± 6.2 mm, respectively). The etoricoxib group (103.4 ± 6.2 mm) expressed significantly less oedema at 7 days after surgery when compared to the placebo group (104.0 ± 5.6 mm) ($P = 0.004$). However, the cumulative effect of the MA–ECE distance did not differ between the three study groups ($P = 0.568$).

The peak MA–NB measurement was significantly increased at 24 h after the surgical procedure in the placebo (118.3 ± 8.7 mm), ibuprofen (114.0 ± 6.8 mm), and etoricoxib groups (113.5 ± 8.5 mm), and reduced significantly in the placebo group after 72 h (115.3 ± 7.0 mm) and in the ibuprofen (108.6 ± 7.5 mm) and etoricoxib groups (110.2 ± 7.7 mm) after 7 days. The cumulative effect of all the MA–NB measurements was significantly reduced in the ibuprofen group (110.9 ± 7.3 mm) and etoricoxib group (111.6 ± 7.9 mm) when compared to the placebo group (114.3 ± 7.9 mm) ($P = 0.007$).

The peak MA–LC measurement was significantly increased 24 h after surgery in the placebo (101.8 ± 9.3 mm), ibuprofen (94.4 ± 7.1 mm), and etoricoxib groups (94.2 ± 8.5 mm) ($P < 0.001$). These peaks were significantly lower in the ibuprofen and etoricoxib groups ($P < 0.001$). The oedema peaks reduced significantly in all groups after the first 72 h (95.5 ± 5.3 mm, 92.7 ± 6.7 mm, and 92.9 ± 7.8 mm, respectively). The cumulative effect of all MA–LC measurements was significantly reduced in the ibuprofen (91.1 ± 7.0 mm) and etoricoxib groups (92.0 ± 7.8 mm) when compared to the placebo group (94.3 ± 8.1 mm) ($P = 0.014$).

The peak MA–SP distance was significantly increased at 24 h after the surgery in the placebo (122.0 ± 9.1 mm), ibuprofen (116.2 ± 6.8 mm), and etoricoxib groups (116.0 ± 8.6 mm) ($P < 0.001$), and the peaks were significantly reduced in the ibuprofen and etoricoxib groups ($P < 0.001$). There was a significant reduction in oedema in the placebo (117.7 ± 7.0 mm) and etoricoxib groups (114.4 ± 7.4 mm) after 48 h. The ibuprofen group (111.3 ± 7.3 mm) demonstrated a reduction in oedema after 7 days. The cumulative effect of all the MA–SP measurements was lower in the ibuprofen group (113.2 ± 7.6 mm) compared to the placebo group (116.0 ± 8.1 mm). No difference in the cumulative effect of all oedema measurements was observed when comparing the three study groups ($P = 0.108$).

Analysis of mouth opening

The group treated with placebo showed a maximum mouth opening value of 32.1 ± 7.0 mm after 24 h, with a significant improvement after 72 h (37.1 ± 8.3 mm) and at 7 days postoperative (43.1 ± 8.0 mm) ($P < 0.001$). The ibuprofen and etoricoxib groups showed maximum mouth opening values after 24 h of 34.4 ± 8.7 mm and 39.2 ± 8.4 mm, respectively, with significant improvements after 7 days (49.2 ± 8.9 mm and 49.9 ± 8.0 mm, respectively) ($P < 0.001$). Mouth opening was significantly greater in the group treated with ibuprofen on day 7 postoperative and in the group treated with etoricoxib at 24 h, 72 h, and 7 days after surgery, when compared to the placebo group ($P = 0.040$). The group treated with etoricoxib (46.5 ± 9.5 mm) demonstrated greater mean mouth opening measurements than the placebo group (40.8 ± 10.0 mm) ($P = 0.001$).

Cytokine profiles

The level of TNF-α in the placebo group showed no statistically significant difference from time 0′ (99.8 ± 123.3 pg/ml) to time 30′ (47.7 ± 42.7 pg/ml, $P = 0.127$); however, the ibuprofen (137.4 ± 130.2 to 32.1 ± 31.6 pg/ml, $P = 0.001$) and etoricoxib groups (88.5 ± 1.0 to 31.7 ± 33.4 7 pg/ml, $P = 0.016$) showed statistically significant reductions in TNF-α from 0 to 30 min after the beginning of the surgical procedure (Fig. 4). There was no significant difference between the three groups at time 0′ ($P = 0.274$) or time 30′ ($P = 0.230$).

No significant variations (Δ) in the levels of IL-1β were observed in the placebo (244.3 ± 99.7 to 268.5 ± 85.1 pg/ml; $P = 0.487$) and etoricoxib groups (246.5 ± 134.3 to 217.5 ± 97.9 pg/ml; $P = 0.593$); however, there was a significant reduction in the levels of IL-1β in the ibuprofen group from time 0′ (322.0 ± 168.8 pg/ml) to time 30′ (253.1 ± 132.9 pg/ml; $P = 0.038$). There was no significant difference in the levels of IL-1β between the three groups at time 0′ ($P = 0.354$) or time 30′ ($P = 0.500$).

Analysis of the correlation between clinical parameters and cytokine levels

Levels of TNF-α at time 0′ ($r = 0.603$) and at time 30′ ($r = 0.451$) were positively correlated with the amount of rescue medication consumed in the placebo group (Table 1). The levels of TNF-α at time 0′ also showed a positive correlation with the VAS at 2 h ($r = 0.461$). In the ibuprofen group, the consumption of rescue medications was positively correlated with levels of TNF-α at time 30′ ($r = 0.546$) and the levels of IL-1β at time 30′ ($r = 0.568$). The level of pain at 7 days after surgery showed a significant negative correlation with the levels of TNF-α at time 30′ ($r = -0.573$) and the levels of IL-1β at time 30′ ($r = -0.501$), whereas the reduction (Δ) in IL-1β levels showed a significant positive correlation with pain levels after 7 days ($r = 0.434$). In the etoricoxib group, the variation (Δ) in TNF-α levels correlated significantly with the consumption of rescue medication ($r = -0.436$), difficulty in mouth opening ($r = -0.477$),

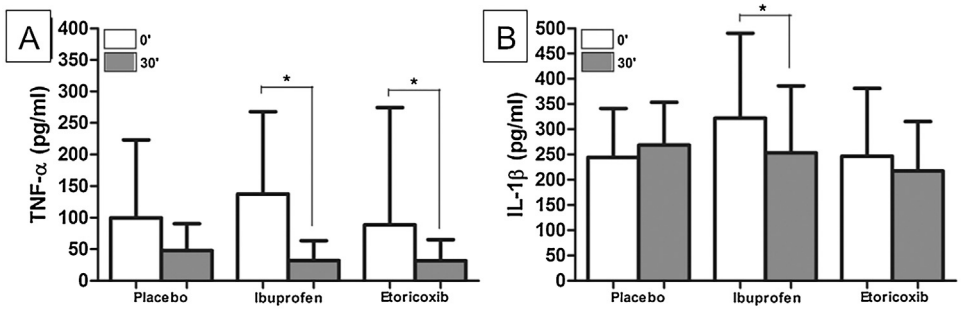

Fig. 4. Assessment of the levels of (A) TNF-α and (B) IL-1β in the placebo, ibuprofen, and etoricoxib groups at postoperative times 0′ and 30′. Data expressed as the mean ± standard deviation (*$P < 0.05$, Wilcoxon test).

Please cite this article in press as: Albuquerque AFM, et al. Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study, *Int J Oral Maxillofac Surg* (2017), http://dx.doi.org/10.1016/j.ijom.2017.05.007

Table 1. Analysis of the correlation between cytokine profiles and their variations (final dosage minus initial dosage) and clinical data for pain.

		TNF-α			IL-1β		
		Time 0′	Time 30′	Δ	Time 0′	Time 30′	Δ
Placebo							
Rescue drug intake	*r*	**0.603**[b]	**0.451**[a]	−0.191	0.411	0.318	−0.336
	P-value	**0.003**	**0.035**	0.394	0.057	0.149	0.127
Mouth opening difficulty	*r*	−0.172	−0.165	−0.040	−0.020	0.056	0.149
	P-value	0.444	0.464	0.861	0.929	0.804	0.507
VAS 0 h	*r*	0.000	0.000	0.000	0.000	0.000	0.000
	P-value	1.000	1.000	1.000	1.000	1.000	1.000
VAS 2 h	*r*	**0.461**[a]	0.238	−0.243	0.183	0.177	−0.131
	P-value	**0.031**	0.287	0.276	0.414	0.430	0.560
VAS 4 h	*r*	0.165	0.093	−0.132	0.069	0.060	0.053
	P-value	0.462	0.680	0.559	0.761	0.790	0.815
VAS 6 h	*r*	0.028	−0.034	−0.195	0.185	0.202	−0.037
	P-value	0.902	0.882	0.384	0.410	0.366	0.871
VAS 8 h	*r*	0.129	0.061	−0.143	0.312	0.193	−0.192
	P-value	0.567	0.788	0.527	0.157	0.390	0.392
VAS 10 h	*r*	0.093	−0.023	−0.189	0.224	0.097	−0.143
	P-value	0.680	0.918	0.399	0.317	0.666	0.527
VAS 12 h	*r*	−0.083	−0.100	−0.115	0.174	0.126	−0.130
	P-value	0.714	0.659	0.609	0.438	0.577	0.565
VAS 24 h	*r*	0.149	0.075	−0.083	−0.113	0.081	0.105
	P-value	0.508	0.740	0.713	0.617	0.719	0.643
VAS 7 days	*r*	0.126	0.113	−0.049	0.088	−0.090	−0.087
	P-value	0.576	0.616	0.828	0.698	0.690	0.701
Ibuprofen							
Rescue drug intake	*r*	0.363	**0.546**[a]	−0.046	0.293	**0.568**[b]	−0.333
	P-value	0.115	**0.013**	0.842	0.210	**0.009**	0.140
Mouth opening difficulty	*r*	0.032	0.071	−0.023	−0.241	0.001	0.216
	P-value	0.895	0.767	0.921	0.306	0.997	0.346
VAS 0 h	*r*	0.000	0.000	0.000	0.000	0.000	0.000
	P-value	1.000	1.000	1.000	1.000	1.000	1.000
VAS 2 h	*r*	−0.221	−0.131	0.192	−0.210	−0.034	0.262
	P-value	0.349	0.582	0.404	0.373	0.887	0.252
VAS 4 h	*r*	0.221	0.141	−0.203	0.028	0.148	−0.060
	P-value	0.349	0.553	0.378	0.906	0.534	0.795
VAS 6 h	*r*	0.191	0.085	−0.171	−0.025	−0.037	−0.001
	P-value	0.419	0.721	0.459	0.918	0.877	0.995
VAS 8 h	*r*	0.077	−0.012	−0.162	−0.056	0.005	−0.007
	P-value	0.748	0.961	0.483	0.815	0.984	0.975
VAS 10 h	*r*	0.221	0.034	−0.426	0.005	0.142	−0.025
	P-value	0.350	0.887	0.054	0.984	0.549	0.914
VAS 12 h	*r*	−0.143	−0.195	0.050	−0.223	0.059	0.231
	P-value	0.546	0.411	0.829	0.344	0.804	0.313
VAS 24 h	*r*	−0.057	−0.310	−0.143	−0.333	−0.031	0.418
	P-value	0.813	0.183	0.538	0.151	0.897	0.059
VAS 7 days	*r*	**−0.446**[a]	**−0.573**[b]	0.100	**−0.501**[a]	−0.362	**0.434**[a]
	P-value	**0.049**	**0.008**	0.666	**0.025**	0.117	**0.049**
Etoricoxib							
Rescue drug intake	*r*	−0.147	−0.362	**−0.436**[a]	−0.027	−0.085	−0.021
	P-value	0.502	0.090	**0.037**	0.901	0.701	0.925
Mouth opening difficulty	*r*	−0.129	−0.387	**−0.477**[a]	0.098	0.040	0.043
	P-value	0.558	0.068	**0.021**	0.657	0.855	0.847
VAS 0 h	*r*	0.000	0.000	0.000	0.000	0.000	0.000
	P-value	1.000	1.000	1.000	1.000	1.000	1.000
VAS 2 h	*r*	−0.246	−0.285	−0.095	−0.307	0.156	0.275
	P-value	0.257	0.187	0.666	0.154	0.478	0.203
VAS 4 h	*r*	0.026	−0.117	−0.399	0.308	−0.090	−0.320
	P-value	0.905	0.595	0.059	0.152	0.684	0.136
VAS 6 h	*r*	−0.080	−0.287	**−0.466**[a]	0.156	0.068	−0.133
	P-value	0.717	0.184	**0.025**	0.476	0.759	0.546
VAS 8 h	*r*	0.078	−0.139	**−0.419**[a]	0.215	−0.102	−0.309
	P-value	0.722	0.526	**0.047**	0.325	0.644	0.152
VAS 10 h	*r*	0.051	0.225	0.035	0.079	−0.246	−0.133
	P-value	0.818	0.301	0.874	0.721	0.258	0.545
VAS 12 h	*r*	0.167	0.189	−0.113	0.350	0.105	−0.248
	P-value	0.446	0.387	0.608	0.102	0.633	0.254

Please cite this article in press as: Albuquerque AFM, et al. Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study, *Int J Oral Maxillofac Surg* (2017), http://dx.doi.org/10.1016/j.ijom.2017.05.007

Table 1 (*Continued*)

		TNF-α			IL-1β		
		Time 0′	Time 30′	Δ	Time 0′	Time 30′	Δ
VAS 24 h	*r*	0.258	0.165	−0.331	0.292	0.046	−0.292
	P-value	0.235	0.451	0.123	0.176	0.833	0.177
VAS 7 days	*r*	−0.140	−0.186	−0.047	−0.304	−0.397	0.035
	P-value	0.525	0.395	0.833	0.159	0.061	0.874

TNF-α, tumour necrosis factor alpha; IL-1β, interleukin 1 beta; VAS, visual analogue scale.
[a] $P < 0.05$.
[b] $P < 0.01$, Spearman correlation.

VAS at 6 h ($r = -0.466$), and VAS at 8 h ($r = -0.419$).

Several significant positive correlations between the oedema data and the levels of TNF-α and IL-1β (at time 0′ and at time 30′) were found: 17 in the placebo group and 0 (none) in the ibuprofen and etoricoxib groups (Tables S1–S5). The number of significant negative correlations between the oedema data and the levels of TNF-α and IL-1β (at time 0′ and time 30′) was 0 in the placebo group, 1 in the ibuprofen group, and 3 in the etoricoxib group. The number of significant negative variations (Δ) between the levels of TNF-α and IL-1β and the oedema values was 5 in the placebo group, 2 in the ibuprofen group, and 1 in the etoricoxib group. The number of significant positive variations (Δ) between the levels of TNF-α and IL-1β and the oedema values was 0 in the placebo group, 6 in the ibuprofen group, and 0 in the etoricoxib group.

There was a significant positive correlation between the level of IL-1β at time 30′ and maximum mouth opening at 24 h ($r = 0.503$) and 72 h ($r = 0.511$) in the placebo group. The levels of TNF-α at time 30′ showed a negative correlation with maximum mouth opening at the initial time point ($r = -0.440$). The levels of TNF-α at time 0′ showed a positive correlation with maximum mouth opening at 7 days postoperative ($r = 0.449$). There was no significant correlation between the levels of TNF-α or levels of IL-1β and the maximum mouth opening values in the groups treated with ibuprofen or etoricoxib (Table 2).

Discussion

This study assessed the effectiveness of the use of pre-emptive analgesia using two orally administered NSAIDs that are commonly prescribed for the control of postoperative pain following oral surgery procedures of mandibular third molar removal. Third molar surgery is usually associated with moderate (40%) to severe pain (60%); thus, this pharmacological model has become the most used in clinical trials involving acute pain[21,30,31]. In addition, this surgical procedure is one of

Table 2. Analysis of the correlation between cytokine profiles and their variations (final dosage minus initial dosage) and clinical data for mouth opening capability.

		TNF-α			IL-1β		
		Time 0′	Time 30′	Δ	Time 0′	Time 30′	Δ
Placebo							
Initial	*r*	−0.186	**−0.440**[a]	−0.258	−0.110	−0.313	−0.135
	P-value	0.406	**0.040**	0.246	0.626	0.156	0.550
24 h	*r*	−0.004	0.090	0.067	−0.002	**0.503**[a]	0.087
	P-value	0.985	0.691	0.767	0.994	**0.017**	0.700
72 h	*r*	0.307	0.364	0.048	0.303	**0.511**[a]	−0.250
	P-value	0.165	0.096	0.833	0.171	**0.015**	0.261
7 days	*r*	**0.449**[a]	0.161	−0.403	0.328	0.145	−0.311
	P-value	**0.036**	0.473	0.063	0.136	0.521	0.158
Ibuprofen							
Initial	*r*	−0.032	−0.082	0.000	0.023	−0.050	−0.082
	P-value	0.892	0.731	1.000	0.922	0.834	0.725
24 h	*r*	0.036	0.003	0.020	0.067	0.125	−0.003
	P-value	0.881	0.989	0.932	0.779	0.601	0.989
72 h	*r*	0.110	0.159	0.120	0.125	0.253	−0.085
	P-value	0.643	0.504	0.606	0.599	0.281	0.713
7 days	*r*	0.028	−0.034	−0.092	0.103	−0.132	−0.164
	P-value	0.907	0.887	0.692	0.667	0.578	0.478
Etoricoxib							
Initial	*r*	−0.201	−0.386	−0.331	0.015	−0.001	−0.011
	P-value	0.357	0.069	0.122	0.944	0.995	0.961
24 h	*r*	−0.041	0.261	0.139	0.197	0.165	−0.067
	P-value	0.852	0.230	0.527	0.368	0.452	0.761
72 h	*r*	0.258	0.309	−0.146	0.134	−0.123	−0.111
	P-value	0.235	0.151	0.506	0.543	0.577	0.616
7 days	*r*	0.193	0.004	−0.277	0.211	0.010	−0.146
	P-value	0.379	0.985	0.200	0.333	0.963	0.506

TNF-α, tumour necrosis factor alpha; IL-1β, interleukin 1 beta.
[a] $P < 0.05$, Spearman correlation.

Please cite this article in press as: Albuquerque AFM, et al. Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study, *Int J Oral Maxillofac Surg* (2017), http://dx.doi.org/10.1016/j.ijom.2017.05.007

the best clinical models to study pain because it presents predictable inflammatory features, and the population studied can be considered more homogeneous because it is usually composed of young, healthy individuals who fully understand all of the information provided. The surgical technique employed is standardized among patients, the surgical time generally does not exceed 40 min, and the procedure is commonly performed under local anaesthesia. Such aspects permit the administration of study medications with a minimal risk of postoperative complications[30]. The present study supports these premises, allowing a more accurate evaluation of the medications studied.

Pharmacologically, NSAIDs act by reducing peripheral and central nociception. The NSAID etoricoxib is considered a selective COX-2 inhibitor. Boonriong et al. showed that when etoricoxib was used pre-emptively in patients undergoing arthroscopic shoulder surgery, it significantly reduced postoperative pain[32]. Others have also demonstrated that the pre-emptive use of etoricoxib 120 mg provides adequate pain control following other types of surgical procedure[32–34]. Costa et al. evaluated the clinical effectiveness of etoricoxib 120 mg using a similar methodology and the same type of surgical procedure as employed in the present study[7]. The comparison of the pre-emptive use of this selective COX-2 inhibitor to placebo allowed the authors to show the effectiveness of etoricoxib 120 mg in the control of postoperative pain. The results obtained in the present study support this finding and others reported in the literature by confirming the effectiveness of etoricoxib when used as pre-emptive analgesia in third molar extraction procedures. In addition, the comparison of the experimental groups to the placebo group in this study showed that the higher the selectivity of the medication, the better the analgesia, oedema, and trismus observed during the postoperative period.

In this study, patients treated with etoricoxib 120 mg showed the greatest improvements in pain intensity, extent of mouth opening, and oedema when compared to those treated with ibuprofen 400 mg. In addition, these two groups expressed better outcomes than the placebo group. In agreement with these results, two recent reviews that measured the postoperative effectiveness of etoricoxib 120 mg showed that 72% of participants from five different studies experienced significant pain relief at 4–6 h after surgery[35,36]. Based on the results found in previous clinical trials, the levels of pro-inflammatory cytokines TNF-α and IL-1β were quantified in this study as a means to measure the clinical outcome. Interestingly, the extent of mouth opening was found not to correlate with the levels of cytokines among patients treated with NSAIDs, while the higher the levels of these cytokines, the higher was the consumption of rescue medication and level of postoperative pain, and the lower the pain scores, the lower were the concentrations of cytokines, and as a result the use of rescue medication was less reported among these individuals in the postoperative period.

A previous study screened pericoronal tissue fragments obtained from third molar surgical sites for the presence of inflammatory mediators and identified a distinct production of COX-1 products and prostaglandin E2 (PGE2), mediated by COX-1 and COX-2[9]. Another study evaluating celecoxib, a selective COX-2 inhibitor, was unable to detect any alterations in the levels of thromboxane B2 following third molar surgery. However, their results showed suppression of PGE2 levels between 120 min and 240 min after third molar removal[37]. According to a study by Khan et al., one can expect a gradual increase in COX-2 at 30 min after the completion of third molar extractions, suggesting that an increase in the levels of prostanoids can contribute to the development of postoperative pain and the acute inflammatory process[38]. Thus, the assessment of these inflammatory mediators as they correlate with postoperative pain in patients undergoing pre-emptive analgesia with NSAIDs may allow a better understanding of the mechanisms involved in these inflammatory processes.

COX-2 is an enzyme commonly expressed in tissues once inflammation occurs and is also constitutively expressed in some tissues. In vitro studies have shown a positive feedback loop between the expression of COX-2 and increased levels of proinflammatory cytokines such as TNF-α and IL-1β in various cell types[39]. In fibroblasts (the main cell type present in tissue samples used to measure cytokines), the expression of COX-2 is directly proportional to the levels of TNF-α and IL-1β, and COX-2 also induces PGE2 production. Thus, a greater inhibition of the COX-2 pathway through the pre-emptive administration of etoricoxib should result in a reduction in TNF-α and IL-1β[40].

In this study, the use of a selective COX-2 inhibitor (etoricoxib) resulted in a reduction in TNF-α with an insignificant change noted in the levels of IL-1β from time 0′ to time 30′ postoperative. Since an increase in COX-2 raises the levels of TNF-α, which in turn induces the formation of IL-1β, an early decrease in TNF-α generated by COX-2 inhibition should precede detectable changes in IL-1β, explaining the present findings. TNF-α is also directly related to the hyperalgesia generated by inflammatory states, acting by two mechanisms: (1) induction of COX-2 and subsequent synthesis of eicosanoids by release of IL-1β, (2) induction of sympathomimetic amine production via IL-8. Depending on the intensity and nature of the stimuli, the release of TNF-α is preceded by the formation of bradykinin[41]. The present study observed that the greater the reduction in the levels of TNF-α from time 0′ to time 30′, the less difficult was mouth opening and the lower were the consumption of rescue medication and pain scores at 6 h and 8 h.

The levels of TNF-α and IL-1β were reduced in the group treated with ibuprofen. Ibuprofen is an NSAID with low COX-2 selectivity, or a lack thereof. Hence, the relative increase in COX-1 selectivity expressed by ibuprofen may be responsible in part for the significant decrease in the levels of these two cytokines. COX-1 is a constitutive enzyme and as such does not need to be induced; hence, the presence of acute inflammatory processes does not modify the levels of COX-1 gene expression[40]. The pre-emptive administration of ibuprofen (a drug with partial COX-2 selectivity) led to a reduction in the levels of TNF-α and IL-1β from time 0′ to time 30′, a relatively short time for such an intense reduction in COX-2 gene expression, but long enough to inhibit the inflammatory process generated by this constitutive enzyme. However, these studies were performed with lung fibroblasts and further studies are needed to confirm these hypotheses.

In conclusion, tissue concentrations of TNF-α and IL-1β and the findings of pain and oedema in mandibular third molar surgeries were inversely associated with the level of COX-2 selectivity of the NSAID used pre-emptively. In addition, patients subjected to pre-emptive analgesia showed a significant reduction in the clinical parameters of pain, trismus, and oedema when compared to the placebo group.

Funding

This research received financial support in the form of a grant (# 477734/2013-8)

from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (MTC/CNPq No. 14/2013).

Competing interests

None.

Ethical approval

This study was approved by the Ethics Committee of Walter Cantídio University Hospital (No. 44058715.4.0000.5045) and was performed in accordance with the Helsinki statements.

Patient consent

Written patient consent was obtained to publish the clinical photograph.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijom.2017.05.007.

References

1. Martin MV, Kanatas AN, Hardy P. Antibiotic prophylaxis and third-molar surgery. *Br Dent J* 2005;**198**:327–30.
2. Benediktsdottir IS, Wenzel A, Petersen JK, Hintze H. Mandibular third molar removal: risk indicators for extended operation time, postoperative pain, and complications. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;**97**:438–46.
3. Moller PL, Sindet-Pedersen S, Pettersen CT, Juhl GI, Dillenschneider A, Skoglund LA. Onset of acetaminophen analgesia: comparison of oral intravenous routes after third molar surgery. *Br J Anaesth* 2005;**94**:642–8.
4. Forbes JA. Oral surgery, advances in pain research and therapy: the design of analgesic clinical trials. In: Max MB, Portenoy R, Laska EM, editors. *Advances in pain research and therapy: the design of analgesic clinical trials*. New York: Raven Press; 1991 . p. 347–74.
5. Saito K, Kaneko A, Machii K, Ohta H, Ohkura M, Suzuki M. Efficacy and safety of additional 200-mg dose of celecoxib in adult patients with postoperative pain following extraction of impacted third mandibular molar: a multicenter, randomized, double-blind, placebo-controlled, phase II study in Japan. *Clin Ther* 2012;**34**:314–28.
6. Averbuch M, Katzper M. Severity of baseline pain and degree of analgesia in the third molar post-extraction dental pain model. *Anesth Analg* 2003;**97**:163–7.
7. Costa FW, Soares EC, Esses DF, Silva PG, Bezerra TP, Scarparo HC, Ribeiro TR, Fonteles CSR. A split-mouth, randomized, triple-blind, placebo-controlled study to analyze the pre-emptive effect of etoricoxib 120 mg on inflammatory events following removal of unerupted mandibular third molars. *Int J Oral Maxillofac Surg* 2015; **44**:1166–74.
8. Van Gool AV, Ten Bosch JJ, Boering G. Clinical consequences and complaints after removal of the mandibular third molar. *Int J Oral Surg* 1977;**6**:29–37.
9. Khan AA, Iadarola M, Yang HY, Dionne RA. Expression of COX-1 and -2 in a clinical model of acute inflammation. *J Pain* 2007; **8**:349–54.
10. Kyrkanides K, Fiorentino PM, Miller JH, Gan Y, Lai Y, Shaftel SS, Puzas JE, Piancino MG, O'Banion MK, Tallents RH. Amelioration of pain and histopathologic joint abnormalities in the Col1-IL-1 XAT mouse model of arthritis by intraarticular induction of μ-opioid receptor into the temporomandibular joint. *Arthrit Rheum* 2007;**56**:2038–48.
11. Vernal R, Velásquez E, Gamonal J, Garcia-Sanz JA, Silva A, Sanz M. Expression of proinflammatory cytokines in osteoarthritis of the temporomandibular joint. *Arch Oral Biol* 2008;**53**:910–5.
12. Chicre-Alcântara TC, Torres-Chávez KE, Fischer L, Clemente-Napimoga JT, Melo V, Parada CA, Tambeli RH. Local kappa opioid receptor activation decreases temporomandibular joint inflammation. *Inflammation* 2012;**35**:371–6.
13. Quinteiro MS, Napimoga MH, Mesquita KP, Clemente-Napimoga JT. The indirect antinociceptive mechanism of 15d-PGJ(2) on rheumatoid arthritis-induced TMJ inflammatory pain in rats. *Eur J Pain* 2012;**16**: 1106–15.
14. Watkins LR, Wiertelak EP, Goehler LE, Smith KP, Martin D, Maier SF. Characterization of cytokine-induced hyperalgesia. *Brain Res* 1994;**654**:15–26.
15. Alstergren P, Ernberg M, Kvarnström M, Kopp S. Interleukin 1β in synovial fluid from the arthritic temporomandibular joint and its relation to pain, mobility, and anterior open bite. *J Oral Maxillofac Surg* 1998;**6**: 1059–65.
16. Alstergren P, Benavente C, Kopp S. Interleukin 1β, Interleukin 1 receptor antagonist, and Interleukin 1 soluble receptor II in temporomandibular joint synovial fluid from patients with chronic polyarthritides. *J Oral Maxillofac Surg* 2003;**61**:1171–8.
17. Cunha FQ, Poole S, Lorenzetti BB, Ferreira SH. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. *Br J Pharmacol* 1992; **107**:660–4.
18. Alstergren P. Cytokines in temporomandibular joint arthritis. *Oral Dis* 2000;**6**:331–4.
19. Emshoff R, Puffer P, Rudisch A, Gabner R. Temporomandibular joint pain: relationship to internal derangement type, osteoarthrosis, and synovial fluid mediator level of tumor necrosis factor-alpha. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000;**90**: 442–9.
20. Ogura N, Tobe M, Sakamaki H, Nagura H, Abiko Y, Kondoh T. Tumor necrosis factor-alpha increases chemokine gene expression and production in synovial fibroblasts from human temporomandibular joint. *J Oral Pathol Med* 2005;**34**:357–63.
21. Ke J, Long X, Liu Y, Zhang YF, Li J, Fang W, Meng QG. Role of NF-kappaB in TNF-alpha-induced COX-2 expression in synovial fibroblasts from human TMJ. *J Dent Res* 2007;**86**:363–7.
22. Kaczmarzyk T, Wichlinski J, Stypulkowska J, Zaleska M, Woron J. Preemptive effect of ketoprofen on postoperative pain following third molar surgery. A prospective, randomized, double-blinded clinical trial. *Int J Oral Maxillofac Surg* 2010;**39**:647–52.
23. Isiordia-Espinoza MA, Pozos-Guillén AJ, Martinez-Rider R, Herrera-Abarca JE, Pérez-Urizar J. Preemptive analgesic effectiveness of oral ketorolac plus local tramadol after impacted mandibular third molar surgery. *Med Oral Patol Oral Cir Bucal* 2011; **16**:776–80.
24. Lustenberger FD, Grätz KW, Mutzbauer TS. Efficacy of ibuprofen versus lornoxicam after third molar surgery: a randomized, double-blind, crossover pilot study. *Oral Maxillofac Surg* 2011;**15**:57–62.
25. Sotto-Maior BS, Senna PM, de Souza Picorelli Assis NM. Corticosteroids or cyclooxygenase 2-selective inhibitor medication for the management of pain and swelling after third-molar surgery. *J Craniofac Surg* 2011; **22**:758–62.
26. Al-Sukhun J, Al-Sukhun S, Penttilä H, Ashammakhi N, Al-Sukhun R. Preemptive analgesic effect of low doses of celecoxib is superior to low doses of traditional nonsteroidal anti-inflammatory drugs. *J Craniofac Surg* 2012;**23**:526–9.
27. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, Elbourne D, Egger M, Altman DG. CONSORT, CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Int J Surg* 2012; **10**:28–55.
28. Pell GJ, Gregory BT. Impacted mandibular third molars: classification and modified techniques for removal. *Dent Digest* 1933; **39**:330–8.
29. Winter GB. *Principles of exodontia applied to the impacted mandibular third molar.* Saint Louis: American Books; 1926.
30. Lustenberger FD, Grätz KW, Mutzbauer TS. Efficacy of ibuprofen versus lornoxicam after third molar surgery: a randomized, double-blind, crossover pilot study. *Oral Maxillofac Surg* 2011;**15**:57–62.
31. Savage MG, Henry MA. Preoperative nonsteroidal anti-inflammatory agents: review

of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;**98**:146–52.
32. Boonriong T, Tangtrakulwanich B, Glabglay P, Nimmaanrat S. Comparing etoricoxib and celecoxib for preemptive analgesia for acute postoperative pain in patients undergoing arthroscopic anterior cruciate ligament reconstruction: a randomized controlled trial. *BMC Musculoskelet Disord* 2010;**11**:246.
33. Liu W, Loo CC, Chiu JW, Tan HM, Ren HZ, Lim Y. Analgesic efficacy of pre-operative etoricoxib for termination of pregnancy in an ambulatory center. *Singapore Med J* 2005;**246**: 397–400.
34. Puura A, Puolakka P, Rorarius M, Salmelin R, Lindgren L. Etoricoxib pre-medication for post-operative pain after laparoscopic cholecystectomy. *Acta Anaesthesiol Scand* 2006;**50**:688–93.
35. Clarke R, Derry S, Moore RA. Single dose oral etoricoxib for acute postoperative pain in adults. *Cochrane Database Syst Rev* 2012;(4):CD004309.
36. Clarke R, Derry S, Moore RA. Single dose oral etoricoxib for acute postoperative pain in adults. *Cochrane Database Syst Rev* 2014;(5):CD004309.
37. 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.
38. Khan AA, Iadarola M, Yang HY, Dionne RA. Expression of COX-1 and -2 in a clinical model of acute inflammation. *J Pain* 2007;**8**:349–54.
39. Crofford LJ. COX-1 and COX-2 tissue expression: implications and predictions. *J Rheumatol Suppl* 1997;**49**:15–9.
40. Diaz A, Chepenik KP, Korn JH, Reginato AM, Jimenez SA. Differential regulation of cyclooxygenases 1 and 2 by interleukin-1β, tumor necrosis factor-alpha, and transforming growth factor-beta1 in human lung fibroblasts. *Exp Cell Res* 1998;**241**:222–9.
41. Ribeiro RA, Vale ML, Ferreira SH, Cunha FQ. Analgesic effect of thalidomide on inflammatory pain. *Eur J Pharmacol* 2000; **391**:97–103.

Address:
Fábio Wildson Gurgel Costa
Federal University of Ceará
Department of Dental Clinic
School of Dentistry
Rua Alexandre Baraúna
949
Rodolfo Teofilo
60430-160 Fortaleza
Ceará
Brazil
Tel/Fax: +55 85 3366 8232
E-mail: fwildson@yahoo.com.br

Please cite this article in press as: Albuquerque AFM, et al. Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF-α and IL-1β in third molar surgery: a triple-blind, randomized, placebo-controlled study, *Int J Oral Maxillofac Surg* (2017), http://dx.doi.org/10.1016/j.ijom.2017.05.007