Biotechnology & Biotechnological Equipment

ISSN: 1310-2818 (Print) 1314-3530 (Online) Journal homepage: https://www.tandfonline.com/loi/tbeq20

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To cite this article: Moises A. Franco-Molina, Edgar Mendoza-Gamboa, Erika E. Coronado-Cerda, Diana Zarate-Triviño, Juan E. Arizpe-Coronado, Pablo Zapata-Benavides, Yareellys Ramos Zayas, Reyes Tamez-Guerra & Cristina Rodríguez-Padilla (2017) Clinical trial evaluating the effectiveness of biocompound IMMUNEPOTENT CRP in the third-molar extraction, Biotechnology & Biotechnological Equipment, 31:1, 182-186, DOI: 10.1080/13102818.2016.1249408

To link to this article: https://doi.org/10.1080/13102818.2016.1249408

BIOTECHNOLOGY & BIOTECHNOLOGICAL EQUIPMENT, 2017
VOL. 31, NO. 1, 182–186
http://dx.doi.org/10.1080/13102818.2016.1249408

Taylor & Francis
Taylor & Francis Group

ARTICLE; MEDICAL BIOTECHNOLOGY

OPEN ACCESS

Clinical trial evaluating the effectiveness of biocompound IMMUNEPOTENT CRP in the third-molar extraction

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ABSTRACT

A controlled, parallel, randomized and comparative trial was carried out to evaluate the anti-inflammatory efficacy of IMMUNEPOTENT CRP versus ibuprofen in patients after third-molar surgery over seven days. The anti-inflammatory efficacy of IMMUNEPOTENT CRP was evaluated using the method of Amin and Laskin, and the analysis of cytokine production (IL-2, IL-4, IL-6, IL-10, TNF-α, INF-γ) in saliva was done by flow cytometry. The swelling process after surgery was significant ($p < 0.05$) and the treatments with IMMUNEPOTENT CRP or ibuprofen controlled this process properly; no difference between the groups was found ($p < 0.05$). Both treatments were shown to modulate the cytokine production. These results demonstrate the anti-inflammatory activity of the natural compound IMMUNEPOTENT CRP and suggest it could be used in clinical dental practice.

ARTICLE HISTORY
Received 20 March 2016
Accepted 13 October 2016

KEYWORDS
Surgery; saliva; inflammation; cytokine; IMMUNEPOTENT CRP

Introduction

Third-molar surgical extraction is the most common traumatic procedure in the field of oral and maxillofacial surgery. Since the area of the third-molar is highly vascularized and is rich in loose connective tissue, this surgical procedure is typically followed by liberation of exudate and subsequent swelling, trismus, pain, etc., for which appropriate treatment is needed [1]. The management of these postoperative symptoms is frequently based on pharmacological manipulation of local and systemic mediators of pain and inflammation [2]. For control of postoperative pain and trismus resulting from lower third-molar surgery, some non-steroidal anti-inflammatory drugs (NSAIDs) are generally used [3,4]. Among NSAIDs used in dentistry, ibuprofen, a non-selective COX inhibitor with a highly effective analgesic and anti-inflammatory action, has been largely studied [5–7]. However, many NSAIDs have side effects, such as gastrointestinal irritation, ulcers and bleeding, and can also aggravate some inflammatory responses [8,9]. Due to the undesirable side effects of steroidal and NSAID medications, there is growing interest in natural compounds, which can reduce pain and inflammation by inhibiting the inflammatory pathways in a manner similar to NSAIDs [10]. Pro-inflammatory cytokines are a measurable marker of post-traumatic pain, fever and inflammation and have been isolated in many body systems, including saliva in the oral cavity. Cytokines, such as tumour necrosis factor alpha (TNF-α), have a wide range of pro-inflammatory and immunomodulatory effects including remodelling of fibroblasts and osteoblasts, but TNF-α can lead to a variety of pathological conditions [8]. Interleukin-6 (IL-6) has been traditionally considered to be a pro-inflammatory cytokine and the production of IL-6 is higher after surgical extraction of lower third molars probably due to trauma caused by extraction [11]. Decreasing the local and systemic cytokines levels with the use of drugs is a well-known method for reducing clinical inflammation [12]. The natural compound IMMUNEPOTENT CRP is a dialysate of a heterogeneous mixture of low-molecular-weight substances released from lymphoid tissue obtained from homogenized bovine spleen. It has been patented in Mexico (NL/a/2004/000058, IMPI) [13] and registered under the trade mark IMMUNEPOTENT CRP. IMMUNEPOTENT CRP is a complementary alternative medicine used for improvement of the immune response in a broad spectrum of diseases such as cancer [14,15], endotoxic shock and

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infections [16]. Our research group has demonstrated that IMMUNEPOTENT CRP increases the total antioxidant activity, catalase, glutathione peroxidase and superoxide dismutase activities and decreases the NO, TNF-α production, cyclo-oxygenase-2 (COX-2) and prostaglandin E2 (PGD2) activities, IκB phosphorylation, NFκB p50 and p65 subunit DNA-binding activity in lipopolysaccharide (LPS)-induced human macrophages [17]. The results obtained *in vivo* have demonstrated that IMMUNEPOTENT CRP improved the survival in LPS-induced murine endotoxic shock [16] and human neonatal sepsis [18]. In the present study, a controlled, parallel, randomized and comparative trial was carried out to evaluate the anti-inflammatory activity of IMMUNEPOTENT CRP versus ibuprofen in patients after third-molar surgery over a seven-day research period.

Subjects and methods

IMMUNEPOTENT CRP

The IMMUNEPOTENT CRP used in this study was produced by the Laboratory of Immunology and Virology, Department of Microbiology and Immunology, Faculty of Biological Sciences, Autonomous University of Nuevo Leon (San Nicolas de los Garza, Nuevo Leon, Mexico). It is a mixture of low-molecular weight molecules (cut-off of 10–12 kDa) obtained by dialysis of disintegrated bovine spleens against water. The IMMUNEPOTENT CRP is then lyophilized and analysed to be free of pyrogens by the Limulus amoebocyte lysate assay (Endotoxin detection kit; MP Biomedicals Inc., Aurora, OH, USA) and determined to be free of bacterial contamination by culturing IMMUNEPOTENT CRP in different culture media and *in vivo* mouse inoculation. The IMMUNEPOTENT CRP obtained from 15 $\times$ 108 leukocytes is defined as 1 unit (1 U).

Patients

The study was approved by the Human Research Ethics Committee of the Faculty of Odontology of the Autonomous University of Nuevo Leon. Informed written consent was obtained from all patients, according to the ethical committee and the Helsinki Declaration of 1975, as revised in 2008. This prospective study included 20 ASA PS1 patients (American Society of Anesthesiology classification, normal healthy patients) [19] with mandibular third-molar impactions class IIB according to the Pell–Gregory scale [20].

The surgery had a limited time of 45 minutes. The patient population was randomly and consecutively selected from an outpatient oral and maxillofacial surgery clinic. Care was taken to maintain that the trial was in compliance with the current CONSORT (Consolidated Standards of Reporting Trials) statement guidelines. Other criteria for inclusion into the study included: age between 18 and 40 years; no systemic disease (ASA I status); patients were not taking any medication before surgery; no allergies to any of the study compounds administered and absence of local or systemic infection. All patients underwent an initial preoperative screening consultation with a single oral and maxillofacial surgery faculty-in-residence and a faculty member. All of the enrolled patients completed the study without any postoperative complications. All extractions were performed by one surgeon and required full-thickness mucoperiosteal flaps and bone removal (performed under irrigation) using an air-driven rotary instrument. A uniform local anaesthetic technique was used which included unilateral inferior alveolar, lingual and long buccal nerve blocks using 2% lidocaine with 1:100,000 epinephrine. The patients were randomly divided into two groups using a random number generated by a computer (10 patients per group). An independent pharmacist dispensed either ibuprofen or IMMUNEPOTENT CRP medications according to computer-generated randomization. The two groups were as follows: group 1 received immediate preoperative ibuprofen 600 mg, which was continued every 8 h postoperatively for 3 days, and group 2 received immediate preoperative 5 U of IMMUNEPOTENT CRP, which was continued every 12 h postoperatively for 3 days. All groups received ketorolac tromethamine 10 mg every 6 h for 3 days and amoxicillin 750 mg every 8 h for 7 days for postoperative pain and antibiotic management. All compounds were administered by oral route.

Measurement of swelling

The measurement of facial swelling was done by the method described by Amin and Laskin [21]. The distance between the lower attachment of the ear lobe to the corner of the mouth, the distance between the lower attachment of the ear lobe to the mandibular symphysis and the vertical distance between the angle of the mandible to the outer canthus of the eye were measured by means of a silk suture. This measurement was done prior to surgery along the natural convexity of the patients' face and the measurements were taken immediately before surgery and on the third and seventh postoperative days. The observation was recorded for statistical analysis and the results were tabulated.

Determination of cytokines

Cytokines were determined as biomarkers reflecting the patients' health status and/or intervention outcomes.

Saliva samples were used as a less invasive and more convenient procedure than blood samples. Briefly, saliva samples (1 mL) were collected from patients before surgery, and on the third and seventh postoperative days. The detritus was eliminated from the saliva by centrifugation (400×*g*) and the samples were stored at −20 °C until cytokine determination. The saliva levels of Th1 and Th2 human cytokines were determined by flow cytometry (Accuri C6, BD Bioscienses, CA, USA) using a CBA Th1/Th2 Cytokine Kit II BD™ (BD Biosciences, Bedford, MA, USA), following the manufacturer's instructions. Cytokines were analyzed by using FCAP array v1.0 software (Soft Flow Inc., Minneapolis, MN, USA). Protein values were converted to NIBSC/WHO protein standards (National Institute for Biological Standards and Control/World Health Organization) for further comparisons.

Statistical analysis

All statistical analyses were done using Statistical Package for Social Science (SPSS 18.0). The differences between the two groups regarding the median facial swelling measurements and median saliva cytokines levels were compared using Mann–Whitney *U*-test and a *p*-value of less than 0.05 was considered significant.

Results and discussion

The third-molar extraction pain model is a recognized model for evaluation of the effect of anti-inflammatory drugs [20]. The technique used in this study to measure the inflammatory processes was the method of Amin and Laskin because it is economical, simple and reproducible and has been used by many researchers [22,23]. There are also several linear methods used, such as facial arcs and callipers [24,25], as well as two-dimensional methods such as photographs, modified face arcs and scans; the use of subjective methods, such as facial arcs and callipers, is common [26]. Furthermore, each technique has advantages and disadvantages depending on the experience of the personnel and the method used. For example, in a study by Villafuerte-Nuñez [27], the proposed technique consists in measuring inflammation through a three-dimensional reconstruction of the area affected by facial edema using the structured light technique by using image-processing and the combination of different devices. The authors pointed out that the main advantages are the scanning speed and the accuracy but concluded that the system allows obtaining accurate results while the illumination of the environment is controlled [27]. Actually, the removal of the third molar is a very common procedure and the secondary effects are swelling, pain and trismus. In a study to evaluate two anti-inflammatory drugs (ibuprofen and/or dexamethasone) in third-molar surgery, no statistically significant advantage was found between treatments, suggesting that ibuprofen could be used as a single anti-inflammatory drug in the management of this procedure [12,28]. In recent studies, submucosal dexamethasone has been used as treatment to control postoperative discomfort, showing a significant reduction of pain, swelling and truisms compared with NSAIDs [29]. The difference in these studies could be the administration route.

Our results showed that the group of patients treated with ibuprofen showed a higher increase (3.26%) in the swelling percentage from day 0 to day 3 ($p < 0.01$) and a significant decrease in the swelling percentage to 1.0% on day 7 ($p < 0.05$) (Table 1). A similar trend was observed in the group of patients treated with IMMUNEPOTENT CRP: they showed a 2.61% increase in the swelling percentage from day 0 to day 3 ($p < 0.05$) and a significant decrease of the swelling percentage to 0.63% on day 7 ($p < 0.05$) (Table 1). However, no significant differences ($p > 0.05$) were found between the swelling percentages after treatment in the two study groups (Table 2). These findings could be associated with the control of pro-inflammatory cytokines. That is why, as next step, we evaluated the levels of some saliva cytokines on day 0, 3 and 7 in the groups treated with ibuprofen or IMMUNEPOTENT CRP. The obtained results, however, showed non-significant differences in the studied cytokine levels between the two types of treatment and the three days of sampling ($p > 0.05$), although high IL-6 production was observed in both treatment groups (Table 3).

Table 1. Intragroup comparisons of the percentage of swelling.

Group	Day 0	Day 3	Day 7
Ibuprofen	0%[c]	3.26%[a]	1.0%[b]
IMMUNEPOTENT CRP	0%[d]	2.61%[e]	0.63%[f]

Note: The percentage of swelling was determined by the method of Amin and Laskin [21].
[a] $p < 0.05$ between day 0 and 3 in the ibuprofen group.
[b] $p < 0.001$ between day 3 and 7 in the ibuprofen group.
[c] $p < 0.001$ between day 0 and 7 in the ibuprofen group.
[d] $p < 0.05$ between day 0 and 3 in the IMMUNEPOTENT CRP group.
[e] $p < 0.05$ between day 3 and 7 in the IMMUNEPOTENT CRP group.
[f] $p < 0.05$ between day 0 and 7 in the IMMUNEPOTENT CRP group.

Table 2. Comparison of the percentage of swelling between groups.

	Ibuprofen	IMMUNEPOTENT CRP	*p* Value[a]
Day 0–0	0%	0%	–
Day 3–3	3.26%	2.61%	0.988
Day 7–7	1%	0.63%	0.447

Note: The percentage of swelling was calculated by the method of Amin and Laskin [21] at different times pre- and post-operatively.
[a] $p < 0.05$ was considered significant.

Table 3. Saliva cytokines production in patients given ibuprofen or IMMUNEPOTENT CRP.

	Ibuprofen (days)			IMMUNEPOTENT CRP (days)		
Cytokines (pg/mL)	0	3	7	0	3	7
INF	0.92 ± 0.24	4.40 ± 1.94	1.46 ± 0.46	1.4 ± 0.52	2.64 ± 0.94	3.56 ± 1.54
TNF	3.95 ± 2.22	8.33 ± 4.77	9.64 ± 4.05	3.91 ± 1.40	5.75 ± 2.54	11.78 ± 6.49
IL-2	0.41 ± 0.26	0.64 ± 0.38	0.383 ± 0.17	0.46 ± 0.19	0.36 ± 0.13	0.60 ± 0.36
IL-4	0.14 ± 0.11	0.20 ± 0.15	0.09 ± 0.03	0.12 ± 0.08	0.09 ± 0.06	0.18 ± 0.11
IL-6	24.02 ± 17.24	438.47 ± 164.32	199.30 ± 39.18	46.81 ± 18.95	355.22 ± 161.73	468.22 ±160.63
IL-10	0.14 ± 0.11	0.99 ± 0.43	0.63 ± 0.36	0.11 ± 0.09	0.28 ± 0.16	0.81 ± 0.43

Note: Saliva cytokines production was determined by flow cytometry.
There were no statistically significant differences between groups ($p > 0.05$).

For the reduction of postoperative inflammation, it is necessary to control the synthesis of cytokines, because they participate in the process of tissue repair, chemotaxis and control of inflammation [30]. As cytokines have a very important role during inflammation, they can be used as a target to control chronic inflammation [30]. The presence of INF-γ, IL-10, IL-4 and IL-6 could play a role in the process of tissue repair, chemotaxis and control of inflammation because the healing process was successful in the patients who participated in our study. Similar results have been reported in the control of cytokines and pro-inflammatory metabolites in other studies of the inflammatory model with ibuprofen [5,6]. In the present study, the inflammatory response caused by cytokines was evaluated in the saliva, which is a representative fluid of the local microenvironment next to the area of the surgery. The flow cytometry technique was used for determination of the saliva cytokine levels because this technique has been found to be more sensitive in the detection of cytokines [31]. Well-known adverse effects of ibuprofen are: nausea, gastrointestinal bleeding, elevated liver enzymes, diarrhoea, constipation, epistaxis, headache, dizziness, rash, salt and fluid retention, and hypertension. Similar to other NSAIDs, ibuprofen may show photo-sensitising properties and also has been implicated in increasing the risk of myocardial infarction particularly in those who use high doses chronically [32]. During our study, the patients of both treatments did not present discomfort about pain related to third-molar surgery. This could probably be due to the administration of ketorolac, as Christensen et al. [33] reported that ketorolac has an analgesic effect in the third-molar surgery, and not anti-inflammatory activity.

Overall, our study showed some promising results about the potential use of IMMUNEPOTENT CRP in third-molar surgery. There are, however, some limitations that need to be mentioned: it was a preliminary trial, with just 10 patients per group. Despite this limitation, our study provides interesting results in the dental surgery field and we are now considering a larger-scale clinical trial.

Conclusions

Our results demonstrated that ibuprofen or IMMUNEPOTENT CRP treatments were efficient in the control of inflammation through cytokines modulation without having side effects in third-molar surgery, suggesting they could be considered appropriate for use in dental clinical practice. Further, larger-scale trials, however, are necessary to corroborate the anti-inflammatory properties and to investigate the wound healing effects.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Laboratory of Immunology and Virology, Faculty of Biological Sciences, Autonomous University of Nuevo Leon (San Nicolas de los Garza, NL, Mexico), in collaboration with CONACYT, 'Red Temática de Inmunología en Cancer y Enfermedades Infecciosas' [grant number 253053].

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