Archives of Oral Biology 84 (2017) 106–111

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Research paper

Anti-inflammatory effect of an adhesive resin containing indomethacin-loaded nanocapsules

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ARTICLE INFO

Keywords:
Non-steroidal anti-inflammatory agents
Indomethacin
Drug carriers
Dental bonding
Drug release

ABSTRACT

Objective: To analyze the anti-inflammatory and analgesic effects of an adhesive resin containing indomethacin-loaded nanocapsules in rat model.

Design: Adhesive resin disks with or without indomethacin-loaded nanocapsules were subcutaneously implanted into right hind paw of rats. A week after surgical procedure, 2% formalin solution was intradermally injected into plantar surface of paw. Nociceptive and inflammatory responses were evaluated by formalin test. Paw edema by pletismometer and mechanical hyperalgesia by von Frey test were performed on day 2, day 4, day 6, day 8, day 10 and day 12 after surgery. IL-6, IL-10, and lactate dehydrogenase (LDH) serum levels were determined by ELISA-sandwich test.

Results: Group containing indomethacin-loaded nanocapsules (NC) presented lower edema in the right hind paw at 24 h after formalin injection than those of the control group (CT) ($P < 0.01$). NC group showed decrease in the nociceptive response in phase I (neurogenic pain) compared to CT group (NC − 66.86 ± 22.83 s X CT − 130.17 ± 35.83s , $P < 0.001$). NC group presented supporting higher intensity of stimulus on days 8 and 12 (24 h and 72 h after formalin injection) ($P < 0.01$ and $P < 0.02$ respectively). The IL-6 serum level was also significantly higher in the NC group than CT group ($p < 0.001$).

Conclusions: These results indicate that an adhesive resin containing indomethacin-loaded nanocapsules has anti-inflammatory and nociceptive activities in a chemical model of acute inflammation. The present investigation confirms an adhesive resin with drug-loaded nanocapsules may be useful for improving therapeutic effect for adhesives to be used in deep cavities.

1. Introduction

Deep carious lesions promote inflammation progress proportionally to the cavity depth (Kassa, Day, High, & Duggal, 2009; Reeves & Stanley, 1966; Wanachantararak et al., 2016), which involves cytokines IL-2, IL-6, IL-8, and IL-10 (Elsalhy, Azizieh, & Raghupathy, 2013). Indirect pulp capping using calcium hydroxide is commonly applied to prevent pulpal inflammatory progress and irreversibility through dentin repair (Weber, Alves, & Maltz, 2011). However, indirect pulp capping has shown no improvement in long-term success rates (Casagrande, Bento, Dalpian, García-Godoy, & De Araujo, 2008; Casagrande, Bento, Dalpian, García-Godoy, & De Araujo, 2010; Falster, Araujo, Straffon, & Nör, 2002). Further, calcium hydroxide presents low compressive strength (Mohammadi & Dummer, 2011) and high solubility, including degradation by acid etching (Heitmann & Utiterbrink, 1995).

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http://dx.doi.org/10.1016/j.archoralbio.2017.09.016
Received 8 November 2016; Received in revised form 15 September 2017; Accepted 19 September 2017

Fig. 1. Experimental design. : Paw edema and Von Frey test. : Interventions: surgery and formalin injection. : Death.

Previous studies have analyzed the modification of capping agents to achieve anti-inflammatory effects (Liu, Jiang, Wang, & Wang, 2013; Louwakul & Lertchirakarn, 2015). An adhesive resin containing indomethacin-loaded nanocapsules has demonstrated controlled release of non-steroidal anti-inflammatory drugs (NSAID) and the permeability of indomethacin through dentin (Genari et al., 2016).

The anti-inflammatory agents commonly used include both steroidal drugs and NSAIDs. One example of a NSAID is indomethacin. The anti-inflammatory effect of NSAIDs is mainly mediated by the inhibition of cyclooxygenases 1 and 2 (COX-1 and COX-2), and they decrease the formation of prostaglandins (Summ & Evers, 2013). Orally administered NSAIDs also have demonstrated an analgesic effect, thus decreasing endodontic treatment-related pain (Lapidus et al., 2016). NSAIDs have the potential to depress the sensory responses of the nociceptive system by central action (Bustamante, Paeile, Willer, & Le, 1996; Jurna & Brune, 1990). The depression of the afferent C-fiber reflex is the main antinociceptive path of NSAIDs (Bustamante et al., 1996; Jurna & Brune, 1990). The analgesic and anti-inflammatory effects of a NSAID are directly related to its concentration at the required site of action. Pharmacokinetic properties, such as half-life rates and time needed to deliver active metabolites, are critical to a NSAID's effects (Bustamante et al., 1996).

The non-specific distribution of a drug leads to its high concentration in no target sites, which in turn leads to low effect and toxicity (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). One method of restricting the drug to the required site is to employ a carrier system. Nanoparticles have received considerable attention as potential drug delivery vehicles over the past few years. The use of a carrier system, which is related to controlled release, modifies the bioavailability of drugs. NSAID-loaded nanocapsules have presented an increase of efficacy, which is associated with a reduction in adverse effects (Bernardi et al., 2009; Guterres, Muller, Michalowski, Pohlmann, & Dalla Costa, 2001).

Animal models of acute inflammation are commonly used to assess the anti-inflammatory properties of agents (Blattes et al., 2017; McCarson, 2015). Intradermal formalin injection into the rat hindpaw is widely used as a nociceptive stimulus in rats, and it is a classical and valid model used to study acute inflammation and pain for different inflammatory diseases (Barth et al., 2016; Bernardi et al., 2009; Dubuisson & Dennis, 1977; Kawamura et al., 2000; McCarson, 2015). The injection of agents causes rapid development of edema and an exacerbated sensitivity to stimuli (McCarson, 2015; Rocha, Fernandes, Quintão, Campos, & Calixto, 2006). Thus, formalin-induced rat paw edema and responses to mechanical and thermal stimuli are widely used to characterize the action mechanisms of new anti-inflammatory drugs or formulations, including NSAIDs (Bernardi et al., 2009; Kawamura et al., 2000; McCarson, 2015).

Therefore, the purpose of this study was to analyze the anti-inflammatory and analgesic effects of a resin containing indomethacin-loaded nanocapsules in acute inflammatory animal models.

2. Materials and methods

2.1. Animals

A total of 20 adult male Wistar rats (55–65 days old; weight 200–250 g) were used. The animals were randomized by weight and housed in groups of three per polypropylene cage (49 cm × 34 cm × 16 cm) with sawdust-covered flooring. All animals were maintained in a controlled environment (22 ± 2 °C) under a standard light-dark cycle (lights-on at 7 a.m. and lights-off at 7 p.m.), with water and chow (Nuvital, Porto Alegre, Brazil) ad libitum. All experiments and procedures were approved by the institutional Animal Care and Use Committee (UFRGS protocol no. 28648) and performed in accordance with the Guide for the Care and Use of Laboratory Animals, 8th ed., 2001 and Law 11794 (Brazil). The experimental protocol complied with the ethical and methodological standards of the Animal Research: Reporting of In Vivo Experiments guidelines (ARRIVE) (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010). The experiment involved only a sufficient number of animals necessary to produce reliable scientific data.

2.2. Experimental design

Rats were acclimated to the maintenance room for 1 week before the experiment began. Animals were divided into two groups: adhesive resin disk without nanocapsules (control − CT), and adhesive resin disk containing indomethacin-loaded nanocapsules (nanocapsule − NC). Each animal surgically received a disk of resin (3 × 1 mm, 0.7 g) with or without nanocapsules into the plantar surface of the right hind paw. A week after the surgical procedure, 0.17 mL/kg of a 2% formalin solution, Formaldehyde P.A.® (Sigma-Aldrich, São Paulo, Brazil) diluted in 0.9% NaCl (saline), was intradermally injected into the plantar surface of the right hindpaw. The rats were killed by decapitation 13 days after surgery. The experimental design of study is shown in Fig. 1. For all procedures, the examiner was blinded to the groups of rats being tested.

2.3. Preparation of nanocapsules

Indomethacin-loaded NCs were prepared by the interfacial deposition of preformed polymer technique (Genari et al., 2016). All reagents were purchased from Sigma Chemical (St. Louis, MO, USA). The organic phase was prepared with Eudragit® S100, poly (MMA-co-MAA) (0.50 g), indomethacin (0.05 g), medium chain triglycerides (0.81 mL) and sorbitan monostearate (0.19 g) dissolved in acetone (125 mL). Under magnetic stirring at 25 °C, the organic phase was added through a funnel to an aqueous phase containing polysorbate 80 (0.385 g) and water (250 mL). Acetone and water excess were eliminated using a rotary evaporator (Rotavapor II, BUCHI, Flawil, Switzerland), a B-740 recirculating chiller (BUCHI, Flawil, Switzerland), and a U-700 vacuum pump (BUCHI, Flawil Switzerland). The IndOH-NC suspension was spray-dried (B-290, BUCHI, Flawil, Switzerland) using hydrophilic-fumed silicon dioxide (Aerosil® 200) as an adjuvant, in the amount of 3% of the suspension content. The inlet temperature in the drying chamber was maintained at approximately 150 ± 4 °C, and the outlet temperature was 107 ± 4 °C. The characterization of NCs is described in Genari et al., (2016). The mean particle size of indomethacin-loaded NCs was 165 nm. Indomethacin content was 7 mg drug/g powder. In 120, an amount of 13.83% of indomethacin was released.