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Development and evaluation of novel biodegradable chitosan based metformin intrapocket dental film for the management of periodontitis and alveolar bone loss in a rat model

Deepak Kumar Khajuria[a,b,*], Omprakash Nandikamba Patil[b], David Karasik[a], Rema Razdan[b]

[a] The Musculoskeletal Genetics Laboratory, Faculty of Medicine in the Galilee, Bar-Ilan University, Safed, Israel
[b] Department of Pharmacology, Al-Ameen College of Pharmacy, Bangalore, India

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ABSTRACT

Objective: The aim of this study was to develop a chitosan-metformin based intrapocket dental film (CMIDF) for applications in the treatment of periodontitis and alveolar bone loss in an rat model of periodontitis.
Design: CMIDF inserts were fabricated by the solvent casting technique. The fabricated inserts were evaluated for physical characteristics such as folding endurance, surface pH, mucoadhesive strength, metformin content uniformity, and release. X-ray diffraction analysis indicates no crystallinity of metformin in presence of chitosan which confirmed successful entrapment of metformin into the CMIDF. Fourier-transform infrared spectroscopy revealed stability of CMIDF and compatibility between metformin and chitosan. Periodontitis was induced by a combination of *Porphyromonas gingivalis*- lipopolysaccharide injections in combinations with ligatures around the mandibular first molar. We divided rats into 5 groups (8 rats/group): healthy, untreated periodontitis; periodontitis plus CMIDF-A (1.99 ± 0.09 mg metformin; total mass-4.01 ± 0.05 mg), periodontitis plus CMIDF-B (2.07 ± 0.06 mg metformin; total mass-7.56 ± 0.09 mg), and periodontitis plus chitosan film (7.61 ± 0.08 mg). After four weeks, mandibles were extracted to evaluate alveolar bone loss by micro-computerized tomography and histological techniques.
Results: Alveolar bone was intact in the healthy group. Local administration of CMIDF resulted in significant improvements in the alveolar bone properties when compared to the untreated periodontitis group. The study reported here demonstrates that novel CMIDF showed good antibacterial activity and effectively reduced alveolar bone destruction in a rat model of experimental periodontitis.
Conclusions: Novel CMIDF showed good antibacterial activity and improved alveolar bone properties in a rat model.

1. Introduction

Periodontitis is an oral disease which is characterized by gingival bleeding, loss in connective tissue connection, development of periodontal pocket and alveolar bone loss. Dental plaque bacteria initiate periodontitis, but abnormal host defenses to bacterial pathogens play an important role in its progression (Kuula et al., 2009). According to National Oral Health Survey, periodontal disease in India increases with age. The pervasiveness was 57%, 67.7%, 89.6% and 79.9% in the age groups 12, 15, 35–44 and 65–74 years, respectively (Shaju, Zade, & Das, 2011). Alveolar bone resorption produces the most serious, irreversible damage associated with periodontitis. Thus, osteogenic and/or anti-resorptive drugs are generally considered significant in the therapy of periodontitis.

Metformin is a widely accepted anti-diabetic drug for type-2 diabetes (Kim et al., 2008; Skamagas, Breen, & LeRoith, 2008). Previous reports suggests that metformin also possess antimicrobial (Ochoa-Gonzalez et al., 2016), antiendometriotic (Zhou et al., 2015), anti-atherogenic (Mamputu, Wiernsperger, & Renier, 2003), antitumor (Della Corte et al., 2016) and antiobesity activity (Kim et al., 2016). A recent report by Cameron et. al., suggests that metformin showed anti-inflammatory effects independently of its effect on glycemic control (Cameron et al., 2016). Studies further illustrated that metformin stimulates genesis of osteoblast cells *in-vitro* (Cortizo, Sedlinsky, McCarthy, Blanco, & Schurman, 2006). Bak et al., reported in a small study that intraperitoneal injection of metformin (10 mg/kg, for 10 days) reduced alveolar bone destruction in ligature-induced periodontitis rat model. Separate *in-vitro* studies showed that metformin

* Corresponding author at: The Musculoskeletal Genetics Laboratory, Faculty of Medicine in Galilee, Bar-Ilan University, H. Szold St. 14, POB 1589, Safed 1311502, Israel.
E-mail address: deepak.kumarkhajuria@gmail.com (D.K. Khajuria).

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increased osteoblast differentiation (Bak et al., 2010). A randomized, controlled clinical trial showed that the local delivery of 1% metformin gel into the periodontal pocket in chronic periodontitis patients stimulated a significant improvement in the intrabony defects (Pradeep et al., 2016). Further, a recent study by Araujo et al., showed that metformin, decreases the inflammatory response, oxidative stress and bone loss in ligature-induced periodontitis in rats (Araujo et al., 2017).

On the other hand, chitosan is an innate polysaccharide which has gained great attention in the field of dentistry because of its bioactive properties like, wound healing, antimicrobial (Dai, Tanaka, Huang, & Hamblin, 2011), anti-inflammatory (Davydova et al., 2016), hypertension control (Park et al., 2009) and tissue regeneration activities (Hurt, Kotha, Trivedi, & Coleman, 2015). It has been established that chitosan has a profound role towards impeding colonization of pathogenic strains (such as *Streptococcus mutans* and *Porphyromonas gingivalis*) on tooth surfaces without disrupting normal oral flora (Husain et al., 2017). Boynuegri et al., demonstrated promising effects of chitosan gel on periodontal regeneration in human patients (Boynuegri et al., 2009). Furthermore, chitosan has high osteoinductivity and osteo-integrability that make it a good candidate for bone regeneration (Ezoddini-Ardakani et al., 2012; Hoemann et al., 2007; Lee et al., 2002).

In the present study, we employed a combination of *Porphyromonas gingivalis*- lipopolysaccharide (*Pg*-LPS) injections combined ligature induced periodontitis animal model (Cochran, 2008; Lee, Lin, Fong, Ryder, & Ho, 2013). As *Pg*-LPS combined ligature-induced periodontitis involves periodontium inflammation and alveolar bone loss, it is possible that chitosan-metformin based intrapocket dental film (CMIDF) might be effective in limiting periodontal inflammation as well as alveolar bone destruction in periodontitis due to the complementary mechanism of action of chitosan and metformin.

Thus, to characterize the pharmacological effect of CMIDF on alveolar bone destruction in periodontitis, we evaluated the effect of CMIDF on *Pg*-LPS combined ligature-induced experimental periodontitis in rats. Further, the antibacterial efficacy of CMIDF was evaluated *in-vitro* on selected strains of *Porphyromonas gingivalis* and *Tannerella forsythia*.

2. Materials and methods

2.1. Materials

Metformin hydrochloride and chitosan (from crab shells) were obtained from Sigma (St. Louis, MO, USA). The forms of LPS used in this study were *Pg*-LPS (InvivoGen, San Diego, CA, USA). The microorganisms used in the study were standard strains of *P. gingivalis* (ATCC 33277) and *T. forsythia* ATCC 43037. All other chemicals used were of analytical grade.

2.2. Fabrication of CMIDF inserts

Casting method was used to prepare metformin loaded chitosan inserts (Barat, Srinatha, Pandit, Anupurba & Mittal, 2007). Chitosan solution was produced by mixing a measured quantity of chitosan in 2% acetic acid. This chitosan solution was filtered by using a muslin cloth to separate insoluble chitin. Glycerine (0.5%) as a plasticizer was added drop wise to the chitosan solution over 45 min with continued stirring. The solution was stirred for a further 2 h, and then allowed to stand overnight before casting into thin films in the Petri dish and dried at room temperature. These films were dipped into glutaraldehyde solution for 15 min for cross-linking. To prepare CMIDF-A and CMIDF-B, two different ratios of metformin and chitosan were taken i.e. 1:75 and 1:125, respectively, as shown in Table 1. Based on our preliminary results (not reported), metformin and chitosan in 1:75 and 1:125 ratio respectively *appeared to be the major factor controlling the release rate of metformin and prolonged biological activity of metformin and chitosan in the* CMIDF. The prepared CMIDF-A and CMIDF-B were divided into small inserts (2 × 1 mm). Dried CMIDF inserts were stored in small amber colored glass bottles.

Table 1
Composition of the fabricated chitosan and CMIDF inserts by casting method in 2% (V/V) glacial acetic acid.

Formulation code	Chitosan (mg cm^{-1})	Metformin hydrochloride (mg per insert)	Glycerine (% m/m)	Glutaraldehyde (% m/m)
CMIDF-A	150	2	0.5	5
CMIDF-B	250	2	0.5	5
Chitosan	250	–	0.5	5

2.3. Characterisation of CMIDF inserts

2.3.1. Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) analysis

FTIR spectroscopy analysis (Nicolet-Nexus 670 FTIR spectrometer, Nicolet Instrument Corporation, Madison, Wisconsin, USA) was used to record the spectra of pure metformin, chitosan film and CMIDF prepared in the potassium bromide in the region of 4000–400 cm^{-1} with 2 cm^{-1} resolution. XRD analysis was used to determine the physical form (crystalline or amorphous) of the individual components present in the CMIDF. The crystallographic structural analysis was carried out by using a Bruker D8 Advance X-Ray powder diffractometer (Bruker, Karlsruhe, Germany) with monochromatic Cu Kα radiation over the 2Θ range of 0–80° at a scan rate of 0.02°/min.

2.3.2. Thickness and weight variation

Film thickness was measured by using a digital micrometer. Three randomly selected films of CMIDF-A and CMIDF-B having 40 mm^2 surface area were used. To determine weight variation, 10 patches from both formulations were weighed separately on an electronic balance (Shimadzu, Kyoto, Japan) and the average weight of the patch was calculated (Cherukuri, Batchu, Mandava, Cherukuri, & Ganapuram, 2017). The individual values allowed were within ± 5% of the mean.

2.3.3. Drug content uniformity

The concentration uniformity of metformin in chitosan was determined by ultraviolet spectrophotometer. Rectangular film inserts (2 × 1 mm) were taken from different areas of the prepared CMIDF-A and CMIDF-B, and placed into a 25 ml volumetric flask. The CMIDF-A and CMIDF-B were weighed and transferred into a 100 ml volumetric flask, which was diluted up to the mark with distilled water. This mixture was stirred for 24 h to allow the total release of the metformin from the CMIDF-A and CMIDF-B. After filtration, the filtrate was assayed using ultraviolet spectrophotometer at 232 nm wavelength (Arayne, Sultana, Zuberi, & Siddiqui, 2009).

2.3.4. Surface pH

For determination of surface pH, CMIDF-A, CMIDF-B or chitosan film was allowed to swell for 2 h on the surface of agar plates prepared in McIlvaine buffer at pH 6.6. The surface pH was measured using pH paper placed on the surface of the wetted CMIDF-A and CMIDF-B patch. A mean of the three readings was recorded (Nafee, Boraie, Ismail, & Mortada, 2003).

2.3.5. Folding endurance

The folding endurance of the CMIDF-A, CMIDF-B or chitosan film was determined by repeatedly folding the film at 180° angle of the plane at the same spot until it breaks or folded to 300 times without breaking. The number of times the CMIDF-A and CMIDF-B folded without breaking is considered as folding endurance (Dixit, Uplana, Patel, Dixit, & Patel, 2010).