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Topical treatment of the buccal mucosa and wounded skin in rats with a triamcinolone acetonide-loaded hydrogel prepared using an electron beam

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ABSTRACT

In this study, a triamcinolone acetonide-loaded hydrogel was prepared by electron beam irradiation and evaluated for use as a buccal mucoadhesive drug delivery system. A poloxamer was modified to have vinyl end groups for preparation of the hydrogel *via* an irradiation cross-linking reaction. Carbopol was introduced to improve the mucoadhesive properties of the hydrogel. The *in vitro* release of triamcinolone acetonide from the hydrogel was examined at 37 °C. To investigate the topical therapeutic effect of triamcinolone acetonide on wounded rat skin and buccal mucosa, the appearance and histological changes were evaluated for 15 days after treatment with saline, triamcinolone acetonide solution, triamcinolone acetonide hydrogel, and blank hydrogel, respectively. Triamcinolone acetonide was released constantly from the gel formulation at 37 °C and reach 100% at about 48 h. After 15 days, in the skin of the group treated with the triamcinolone acetonide-loaded hydrogel, the wound was almost completely free of crust and a number of skin appendages, including hair follicles, had formed at the margins of the tissue. Moreover, the inflammatory response in the buccal mucosa was milder than that in the other groups, and the wound surface was completely covered with regenerating, hyperkeratotic, thickened epithelial cells. Our results indicate that the triamcinolone-acetonide hydrogel showed sustained drug release behavior, while causing no significant histopathological changes in buccal and skin tissues. Therefore, this hydrogel system may be a powerful means of drug delivery for buccal administration with controlled release and no tissue irritation.

1. Introduction

The buccal administration of drugs has several advantages over oral administration, including the avoidance of presystemic metabolism in the gastrointestinal tract and hepatic first-pass elimination (Shinkar et al., 2012). The buccal mucosa has a number of advantages, including high permeability and high blood flow (Patel et al., 2012), and is easily accessible for both the application and removal of a drug delivery device. Given these advantages, the buccal mucosa has been investigated as a potential site for the controlled delivery of hydrophilic macromolecules as well as small compounds.

Poloxamers are thermosensitive triblock copolymers composed of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly(propylene oxide) (PPO) that are classified according to their composition ratio and molecular weight. Among poloxamers, poloxamer 407 has been used in topical formulations for drug delivery due to its thermosensitive properties, low toxicity, high solubilizing capacity, and good release properties (Fusco et al., 2006; Shin et al., in press). The aqueous solutions (>18% w/v) of poloxamer 407 are a low viscosity solution (sol) at room temperature, but undergo a reversible sol–gel transition above a critical temperature, during which micelles are formed through hydrophobic interactions between the PPO blocks *via* the breakage of hydrogen bonds (Alexandridis and Hatton, 1995) and become packed together (Chung et al., 2008; Han et al., 2003). However, poloxamer-based hydrogels are biodegradable in the presence of excess water due to dissociation of the consistently packed micelles (Chun et al., 2004; Lee et al., 2004). Therefore, they show very weak mechanical properties and a phase inversion due to dilution by physiological fluids or foods when attached to the buccal mucosa (Chung et al., 2008; Kweon et al., 2003). To overcome these problems, it is necessary to enhance the physical stability and mechanical strength of hydrogels by chemical cross-linking.

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However, poloxamer itself cannot be cross-linked since it has no functional group to irradiation. Therefore, many researchers have modified poloxamer for cross-linking reaction to reinforce the gel's physical strength and mechanical properties (Baek et al., 2012; Choi and Yoo, 2010; Chun et al., 2004; Lee and Tae, 2007).

In general, hygrogels are prepared by the cross-linking of hydrophilic polymers (Hennink and van Nostrum, 2002). Most chemical cross-linking procedures make use of a cross-linking agent and initiator, which may remain as toxic residuals even after the reaction. From this perspective, cross-linking induced by radiation offers several advantages, including no need for cross-linking agents, initiators, and the removal of residual moieties, as well as simultaneous sterilization through the cross-linking process, which makes the product suitable for medical applications (Hennink and van Nostrum, 2002; Lugao and Malmonge, 2001). Moreover, the phase of the polymers is unimportant (solid or liquid), and the irradiation of samples for cross-linking is independent on temperature. Common cross-linking processes require long reaction times, while cross-linking by irradiation takes only a short time. As physical properties can be controlled by modulation of the strength and time of irradiation and hydrogels with various types and shapes can be fabricated using molds, radiation technology has been extensively used for both biomedical and other industrial applications (Baek et al., 2012; Choi et al., 2007; Lugao and Malmonge, 2001).

Triamcinolone acetonide, a long-acting synthetic glucocorticoid, has been used for the treatment of oral inflammatory disease by the parenteral route (González-García et al., 2006). Due to its therapeutic use in diseases of the oral mucosa, triamcinolone acetonide has attracted a great deal of research interest with regard to administration through the buccal mucosa, both *in vitro* (Shin and Kim, 2000; Ungphaiboon and Maitani, 2001) and *in vivo* (Ali et al., 1998; Shin et al., 2000).

Here, we prepared a triamcinolone acetonide-loaded hydrogel formulation by radiation-induced cross-linking to enhance the physical stability and mechanical strength of typical poloxamer based hydrogels. The release of triamcinolone acetonide from the hydrogel was investigated. Moreover, histological changes in wounded skin and the buccal mucosa were compared for 15 days after application of the hydrogel to the dorsal skin and buccal mucosa of rats.

2. Materials and methods

2.1. Materials

Pluronic F127 (poloxamer 407, MW 12600) was a gift from BASF Corp. (Ludwugschafen, Germany). Carbopol 934P was obtained from Wako Pure Chemical Industries (Osaka, Japan). Triamcinolone acetonide, acryloyl chloride, and triethylamine were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of the hydrogel formulation

Thermoreversible hydrogels were prepared using the cold method (Shin et al., in press). Briefly, diacrylated poloxamer (D-Pol) and triamcinolone acetonide were dissolved in distilled water as described previously (Baek et al., 2012). The D-Pol was synthesized by the addition of an acryloyl group to poloxamer 407 (Pol) according to a previously reported method (Baek et al., 2012). To increase the mixture's mucoadhesive properties, carbopol was added to the D-Pol solution. The concentrations of D-Pol and carbopol were 20 and 1%, respectively. The formulation included triamcinolone acetonide at a concentration of 1 mg/mL. The mixture solution was kept at 4 °C until a clear solution was obtained. After gelation 37 °C, 3 mL of the prepared gel formulation were placed in Petri dishes ($35\ mm^2 \times 10\ mm$) and covered with a lid. Irradiation with an electron beam was performed using a linear accelerator (UELV-10-10S, NIIFFA, Moscow, Russia); the dose of irradiation was 25 kGy for 2.5 h (10 kGy/h). After irradiation, an *in vitro* release test and *in vivo* histological observations were performed.

2.3. In vitro release

In vitro drug release from the hydrogel was investigated at 37 °C for 48 h using a Franz diffusion cell (Logan Instruments, Somerset, NJ, USA). Samples were placed in Franz diffusion cells with a permeation area of 0.785 cm^2, and 10 mL of PBS (pH 7.4) were added to the acceptor chamber. For the cross-linked polymer samples, additional swelling time was not considered because the samples were not subjected to drying. After preincubation for 20 min, a formulation equivalent to 0.3 mg of triamcinolone acetonide was placed in the donor chamber. At predetermined time points (0.5, 1, 2, 4, 8, 12, 24, and 48 h), 1 mL of sample was withdrawn from the acceptor compartment, and the sampled volume was replaced with PBS (pH 7.4). The withdrawn samples were filtered and analyzed. The amount of drug was determined by UV–visible spectrophotometry at 240 nm.

2.4. Animal experiments

Twenty-six Sprague–Dawley rats obtained from Orient Bio Inc. (Sungnam, Korea) were maintained under specific pathogen-free conditions and provided with water and standard chow (Jeil Feed Co., Daejeon, Korea) *ad libitum*. All experimental procedures were performed in accordance with NIH Guidelines for the Care and Use of Laboratory Animals.

For the skin model, 18 rats were anesthetized with xylazine (2–5 mg/kg) and ketamine (40–50 mg/kg) administered intramuscularly. After shaving and cleaning with a tincture of iodine, the backs of the animals were wounded twice on each side using a biopsy punch 6 mm in diameter (Miltex, Inc., York, PA) on both sides of the spinal cord (Yamada et al., 2012). The dorsal skin samples were excised and removed with surgical scissors and forceps. Over 3 days, the wounds were dressed with normal saline (control), 0.1% triamcinolone acetonide solution, 0.1% triamcinolone acetonide-loaded hydrogel, or blank hydrogel for 8 h daily under moist and sterile conditions. On the days 1, 2, 5, 8, 11, and 15, scar tissues were excised to investigate the histopathological features.

For the buccal model, eight rats were anesthetized as described above. Ulceration was induced by excision of the mucosal membrane using a biopsy punch 4 mm in diameter (Miltex) on the left cheek (Cavalcante et al., 2011). The animals were observed daily and assigned randomly to four groups, which were treated with normal saline, 0.1% triamcinolone acetonide solution, 0.1% triamcinolone acetonide-loaded hydrogel or blank hydrogel, respectively, for 8 h daily for 2 days and sacrificed on the days 2 and 8.

2.5. Histological study

Lesions from the dorsal skin and oral cavity obtained by necropsy were fixed in formalin and embedded in paraffin for routine histological processing. Sections (4-μm thick) obtained from each paraffin block were stained with hematoxylin and eosin.

3. Results and discussion

3.1. Preparation of drug-loaded hydrogel formulation

In this study, poloxamer hydrogel was prepared by electron beam irradiation and evaluated for potential application in the