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Development of a novel triamcinolone acetonide-loaded spray solution for the treatment of stomatitis

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To develop a novel triamcinolone acetonide (TAA)-loaded spray for the treatment of stomatitis, several spray solutions were prepared using various amounts of TAA, Eudragit® L100 (Eudragit L) and PEG 400, and 100 ml ethanol. Their viscosity and spraying potential were investigated, with the result that the spraying threshold was 9.5 cP. The effect of PEG 400 on the properties of films formed after spraying was assessed. Its anti-inflammatory effect in mice was evaluated and compared to a commercial product. As the PEG 400 concentration increased, the film elongation and washability by the saliva solution increased, and tensile strength decreased. PEG 400 had little effect on mucoadhesive force and drug release. The TAA-loaded spray solution containing TAA, Eudragit L, PEG 400 and ethanol at the ratio of 1:6:3:100 (w/w/w/v) was easy to spray onto stomatitis lesions in the mouth *via* a spraying vessel incorporating a long straw. After spraying, the TAA-loaded spray formed a film with suitable elongation, tensile strength and washability that attached onto the mucosal membrane and released the drug. Moreover, it had excellent anti-inflammatory properties, similar to those of the commercial product. Thus, this novel TAA-loaded spray solution was easy to administer, had good film properties and excellent anti-inflammatory efficacy, and is therefore a potential candidate for the treatment of stomatitis.

1. Introduction

Stomatitis is a relatively common chronic dermatological disease that frequently distresses the oral mucosa. Although the cause of stomatitis has not yet been identified, current evidence indicates that it is an immunologically mediated mucocutaneous disorder (Hanawa et al. 2004). The symptoms of stomatitis comprise distinct, painful, thin and persistent ulcers measuring 3 mm to less than 3 cm in diameter. The lesions tend to remain for 2 to 3 weeks. Moreover, recurrences are common following shock, allergy, emotional anxiety and hormonal deviations (Vyas et al. 2006). Stomatitis can be particularly painful in the oral mucosa, where it can interfere with eating, speaking and swallowing (Zacharias et al. 2011). Among the numerous treatments for stomatitis, triamcinolone acetonide (TAA), a long-acting synthetic glucocorticoid, has recently been used to treat inflammatory conditions including stomatitis (Peng et al. 2010; Sabzevari et al. 2013). Various TAA-loaded pharmaceutical formulations such as ointments (Choi et al. 2013; Paulo et al. 2000), gels (Shin et al. 2000; Yang et al. 2005; Yang et al. 2006), tablets (Ali et al. 1998) and patches (Chun et al. 2003) have been studied. The ointment and buccal tablet formulations have been given the brand names Oramedy® (Dongkook ***Pharm. Co.;*** Suwon, South Korea) and Aftach® (Dongwha ***Pharm. Co.;*** Yong-In, South Korea), respectively. However, these formulations are difficult to apply to the oral mucosa and to attach to the stomatitis lesions for a sufficient period of time, because they are easily removed by salivation, tongue movement and swallowing (Choi and Kim 2000; Shin et al. 2000; Yang et al. 2005).

With the aim of overcoming these problems, a novel triamcinolone acetonide-loaded spray solution was developed for this study. Several spray solutions were prepared using various amounts of TAA, Eudragit® L100 (Eudragit L) and PEG 400, and 100 ml ethanol, and then their viscosity and spraying potential were measured. The effect of PEG 400 on the properties of films formed after spraying these solutions using a spraying device was investigated. Their anti-inflammatory efficacy in inflammation-induced mice was also evaluated compared to that of a commercial ointment.

2. Investigations, results and discussion

In this study, a spraying vessel incorporating a long straw was used to spray the TAA-loaded solutions. This straw was similar in shape to the NM17 Actuator (Presspart Manufacturing Co., Lancashire, UK). By spraying using this vessel, only the specific wound areas were treated, avoiding spraying any unaffected regions. When using the vessel without a straw or with a relatively short one, it was difficult to apply accurately and the spray was deposited on areas around the lesion. In the development of TAA-loaded spray solutions, Eudragit L (Kulthe et al.

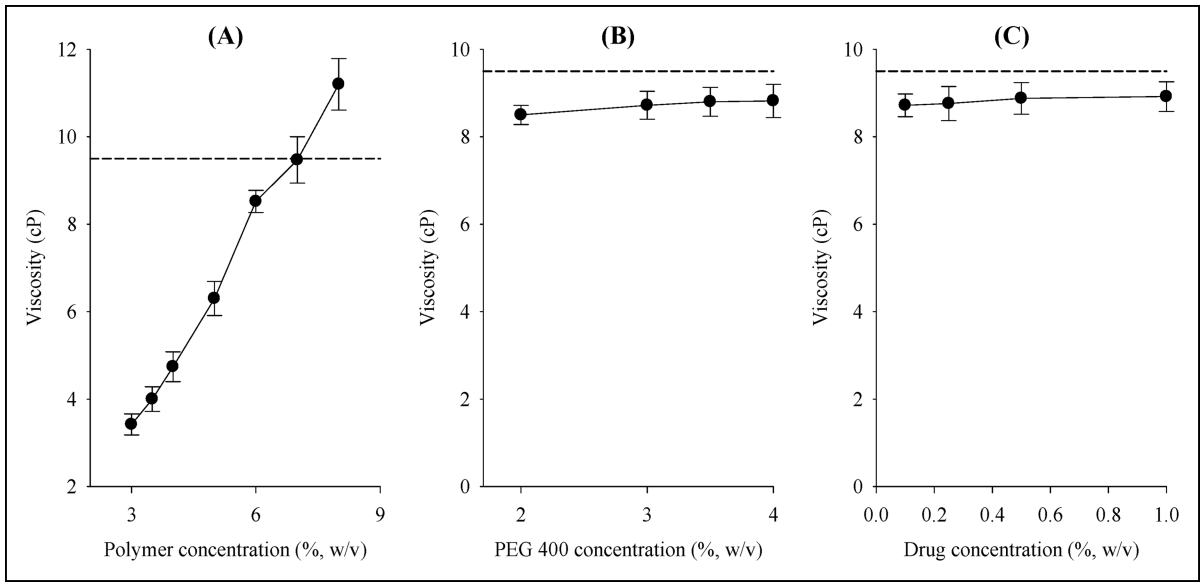

Fig. 1: Effect of ingredients on the viscosity of TAA-loaded spray solution: (A) Eudragit L; (B) PEG 400; (C) drug. Each value represents the mean ± S.D. (n = 3).

2013; Park et al. 2012) and PEG 400 (Bruce et al. 2011; Kianfar et al. 2012) were used as a film-forming polymer and plasticizer, respectively. Eudragit L, a copolymer of methacrylic acid and methyl methacrylate, gives tremendous mucoadhesive characteristics and extraordinary viscosity when dissolved in solution (Choi et al. 2000; Yun et al. 1999).

In this study, solutions containing 3–8% Eudragit L in ethanol were prepared and their ease of spraying was investigated. The ethanol solution containing 8% Eudragit L was hard to spray. Ethanol solutions containing less than 7% Eudragit L could be sprayed. However, at higher concentrations spraying was impossible due to the relatively high viscosity. The viscosity of the ethanol solution containing 7% Eudragit L was about 9.5 cP, which was taken as the threshold for spraying in this study.

Figure 1 shows the influence of Eudragit L, PEG 400 and drug on the viscosity of the spray solution. The ethanol solutions containing 3–8% Eudragit L were manufactured and their viscosities were assessed, indicating that Eudragit L had a large effect on the viscosity of the ethanol solution (Fig. 1A) (Kulthe et al. 2013; Park et al. 2012). Secondly, to evaluate the effect of PEG 400 on the viscosity, the solutions were also prepared with 6% Eudragit L and 0–4% PEG 400, and their viscosity was investigated. PEG 400 had a small effect on viscosity, but this was not significant (Fig. 1B). Finally, the viscosities of the TAA-loaded spray solutions prepared with 6% Eudragit L, 4% PEG 400 and 0–1% TAA were also determined, revealing that the drug had little effect on the viscosity of the spray solution (Fig. 1C). All ethanol solutions containing less than 7% Eudragit L could be sprayed, as they were below the spraying threshold of 9.5 cP. However, in this study, the concentration of Eudragit L was fixed at 6%, to avoid being too close to the threshold value. Figure 2 shows the effect of PEG 400, a plasticizer, on the mechanical properties of the TAA-loaded film. Plasticization of the polymer decreased the polymeric intermolecular interfaces, improving the freedom of movement of the polymeric molecules. Consequently, the deformability of the film increased (Bruce et al. 2011). As the PEG 400 concentration increased, the film elongation increased (Fig. 2A), particularly above 3% PEG 400. The tensile strength was not significantly affected up to 3% PEG 400, above which it diminished dramatically (Fig. 2B). Biocompatible films such as buccal film and epidermal film must have excellent elasticity and strong tensile

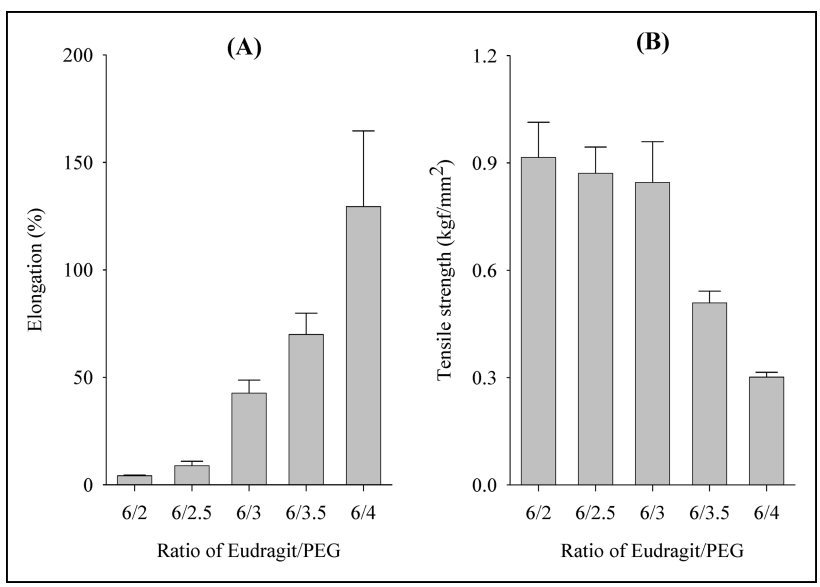

Fig. 2: Effect of PEG 400 on the elongation (A) and tensile strength (B) of TAA-loaded spray film. Each value represents the mean ± S.D. (n = 3).

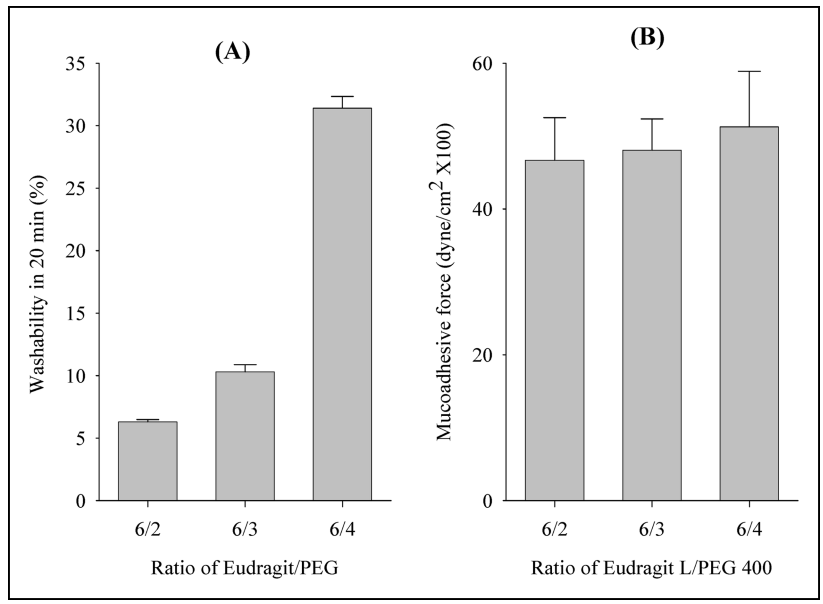

Fig. 3: Effect of PEG 400 on the washability (A) and mucoadhesive force (B) of TAA-loaded film.

strength in order to retain their film form (Choi et al. 2013: Lim et al. 2010). However, because elasticity and tensile strength are opposing properties, the films have anything to give not highest but suitable mechanic properties. Thus, among the formulations tested in this study, the TAA-loaded spray prepared with 3% PEG 400 produced the film with the most suitable elasticity and tensile strength.

Washability and mucoadhesion are two important factors in the development of TAA-loaded films. When sprayed onto the buccal or tongue membrane, the film can spread with adhesion onto the mucous membrane, and needs to remain in place for a sufficient amount of time to protect the lesion. Furthermore, the film must not be rapidly washable by the saliva solution or become detached until the drug is entirely released or absorbed in the mouth (Yong et al. 2001). In other words, rapid washability by the simulated saliva solution and detachment from the mucous membrane reduce drug efficacy. Figure 3A demonstrates how the washability of the film was affected as a function of increasing PEG 400. PEG 400 greatly increased the film's washability by the simulated saliva solution over 20 min. PEG 400, a water-soluble plasticizer, dissolves in the saliva, but Eudragit L, a water-insoluble polymer, does not dissolve.

The film prepared with Eudragit L demonstrated a strong mucoadhesive force exceeding 4000 dyne/cm^2 (Fig. 3B). Since the mucous membranes consist of oligosaccharide chains containing sialic acid, polymers with hydrophilic groups can bind strongly to these oligosaccharide chains, resulting in a strong mucoadhesive force (Choi et al. 1998; Yun et al. 1999). Eudragit L exhibits a strong mucoadhesive force due to its carboxylic groups, which can form hydrogen bonds with the sialic acid of the oligosaccharide chains on the mucous membranes (Kulthe et al. 2013; Park et al. 2012). It was predicted that, like Eudragit L, the PEG 400 plus ethylene glycol formulation might produce an excellent mucoadhesive force due to its capacity to form hydrogen bonds (Kianfar et al. 2012). However, in this study, PEG 400 had little effect on the mucoadhesive force of the film. Indeed, the film containing PEG 400 with ethylene glycol showed a slight, but not significant, improvement in this

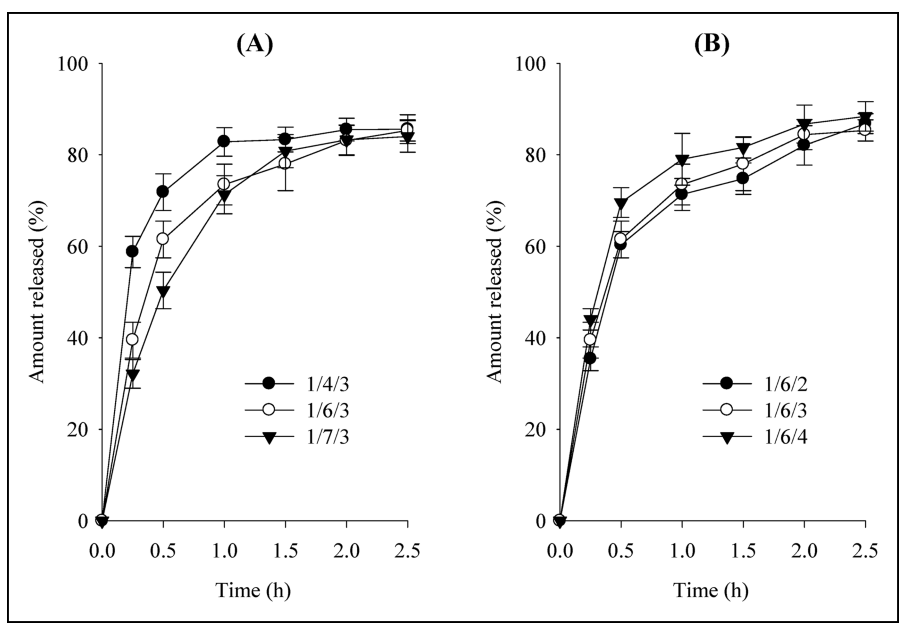

Fig. 4: Effect of Eudragit L (A) and PEG 400 (B) on drug release. Each value represents the mean ± S.D. (n = 6). TAA-loaded films were composed of TAA, Eudragit L and PEG 400.

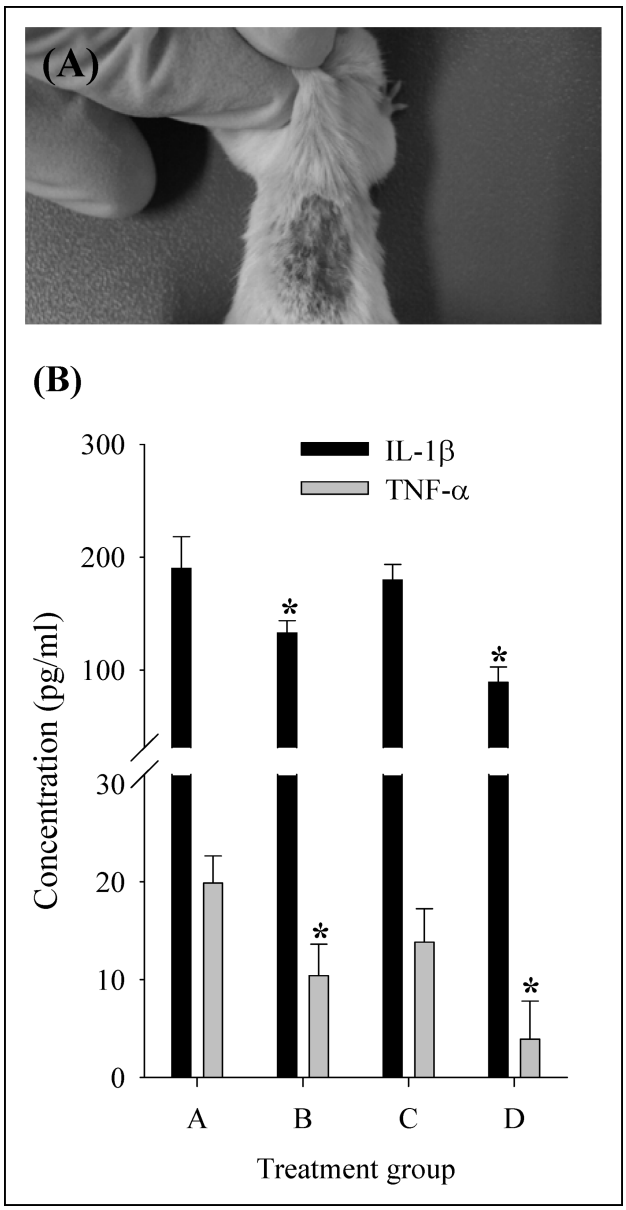

Fig. 5: Degree of inflammation after administering 1-chloro-2,4-dinitro-benzene to mice (A) and anti-inflammation efficacy (B). Groups A, B, C and D received no treatment (control), commercial ointment, spray solution vehicle without drug and TAA-loaded spray solution, respectively. The TAA-loaded spray solution was composed of TAA, Eudragit L, PEG 400 and ethanol at the ratio of 1:6:3:100 (w/w/w/v). * Significantly lower than group A (control) ($p < 0.05$).

property. In the development of TAA-loaded films, the mucoadhesive force of the TAA-loaded films was dependent upon the amount of Eudragit L. Moreover, PEG 400 had an effect not on mucoadhesive force but on the washability of spray films.

Figure 4 shows the drug-release profiles from the films in the simulated saliva solution at 36.5 °C. As the Eudragit L content increased, the slower was the rate of drug release from the film (Fig. 4A). This retardation of the release rate was due to its water-insoluble property. In contrast, PEG 400 increased the release rate, although not significantly (Fig. 5B). The rate of release of drug from all films was about 70% at 1 h, suggesting that the films had excellent release properties. Generally, non-injectable commercial pharmaceutical products show more than 70% dissolution of the drug at 1 h (Cho and Choi 2013; Kim et al. 2011).

Based on these findings, the formulation containing TAA, Eudragit L, PEG 400 and ethanol at the ratio of 1:6:3:100 (w/w/w/v) was selected as the optimal TAA-loaded spray solution because this formulation produced a film with suitable elongation, tensile strength and washability, strong mucoadhesive force, and good release properties.

Figure 5 compares inflammatory parameters such as IL-1ß and TNF-α measured after treating inflammation-induced mice with four different preparations. The inflammation was induced by the epicutaneous application of 1-chloro-2,4-dinitro-benzene (Fig. 5A) (Koziorowski et al. 2012; Zhang et al. 2008). In this study, IL-1ß and TNF-α values in serum were used as inflammatory indices. It was reported that the levels of proinflammable cytokines such as TNF-α and IL-1ß were markedly elevated in the serum of patients with inflammatory conditions (Koziorowski et al. 2012). The IL-1ß and TNF-α values measured in group C, which was treated with the spray solution vehicle without drug, did not differ significantly from those measured in group A (control; no administration) (Fig. 5B). Group B (132.94 ± 10.80 and 10.40 ± 3.23, respectively) and D (89.33 ± 13.43 and 3.92 ± 3.89, respectively), which were treated with the commercial ointment and TAA-loaded spray solution, respectively, showed significantly lower values compared to the control (190.10 ± 28.15 and 19.88 ± 2.79, respectively). Furthermore, values in group D, which was treated with the spray solution, were lower than those in group B, which was treated with the commercial ointment. However, the difference was not significant. Our results suggested that the TAA-loaded spray had excellent anti-inflammatory properties similar to those of the commercial ointment.

In conclusion, the TAA-loaded spray solution composed of TAA, Eudragit L, PEG 400 and ethanol at the ratio of 1:6:3:100 (w/w/w/v) was easy to administer, had good film properties and excellent anti-inflammatory efficacy, and is thus a potential candidate for the treatment of stomatitis.

3. Experimental

3.1. Materials

Triamcinolone acetonide (TAA) was purchased from Hwail Chem. Co. (Seoul, South Korea). Eudragit® L100 (Eudragit L) and 1-chloro-2,4-dinitro-benzene were supplied by Röhm Pharma Co. (Darmstadt, Germany) and Sigma-Aldrich Co. (Milwaukee, WI, USA), respectively. The TAA-loaded commercial ointment (Oramedy®) was purchased from Dongkook Pharm. Co. (Suwon, South Korea). The spraying vessel incorporating a long straw used in this study was supplied by Kyungnam Pharm. Co. (Euiryung, South Korea). Ethanol (95%) and polyethylene glycol 400 (PEG 400) were of USP grade. All other chemicals were of reagent grade and were used without further purification.

3.2. Animals

All animal procedures followed the Guiding Principles in the Use of Animals in Toxicology, as approved in 1989, reviewed in 1999 and edited in 2008 by the Society of Toxicology (SOT, 2008). In addition, the code of conduct for the animal studies was approved by the Institute of Laboratory Animal Resources of Yeungnam University. Male Golden hamsters weighing 100 + 10 g and female mice weighing 16.5 + 1.5 g were purchased from Charles River Company Korea (Orient, Seoul, South Korea) and Biotoxtech Company (Seoul, South Korea), respectively.

3.3. Preparation of TAA-loaded spray solutions

The TAA-loaded spray solutions were prepared by entirely dissolving TAA (0–1 g), Eudragit L (0–8 g) and PEG (0–4 g) in 100 ml ethanol at 25 °C with gentle stirring. Each spray solution was placed in a spraying vessel attached to a long straw, and its spraying potential was examined by visual observation. Its viscosity was then checked using a Brookfield viscometer (LVDV-II + Pro; Brookfield, IL, USA) with a spindle (#18) speed of 100 rpm, resulting in determination of the spraying threshold.

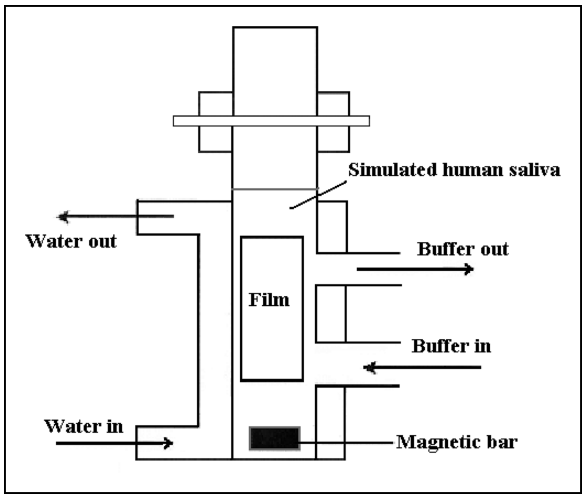

Fig. 6: Film-washing device.

3.4. Film properties

3.4.1. Formation of TAA-loaded spray film

Each spray solution (15 ml) was poured into a Petri dish, in which a Teflon sheet was attached to the bottom using cyanoacrylate adhesive. The film was dried at 70 °C for 2 h and lifted off the Teflon sheet, leading to a TAA-loaded film.

3.4.2. Mechanics

The tensile strength and elongation of TAA-loaded films were evaluated using a tensile test machine (Instron 4464, UK). Each film was cut into a defined dog-bone shape (6 cm long, 2 cm wide at the end and 1 cm wide in the centre). A mechanical study was carried out at a stretching rate of 10 mm/min with a pre-load of 0.5 N to find the highest load of each film. Immediately prior to evaluation, the film thickness was measured using a digital calliper (CD-15CPX; Mitutoyo Co., Japan).

3.4.3. Washability

The washability study was performed using a simulated saliva solution with a Franz cell apparatus and peristaltic pump. The simulated saliva solution was prepared by dissolving 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8.00 g NaCl in 1 L of distilled water, and adjusting pH to 6.75 with phosphoric acid (Choi et al. 2000). The square pieces (2 × 2 cm) of TAA-loaded spray films, as prepared above, were placed into the simulated saliva solution in a Franz cell apparatus (Fig. 6), stirred at 10 rpm using a magnetic bar for 20 min, and desiccated at 70 °C under vacuum for 12 h (W). The washability of the film was determined as follows: Washability % = $(W_o\text{-}W)/W_o$ *100, where W and W_o are the weights of the film at 0 and 20 min, respectively.

3.4.4. Mucoadhesive force

The mucoadhesive force of the films was checked using the measuring equipment shown in Fig. 7 (Choi et al. 1998; Yun et al. 1999). In detail, a cross-section of tissue was cut from the fundus of the hamster cheek pouch and clipped with the mucosal side (E) out onto a glass vial (C) using a rubber band and an aluminium cap. One vial (C) with a section of tissue was attached to the modified balance (A) and another vial was positioned on a height-adjustable pan (F). Each formulation was sprayed onto the rubber band of the other vial without spraying the aluminium cap and dried using a hair dryer until the film (D) had formed. Then, the height of the vial was moved accordingly to place the film between the mucosal tissues of both vials. The weights (B) were increased until the two vials separated. Mucoadhesive force reflects the detachment stress (dyne/cm^2), which was the lowest weight that separated the two vials.

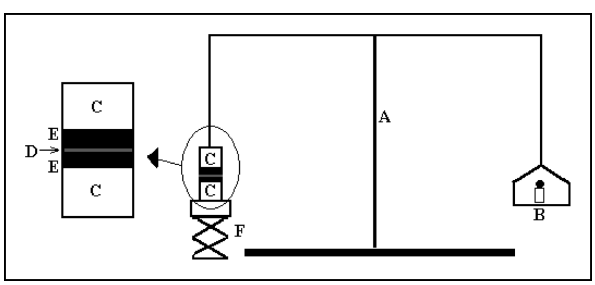

Fig. 7: Mucoadhesive force-measuring device: (A) modified balance; (B) weights; (C) glass vial; (D) sprayed film; (E) hamster cheek pouch; (F) height-adjustable pan.

3.5. Drug release

In-vitro drug-release studies of the TAA-loaded films, as prepared above, were conducted at 36.5 °C using the paddle method at 50 rpm with 400 ml of the simulated saliva solution as a dissolution medium in a dissolution tester (Shinseang Instrument Co.; Hwasung, South Korea). One millilitre of sample was taken from the dissolution medium at predetermined intervals, and filtered through a syringe filter (pore size, 0.45 (m; diameter 25 mm; Whatman Co., Breda, Netherlands). The drug concentration in the resulting solution (20 (l) was checked by HPLC (Hitachi; Tokyo, Japan) using an Inertsil ODS-3 C_{18} column (GL Science, 0.5 (m, 15 cm x 0.46 cm i.d.) and a UV detector (Model L-7450). The mobile phase consisted of methanol and water (70/30, volume ratio). The eluent was observed at 242 nm with a flow rate of 1 ml/min.

3.6. Anti-inflammatory efficacy

Forty female mice, with flanks shaved using a small animal clipper one day prior to sensitization, were randomly divided into four groups. The mice were sensitized by the epicutaneous application of 100 (l of 1% 1-chloro-2,4-dinitro-benzene onto the shaved flanks on day 1, 3, 5 and 7. Each mouse was restrained for 3–5 s to permit some of the solvent to evaporate on the skin. The test materials were applied every day from day 8 to day 14. Group A received no treatment (control). Group B was given 1 g of commercial ointment equivalent to 1 mg TAA. Group C and D were administered with 100 (l of the vehicle [Eudragit L/PEG 400/Ethanol (6:3:100, w/w/v)] and TAA-loaded spray solution [TAA/Eudragit L/PEG 400/Ethanol (1:6:3:100, w/w/w/v)] at the drug dose of 1 mg, respectively. The mice were sacrificed on day 15, and the heart blood was collected. The IL-1ß and TNF-α values in serum were measured using enzyme-linked immunosorbent assay (ELISA) kits plus commercially available reagents according to the manufacturer's instructions (Bio-Tek Instruments Inc.; Winooski, VT, USA) (Koziorowski et al. 2012; Zhang et al. 2008).

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References

Ali J, Khar RK, Ahuja A (1998) Formulation and characterisation of a buccoadhesive erodible tablet for the treatment of oral lesions. Pharmazie 53: 329–334.

Bruce HF, Sheskey PJ, Garcia-Todd P, Felton LA (2011) Novel low-molecular-weight hypromellose polymeric films for aqueous film coating applications. Drug Dev Ind Pharm 37: 1439–1445.

Choi YK, Din FU, Kim DW, Kim YI, Kim JO, Ku SK, Ra JC, Huh JW, Lee JI, Sohn DH, Yong CS, Choi HG (2013) Amniotic membrane extract-loaded double-layered wound dressing: evaluation of gel properties and wound healing. Drug Dev Ind Pharm, in press.

Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK and Kim CK (1998) Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. Int J Pharm 165: 33–44.

Choi HG, Jung JH, Yong CS, Rhee CD, Lee MK, Han JH, Park KM, Kim CK (2000) Formulation and *in vivo* evaluation of omeprazole-loaded buccal tablet. J Control Rel 68: 405–412.

Choi HG, Kim CK (2000) Development of omeprazole-loaded buccal tablets with stability enhancement in human saliva. J Control Rel 68: 397–404.

Choi SG, Baek EJ, Davaa E, Nho YC, Lim YM, Park JS, Gwon HJ, Huh KM, Park JS (2013) Topical treatment of the buccal mucosa and wounded skin in rats with a triamcinolone acetonide-loaded hydrogel prepared using an electron beam. Int J Pharm 447: 102–108.

Cho KH, Choi HG (2013) Development of novel bepotastine salicylate salt bioequivalent to the commercial bepotastine besilate in beagle dogs. Drug Dev Ind Pharm 39: 901–908.

Chun MK, Kwak BT, Choi HK (2003) Preparation of buccal patch composed of carbopol, poloxamer and hydroxypropyl methylcellulose. Arch Pharm Res. 26: 973–978.

Hanawa T, Masuda N, Mohri K, Kawata K, Suzuki M, Nakajima S (2004) Development of patient-friendly preparations: preparation of a new allopurinol mouthwash containing polyethylene(oxide) and carrageenan. Drug Dev Ind Pharm 30: 151–161.

Kianfar F, Chowdhry BZ, Antonijevic MD, Boateng JS (2012) Novel films for drug delivery via the buccal mucosa using model soluble and insoluble drugs. Drug Dev Ind Pharm 38: 1207–1220.

Kim YI, Kim KS, Suh KH, Shanmugam S, Woo JS, Yong CS, Choi HG (2011) New clopidogrel napadisilate salt and its solid dispersion with improved stability and bioequivalence to the commercial clopidogrel bisulphate salt in beagle dogs. Int J Pharm 415: 129–139.

Koziorowski D, Tomasiuk R, Szlufik S, Friedman A (2012) Inflammatory cytokines and NT-proCNP in Parkinson's disease patients. Cytokine 60: 762–766.

Kulthe SS, Bahekar JK, Godhani CC, Choudhari YM, Inamdar NN, Mourya VK (2013) Modulated release of 5-fluorouracil from pH-sensitive and colon targeted pellets: an industrially feasible approach. Drug Dev Ind Pharm 39: 138–145.

Lim SJ, Lee JH, Piao MG, Lee MK, Oh DH, Hwang du H, Quan QZ, Yong CS, Choi HG (2010) Effect of sodium carboxymethylcellulose and fucidic acid on the gel characterization of polyvinylalcohol-based wound dressing. Arch Pharm Res 33: 1073–1081.

Park CW, Kim JY, Rhee YS, Oh TO, Ha JM, Park ES (2012) Preparation and *in vivo* evaluation of spray dried matrix type controlled-release microparticles of tamsulosin hydrochloride for orally disintegrating tablet. Drug Dev Ind Pharm 38): 1179–1187.

Paulo Filho W, Ribeiro JE, Pinto DS (2000) Safety and efficacy of *Eupatorium laevigatum* paste as therapy for buccal aphthae: randomized, double-blind comparison with triamcinolone 0.1% orabase. Adv Ther 17: 272–281.

Peng C, Liu C, Tang X (2010) Determination of physicochemical properties and degradation kinetics of triamcinolone acetonide palmitate *in vitro*. Drug Dev Ind Pharm 36: 1469–1476.

Sabzevari A, Adibkia K, Hashemi H, De Geest BG, Mohsenzadeh N, Atyabi F, Ghahremani MH, Khoshayand MR, Dinarvand R (2013) Improved anti-inflammatory effects in rabbit eye model using biodegradable poly beta-amino ester nanoparticles of triamcinolone acetonide. Invest Ophthalmol Vis Sci 54: 5520–5526.

Shin SC, Kim JY, Oh IJ (2000) Mucoadhesive and physicochemical characterization of Carbopol-Poloxamer gels containing triamcinolone acetonide. Drug Dev Ind Pharm 26: 307–312.

Society of Toxicology (SOT) (2008) Guiding principles in the use of animals in toxicology: www.toxicology.org/AI/FA/guidingprinciples.pdf

Yang JH, Kim DK, Kim TY, Kim GY, Shin SC (2005) Anti-inflammatory effects by transdermal application of triamcinolone acetonide gel using phonophoresis in rats. Int J Pharm 302: 39–46.

Yang JH, Kim DK, Yun MY, Kim TY, Shin SC (2006) Transdermal delivery system of triamcinolone acetonide from a gel using phonophoresis. Arch Pharm Res 29: 412–417.

Yong CS, Jung JH, Rhee JD, Kim CK, Choi HG (2001) Physicochemical characterization and evaluation of buccal tablets containing omeprazole. Drug Dev Ind Pharm 27: 447–455.

Yun MO, Choi HG, Jung JH and Kim CK (1999) Development of thermoreversible insulin liquid suppository with sodium salicylate. Int J Pharm 189: 137–145.

Vyas SP, Rawat M, Rawat A, Mahor S, Gupta PN (2006) Pegylated protein encapsulated multivesicular liposomes: a novel approach for sustained release of interferon alpha. Drug Dev Ind Pharm 32: 699–707.

Zacharias LC, Estrago-Franco MF, Ramirez C, Kenney MC, Takahashi WY, Seigel GM, Kuppermann BD (2011) The effects of commercially available preservative-free FDA-approved triamcinolone (Triesence®) on retinal cells in culture. J Ocul Pharmacol Ther 27: 143–150.

Zhang X, Song Y, Ci X, An N, Ju Y, Li H, Wang X, Han C, Cui J, Deng X (2008) Ivermectin inhibits LPS-induced production of inflammatory cytokines and improves LPS-induced survival in mice. Inflamm Res 57: 524–529.