—Regular Articles—

Controlled Indomethacin Release from Mucoadhesive Film: *In Vitro* and Clinical Evaluations

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To develop a film formulation allowing controlled release for long-term analgesia, we selected ethyl cellulose (EC) as a novel additive, prepared a film formulation using indomethacin (IM film), and evaluated it *in vitro* and clinically. In the *in vitro* experiments, the effects of the EC concentration on the release rate of IM and on the adhesion force to the mucous membrane were investigated. The addition of 10% EC resulted in more sustained slow release compared with no EC, and the adhesion of the film with 10% EC added was similar to that of films containing carboxyvinyl polymer, which we reported previously showed significantly increased adhesion. A two-layered film consisting of an adhesive layer with 2% or 1% IM and 10% EC and a nonadhesive layer with 2% polyethylene glycol as a softening agent, was investigated for clinical use. Film consisting of an adhesive layer with 2% IM and 10% EC exhibited rapid onset of potent analgesia and was expected to prolong the duration of analgesia. These results suggest that IM film with EC added may be useful clinically, since it shows both immediate analgesic effects and prolonged duration of release.

Key words——controlled release; mucoadhesive film; analgesic; ethyl cellulose; indomethacin

INTRODUCTION

Oral mucosal pain can affect the activities of daily living such as eating and sleeping and may result in disorders that significantly reduce patient quality of life (QOL). In the field of oral surgery, conditions involving oral pain are common, including oral mucositis, periodontal disease, tooth extraction, hemodia, and glossitis. These conditions are induced by numerous causes, such as 1) physical contact with a sharp tooth or artificial denture; 2) heat injury or burns from chemical agents; 3) mucosal infection; and 4) oral mucositis or oral ulcers resulting from chemotherapy or radiation therapy.

Although oral nonsteroidal antiinflammatory drugs (NSAIDs) are administered to relieve pain,[1–4] there have been several reports on the risks of systemic side effects with oral NSAIDs, including gastrointestinal disorders.[5,6] Thus external formulations that decrease the risk of side effects and allow the rapid onset of analgesia are an attractive alternative.

External formulations that relieve oral pain include films, sprays,[7] ointments, and mouthwashes. Film formulations can particularly improve patient QOL because of better localization and drug retention times, as well as protective coverage of the affected site. We previously prepared film formulations containing indomethacin (IM) as an analgesic.[8] Although the novel film containing carboxyvinyl polymer (CP) and polyethylene glycol (PEG) to enhance the adhesive effects and improve comfort, respectively, improved adhesion to the affected site, the formulation also showed markedly rapid drug release. Ethyl cellulose (EC) was therefore investigated in an effort to maintain long-term analgesic effects.

EC is a water-insoluble polymer used in waterproof films in oral surgery,[9] is an additive in controlled-release tablets and capsules, and has been a focus of research for improving the controlled release of drugs.[10–12] Various film formulations exhibiting controlled drug release which include additives other than EC have been reported,[10–13] indicating the increasing

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importance of research in this field.

In this study, the effects of various amounts of EC in film formulations on the controlled release of the drug were first investigated by monitoring IM retention. Next, other properties of EC were assessed. After storage stability had been confirmed, the effectiveness of this film formulation was evaluated in clinical use.

MATERIALS AND METHODS

Film Preparation The layer adhering to the mucous membrane was composed of hydroxypropyl cellulose (HPC, Wako Pure Chemical Industries, Osaka, Japan) as a base component, IM (0.5%, 1%, or 2%) as the medication, and EC (2%, 5%, or 10%) (reagent grade, 10 cP, Wako Pure Chemical Industries) or CP (0.3%)[8] (Hivis Wako 105, Wako Pure Chemical Industries). Ingredients were dissolved in ethanol, 0.9 g of HPC was added, and then ethanol was added to give a final volume of 30 ml. Using a graduated pipette with a flow rate of 10 ml/4 min, 10 ml of the solution was cast in a flat 75-mm diameter Teflon dish. This was followed by drying overnight on a clean bench. For the second layer, 2% PEG[14] as a softening agent and HPC dissolved in ethanol were cast as described above. No drug precipitation was noted.

Each application was assumed to use 1 cm^2 of film with 0.5%, 1.0%, or 2.0% IM (IM contents: 0.03 mg/cm^2, 0.06 mg/cm^2, or 0.12 mg/cm^2, respectively); 2%, 5%, or 10% EC (EC contents: 0.12 mg/cm^2, 0.3 mg/cm^2, or 0.6 mg/cm^2, respectively), or 0.3% CP (CP contents: 0.036 mg/cm^2) and 2% PEG (PEG content: 0.24 mg/cm^2). Concentrations of IM or additives were set as reported previously.[13,15]

***In Vitro* Release of IM** Six types of film (1% IM, 1% IM+2% EC, 1% IM+5% EC, 1% IM+10% EC, 2% IM+5% EC, 2% IM+10% EC) were prepared and cut into circles 21 mm in diameter. A piece of film was placed in the center of a membrane filter (type HA, pore size 0.45 μm, Millipore, Billerica, MA, USA) in a vertical-diffusion cell system (Hanson Research, Chatsworth, CA, USA) filled with 15 ml of phosphate-buffered saline (PBS) 0.1 M and kept at 37°C. The units used in this study had an effective diffusion area of 21 mm in diameter and a receptor compartment volume of 15 ml. The solvent was maintained at 37°C and continuously stirred with a magnetic bar. Sample aliquots were removed through the sampling port using a syringe at 5, 10, 15, 30, 60, 120, and 360 min and replaced with an equivalent volume of fresh solvent. The amount of IM diffusing into the collected samples was measured using an HPLC system. The HPLC system included two LC-10AD *vp* pumps, an SPD-10A *vp* ultraviolet detector, and an SIL-10AD *vp* autosampler (Shimadzu, Kyoto, Japan). Analysis was performed as reported previously[8] on an octadecylsilica (ODS) column (150 mm×4.6 mm i.d.) with a 5-μm particle size (Wakopak, Wakosil-II 5C18, Wako Pure Chemical Industries). The mobile phase of the assay consisted of sodium monophosphate buffer 0.1 M and sodium acetate buffer 0.2 M (8 : 2 vol/vol) at a flow rate of 1.0 ml/min. Standard solutions were prepared for each assay at 0.25, 0.5, 2, 20, and 40 μg/ml.

***In Vitro* Adhesion Tests** The experimental film consisted of three concentrations (0%, 5%, and 10%) of EC, 0.3% CP as an adhesion agent, and HPC as a base component and was cut into 2-cm squares. After the experimental film was placed in the center of nonwoven cloth (4C cloth, FK900-0138 EVA80, Kuraray Kuraflex, Tokyo, Japan) cut into 3-cm×10-cm pieces, it was wet with 39.6 μl of PBS, folded in half, and 500 g of flat weight was placed on the cloth for 5 s and then removed. Five minutes after the experimental film was placed in the center of the cloth, one end of half of the cloth was pulled at a speed of 300 mm/min. The maximum force [kilogram-force (kgf)] of peeling was measured at an angle of 90° with a digital force gauge (ZP-50N, Imada, Aichi, Japan) by adjusting the slide system. The volume of PBS applied to the wet nonwoven cloth was calculated from the volume of saliva secreted in a Saxon test in a fixed time so that it would adequately permeate the entire experimental film.[16] The experimental results were compared using Scheffe's multiple-comparison tests.

Stability Test A 2% IM-10% EC film formulation was prepared. Films were cut into 1-cm×1-cm pieces, packed for clinical use, and then stored under one of three conditions: at 37°C; at room temperature; and at 4°C with shading. Films were stored for 0, 7, or 28 days. The amount of IM in the films was measured after dissolving in 5 ml of 0.1 M phosphoric acid buffer solution (pH 7.0) using the HPLC system described above.

Clinical Evaluation The film composition used in the clinical evaluation is described in Table 1. Clin-

ical evaluation was conducted between November and December 2004 in 48 patients (age: 22–83 years; 17 men, 31 women) who visited the the Maxillo-facial Surgery Department of Teikyo University Hospital. Patients who had oral pain and provided written informed consent to participate were randomly allocated into three groups (control, 1% IM, and 2% IM) and treated in a double-blind manner. The analgesic efficacy of the films was evaluated 1, 3, and 5 min after application. The pain ratio ($\underline{X}$) was evaluated using the Visual Analogue Scale method, with pain before using the film scored as 100%. The pain relief ratio was set at 100-$\underline{X}$. Pain relief of 50% or greater was judged as effective, while less than a 50% reduction in pain was considered ineffective. In addition, patients were interviewed about their degree of pain, type of pain, presence of hypoalgesia, film taste, film texture, and film softness. The duration of the analgesic effects was set based on the time when pain recurred.

Table 1. Composition of the Film Used in Clinical Evaluation

Sample	Sample component		
	Mucosal adhesion layer		Support layer
	IM (%)	EC (%)	PEG (%)
Control	—	—	2
1% IM	1	10	2
2% IM	2	10	2

The three types of film were composed of two layers.

Patients who judged the experimental film as effective and could evaluate pain after receiving treatment at the hospital made a note of the time when oral pain was felt again. Notes were collected during the next consultation. Patients in whom the pain did not return or did not improve with initial treatment, or who could not evaluate the duration of relief due to their general medical condition, were considered unevaluable. At the next consultation, the incidence of side effects and treatment efficacy were evaluated in an interview. Pain relief ratios were compared using Scheffe's multiple-comparison tests based on ANOVA. This study protocol was approved by the Committee for Medicinal Products of Teikyo University Hospital.

RESULTS AND DISCUSSION

Efficacy of EC in Controlled Drug Release IM released from the four film formulations (0%, 2%, 5%, and 10% EC) was compared. At 1 and 2 h, 5% EC and 10% EC, respectively, showed significantly lower cumulative release of IM ($p<0.01$, Fig. 1). Decreased release of IM was observed with increasing EC concentration, suggesting that IM release was controlled by EC. However, the controlled release of IM would likely delay the analgesic effects, and thus the IM concentration in the film was increased to 2%. The IM concentration was then investigated in three formulations (1% IM, 2% IM+5% EC, and 2% IM +10% EC). In the 1% IM and 2% IM+10% EC

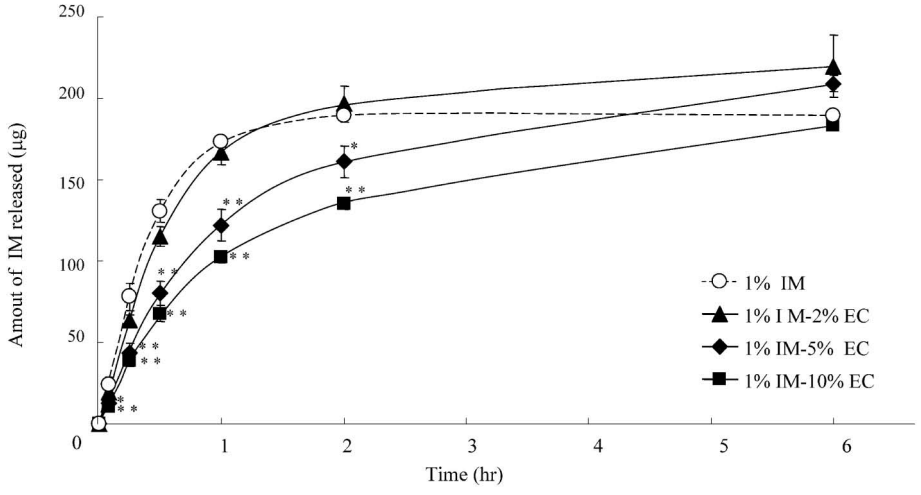

Fig. 1. Controlled Release of IM with EC Added

Films prepared for measuring the amount of IM released contained approximately 208 μg of indomethacin. Each sample was checked four times. The data shown are mean±S.D.
$^{*}p<0.05$, $^{**}p<0.01$ *vs* 1% IM, Scheffe's F-test.

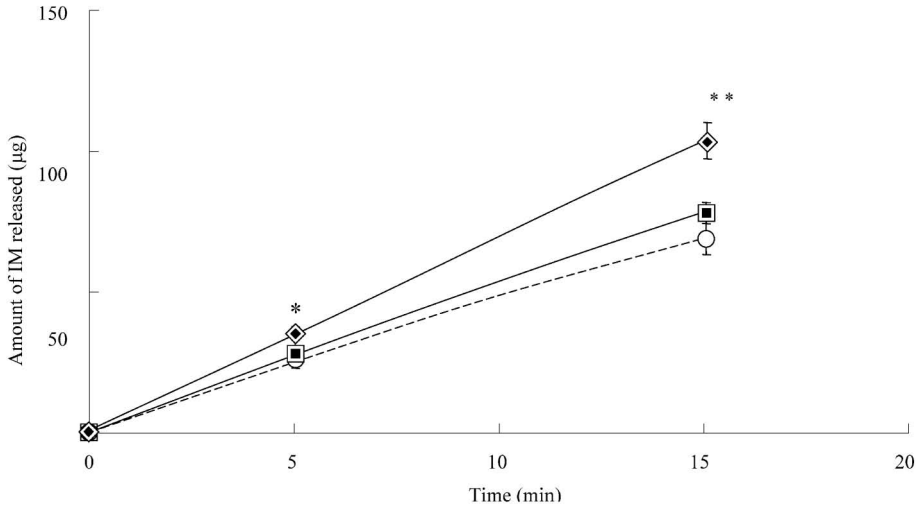

Fig. 2. Comparison of the Amount of IM Released between 1% IM-HPC, 2% IM-5% EC-HPC and 2% IM-10% EC-HPC during Initial Application

Mean±S.D. ($n=4$). $^{*}p<0.05$, $^{**}p<0.01$ *vs* 1% IM-HPC; Scheffe's F-test.

formulations, almost the same amount of IM was released at 5 and 15 min, respectively (Fig. 2). The 2% IM film with EC exhibited rapid release, which was comparable to the 1% IM film formulation without EC, and was expected to have an immediate analgesic effect. The clinical film formulation was thus set at 10% EC to minimize film hardness and 2% IM to ensure immediate analgesic effects.

Efficacy of EC in the Adhesion Test From the results of the *in vitro* adhesion test, film containing 5% EC, 10% EC, and 0.3% CP showed greater adhesion compared with control film ($p<0.01$). The addition of 5% and 10% EC showed greater adhesion, but the adhesion was approximately the same adhesive force as 0.3% CP, which previously showed significantly higher adhesive force (Fig. 3), but EC was also found to increase the adhesion force.

Optimization of Storage Conditions and Expiration Date Although decreases in IM amounts in 1% IM film and 2% IM film were seen after 4 weeks under all conditions examined (4℃, 99.3±1.3% and 99.7±0.8%; room temperature, 99.8±2.3% and 100.0±1.8%; 37℃, 98.0±1.5% and 99.4±3.3%), the amount of IM remained at almost 100% for 4 weeks after preparation (Table 2). Thus the storage conditions and expiration dates for clinical use were determined to be storage with shade, preferably in a refrigerator, for no more than 4 weeks.

Patient Background The patients who participated in the clinical evaluation included: 16 with mucostitis; 5 with pain from surgery including tooth

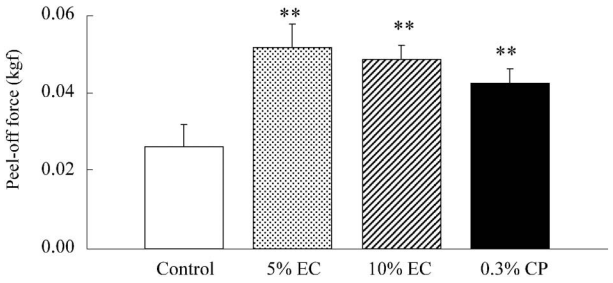

Fig. 3. Peel-off Force of Samples

Peel-off force was measured with samples cut into 2-cm×2-cm squares. Data are mean±S.D. of six experiments.
$^{**}p<0.01$ *vs* control, Tukey-Kramer test.

Table 2. Indomethacin Stability in IM Film

a) 1% IM

Storage condition	Time after preparation (days)		
	0	7	28
4℃	100.0±1.8	99.5±0.5	99.3±1.3
Room temperature	100.0±1.8	99.6±1.8	99.8±2.3
37℃	100.0±1.8	97.3±0.5	98.0±1.5

b) 2% IM

Storage condition	Time after preparation (days)		
	0	7	28
4℃	100.0±2.9	99.7±2.5	99.7±0.8
Room temperature	100.0±2.9	97.9±3.8	100.0±1.8
37℃	100.0±2.9	98.5±1.8	99.4±3.3

Each sample was determined four times. Data are mean±S.D.

extraction; 11 with decubitus ulcer; 12 with injury to the oral mucous membrane (6 glossitis and 6 periodontal disease); 2 with dental caries; and 2 with xerostomia.

Clinical Evaluation Among the 48 patients, 47 were able to evaluate pain relief, and efficacy was noted in 72% (34 of 47). Both IM concentrations showed good maximum pain relief ratios (1% IM, 83.78±19.45%; 2% IM, 93.00±11.10%).

Onset of Analgesic Effects Both IM concentrations yielded rapid analgesic effects (1% IM, 2′47″±1′52″; 2% IM, 1′58″±1′35″) (Table 3). The rapid appearance of analgesic effects of both 1% and 2% IM was particularly marked in stomatitis (1′17″±0′46″) and decubitus ulcer (1′48″±1′47″) (data not shown).

Maximum Pain Relief by Patient Condition Maximum pain relief ratios were seen in aphtus stomatitis (98.1±4.9%), although the ratios were low for dental caries and gingivitis (Table 4). Changes in pain relief ratio at 1, 3, and 5 min are shown in Tables 5 and 6.

When maximum pain relief ratios were compared by patient condition, 2% IM showed immediate effects that appeared in 1 min in aphtus stomatitis and decubitus ulcer, analgesic effects appeared more slowly and were weaker in patients with dental caries or gingivitis.

Duration of Analgesia and Side Effects The duration of analgesia ranged from less than 1 to 6 h. After analgesia had been achieved, pain disappeared in 3 patients (Table 7). In addition, patient reactions to the films were satisfactory, with no objections to film taste, texture, or softness. The duration of analgesia also showed great individual differences and it was difficult to determine whether the prolongation of analgesia was due to the addition of EC. Therefore more examinations will be needed to determine

Table 3. Comparison of Maximum Pain Relief Ratios

Sample	No. of patients	Maximum pain relief ratio (%)	Pain relief rate
1% IM	9	83.78±19.45	2′47″±1′52″
2% IM	23	93.00±11.10	1′58″±1′35″

Data are mean±S.D.

Table 4. Comparison of Maximum Pain Relief Ratios between 1% and 2% IM at each condition

Patient condition	Maximum pain relief ratio (%)			
	2% IM	*n*	1% IM	*n*
Aphthous stomatitis	98.1±4.9*	7	54.3±50.6	3
Stomatitis	40.0	2	—	
Decubitus ulcer	93.8±11.3	5	66.8±47.1	4
Glossitis	80.3±24.8	6	—	
Gingivitis	48.7±50.1	3	62.0	2
Inflammation	100	1	48.0	1
Extraoral flare	100	1	—	
Post exodontia	52.7±26.8	3	—	
Dental caries	42.5	2	—	
Xerostomia	0	1	100	1
Post suture removal	—		100	1

* $p<0.05$ *vs* 1% IM, Scheffe's F-test.

Table 5. Pain Relief Ratio (%) 1, 3, and 5 Min after 2% IM Film Application

Patient condition	*n*	Pain relief ratio (%) at each time		
		1 min	3 min	5 min
Extraoral flare	1	100	100	100
Inflammation	1	83.0	83.0	100
Aphthous stomatitis	6	89.0±26.9	96.0±9.8	97.8±5.3
Decubitus ulcer	5	71.2±27.5	83.8±23.1	93.8±11.3
Glossitis	6	50.3±45.2	64.5±39.9	80.3±24.8
Stomatitis	3	33.3±57.7	33.3±57.7	60.0±52.9
Post exodontia	3	14.3±24.8	25.0±22.3	52.7±26.8
Gingivitis	2	29.0	41.0	50.0
Dental caries	3	18.3±25.9	43.7±35.6	43.7±35.6
Xerostomia	1	0	0	0

Table 6. Pain Relief Ratio (%) 1, 3, and 5 Min after 1% IM Film Application

Patient condition	*n*	Pain relief ratio (%) at each time		
		1 min	3 min	5 min
Xerostomia	1	100	100	100
Decubitus ulcer	4	62.3±48.0	62.3±48.0	66.8±47.1
Post suture removal	1	45.0	100	100
Inflammation	1	34.0	48.0	48.0
Aphtus stomatitis	3	29.3±50.8	33.3±57.7	54.3±50.6
Gingivitis	2	24.0	51.5	62.0

Table 7. Duration of Analgesic Effect

No.	Age (y)	Gender	Time until appearance of analgesic effect (min)	Duration of analgesic effect	Maximum pain relief ratio (%)	Condition	Film application site
1	68	M	1	Pain disappearance	100	Aphthous stomatitis	Buccal mucosa
2	64	F	1	1 h	100	Aphthous stomatitis	Glossa
3	—	M	1	>6 h	100	Gingivitis	gingiva
4	23	M	1	4 h 52 min	100	Aphthous stomatitis	Gingiva
5	81	F	1	5 h 5 min	100	Decubitus ulcer	Glossa
6	76	F	3	1 h	78	Pus retention and inflammation from infectious disease	Lower jaw
7	61	F	3	3 h	87	Aphthous stomatitis	Labrum
8	61	F	1	Pain disappearance	100	Aphthous stomatitis	Glossa
9	83	F	5	6 h 40 min	60	Glossitis	Glossa
10	20s	F	3	Pain disappearance	87	Glossitis	Glossa
11	73	F	1	1 h	100	Decubitus ulcer	Glossa

whether the addition of EC prolongs the duration of analgesia significantly. There were few adverse events reported in clinical use and none was serious.

A two-layered film comprising a 2% IM+10% EC layer and a PEG layer resulted in immediate onset of analgesic effects and excellent clinical pain relief. The IM content of this formulation is only 1/200 of the standard oral dose and can thus likely be used in patients who are unable to receive oral analgesics, thus improving patient QOL. EC has been used in controlled-release tablets and widely studied as a release-controlling polymer. However, to the best of our knowledge, this is the first report of EC used in film formulations for application to the oral mucous membrane. The present results suggest that EC, which has moderate adhesion to the mucous membrane, exhibits both immediate drug effects and prolonged duration of release.

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