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Preparation and Clinical Application of 2% Diflunisal Oral Ointment for Painful Lesions of the Oral Mucosa

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We previously reported the development and clinical efficacy of a 2% aspirin oral ointment and 2% ethenzamide oral ointment as hospital preparations for painful lesions of the oral mucosa. This study investigated methods of preparing a more stable oral ointment with a more effective analgesic action, using diflunisal, another salicylic acid derivative, with an analgesic effect stronger than that of aspirin. A two-percent diflunisal oral ointment was prepared similarly to the aspirin ointment using plastibase and CMC–Na as the ointment base. From the results of spreadability measurement, a CMC–Na content of 20% was considered appropriate. The stability of diflunisal in 2% diflunisal oral ointment stored at 5°C, 20°C and 30°C, was determined using HPLC, and a high stability of diflunisal at room temperature for more than 100 days was confirmed. We also investigated its antinociceptive effect using the Randall-Selitto paw pressure test in rats, which showed that 2% diflunisal oral ointment was as effective as 2% aspirin oral ointment. On clinical application of 2% diflunisal oral ointment to 8 patients with painful oral mucous diseases, it was found to be significantly ($p=0.014$) more effective than 2% aspirin oral ointment. The results of this study demonstrated that 2% diflunisal oral ointment is a clinically useful analgesic for painful oral lesions.

Key words——diflunisal oral ointment; lesions of the oral mucosa; hospital preparation; clinical application

INTRODUCTION

In diseases of the oral mucosa such as ulcerative stomatitis and recurrent aphthous stomatitis, healing may require several weeks or months. Many patients experience pain that makes it difficult to eat, sleep or even talk, and a good pain-relieving treatment with prolonged effect is required.[1,2]

Several ointments or patch preparations containing steroids (Kenalog® oral ointment, Dexaltin® oral ointment, Aphthatch® and Aphthaseal®) are used for external application to the oral cavity. However, there have been no reports of these ointments being markedly effective in relieving pain. Furthermore, the analgesic effects of surface anesthesic drugs such as Xylocaine Jelly® and Xylocaine Viscose® show limited duration.

We previously reported the development of 2% aspirin oral ointment for painful oral lesions and demonstrated the clinical efficacy in a placebo-controlled study.[3,4] Two percent aspirin oral ointment showed a rapid effect with essentially no side effects. Although aspirin oral ointment has been used for many patients in our hospital, the stability of aspirin in an ointment form has not been sufficient for patients to use at home and storage in a refrigerator is required. Later, we developed 2% ethenzamide oral ointment, which was stable at room temperature but the clinical effect was not greater than that of 2% aspirin oral ointment.[5] Therefore, the present study developed a stable oral ointment with a more effective analgesic action. For this purpose, we used diflunisal, another salicylic acid derivative. Diflunisal is reported to have an analgesic effect 3–8 times greater and an anti-inflammatory effect 1.5 times greater than aspirin.[6,7] Furthermore, its analgesic effect has been reported to be of long duration.[8,9] We prepared diflunisal oral ointment and evaluated its stability, analgesic effect in a rat model, and clinical efficacy in patients with painful oral mucous diseases.

MATERIALS AND METHODS

1. Reagents The reagents used were as follows: diflunisal (Sigma Co., St. Louis, MO, USA); plastibase (Taisho Co., Tokyo, Japan) as an ointment base; carmellos sodium (CMC–Na) (Wako Pure Chemicals Co., Osaka, Japan) as an adhesive; liquid paraffin (Maruishi Co., Osaka, Japan) as a

solvent and acetonitrile for high-performance liquid chromatography (HPLC) (Wako Pure Chemicals Co.). Kenalog® oral ointment (Bristol-Myers Squibb Co., Tokyo Japan) and Dexaltin® oral ointment (Nippon Kayaku Co., Tokyo Japan) were purchased from the local distributor. All other reagents were commercially available or analytical-grade products.

2. Preparation of Oral Ointments Preliminary clinical application was performed by an oral surgeon to establish the optimal concentration of diflunisal. When diflunisal 0.5%, 1%, 2% and 5% were tested, the duration of the analgesic effect of 2% diflunisal oral ointment was the same as that of 5%, and the effect was more prolonged than that of 0.5% and 1%. Thus, 2% diflunisal oral ointment was used in this study.

Two percent diflunisal oral ointments containing 20% or 40% CMC-Na were prepared as follows. Two g of diflunisal crystals were ground in a mortar, then 2 g of liquid paraffin was added and mixed well to make a diflunisal suspension. Then about 20 g of plastibase was gradually mixed in. Twenty g or 40 g of CMC-Na was gradually added, then more plastibase was added to make 100 g of ointment. We also prepared 0.5% diflunisal ointment containing 20% CMC-Na in the same way. Two percent aspirin oral ointment was prepared according to the previously reported method.[3] Briefly, aspirin crystals (2 g) were ground to a fine powder in a mortar, with a few drops of ethanol added then allowed to evaporate. Plastibase (78 g) was added and mixed well. CMC-Na (20 g) was then gradually added, and the mixture was ground to a uniform consistency.

3. Evaluation of the Spreadability of 2% Diflunisal Oral Ointments Spreadability is one of the important indices of the ease of the use of ointments. We measured the spreadability of 2% diflunisal oral ointment containing 20 or 40% CMC-Na in the base and evaluated the optimal base for diflunisal ointment.

Spreadability of the 2% diflunisal oral ointment was measured using the diameter of the spread from 10 sec to 1000 sec with a spreadmeter (JIS; made by Rigo Co. Tokyo Japan).[10] The measurement was performed at 25±2℃, and spreadability was determined as the mean value of 3 trials. Two percent diflunisal oral ointments were prepared and stored at 5℃, 20℃ and 30℃ for 0, 1, 7, 14, 21 and 28 days. For comparison, spreadability was also calculated for Kenalog® oral ointment and Dexaltin® oral ointment.

4. Stability of Diflunisal in 2% Diflunisal Oral Ointments The residual diflunisal concentration in 2% diflunisal ointments containing 20% CMC-Na, stored at 5℃, 20℃ and 30℃, were measured using HPLC at 1 day, 7 days, 14 days, 28 days, 56 days and 100 days after preparation. The HPLC apparatus consisted of the following components: a NSI-33R pump (Shimadzu Ltd., Kyoto, Japan), a KHP-UI-130A sample injector (Kyowa Seimitsu, Tokyo, Japan), a TSK ODS-80TM reverse-phase column (4.6 mm (150 mm I.D., Toso Co., Tokyo, Japan), a SPD-6A ultraviolet absorption detector (Shimadzu Co., Kyoto, Japan), and a HITACHI 561 recorder (Hitachi Ltd., Tokyo, Japan).

For testing, 50 mg of the preserved ointments were added to 10 ml of ethanol containing flufenamic acid (50 μm) as an internal standard. After vigorous mixing, the sample was sonicated for 20 min, 500 μl of the resultant suspension was then added to 500 μl of water, mixed well and filtered with a Dismic™ 3 JP cartridge (0.5 μm, Advantec Toyo Co., Tokyo, Japan). Twenty μl of the filtrate was analyzed by HPLC. The mobile phase was 0.1% trifluoroacetic acid in a mixture of acetonitrile-water, 45 : 55, by volume. The flow rate of the mobile phase was 1.0 ml /min and the HPLC column was operated at room temperature. The eluate was monitored at 251 nm. Diflunisal contents in the ointments were calculated from their peak height ratios to the internal standard using a calibration curve. The mean of 4 estimates was used for calculation. The remaining diflunisal concentration in the ointment immediately after preparation was normalized as 100%.

5. Measurement of Diflunisal Absorption by the Oral Mucous Membrane Measurement of diflunisal absorption by the oral mucous membrane was carried out using a test patch made of adhesive plastic film with a 10-mm cotton disk inside (Patch tester TORII, Torii Co., Tokyo, Japan). Six healthy hospital pharmacists participated as subjects. Fifty mg of 2% diflunisal oral ointment containing 20% CMC-Na was applied to the cotton part of the test patch. The test patches were affixed to the mucous membrane inside of the subject's cheek, and then detached immediately or at 10, 30 and 60 min. The ointment that remained attached to the mucous membrane was collected using a scraper, and combined with the recovered test patch. Then, the quantity of

diflunisal in the test patch was measured using HPLC. When the patch was detached immediately after affixation to the mucous membrane, the remaining diflunisal content in the ointment was normalized as 100%. No leakage of ointment from the patch into the space other than the mucous membrane was confirmed by the method reported previously. In brief, 1% gentian violet solution was added as a coloring agent at a ratio of 3 drops to 10 g of ointment, and the ointment was mounted on the test patch and applied to the mucous membrane. No leakage of dye from the test patch to the surrounding mucous membrane was observed.

6. Antinociceptive Test in Experimental Hyperalgesia Male Wistar rats ($n=6$, supplied by NRC Haruna, Gunma, Japan) weighing 200–230 g were used. The nociceptive threshold against pressure stimuli on the hind paw was determined using the Randall-Selitto method (Analgesy-Meter, Ugo Basile Co., Milan, Italy).[11] Rats showing a basal nociceptive threshold between 75 g and 95 g were selected and 0.1 ml of 20% brewer's yeast (Tanabe Co., Osaka, Japan) suspension in saline was injected under the plantar skin of the left hind paw. Animals did not show any weight loss or apparent change in grooming behavior during the experimental period. After 5 days, 1 g of 2% or 0.5% diflunisal oral ointment, 2% aspirin oral ointment or control basal ointment was applied to the hind paw and wrapped with cotton and a latex sac. Basal oral ointment (containing 20% CMC–Na) was used as a control. Application of ointment was renewed after every measurement of nociceptive threshold until 3 h. Nociceptive thresholds were measured just before the application (0 h) of ointment and at 1 h, 2 h, 3 h, 4 h and 5 h after the application. At each time point, the average of three estimations was taken as a threshold value. All threshold values of each rat were normalized to 1 by the value of that rat at 0 h. Threshold measurements were performed in a blind manner in which the measurer was not aware of the ointment applied. Animal experiments were performed according to the guidelines stated in The Guide for Animal Experimentation, Faculty of Medicine, University of Tokyo.

7. Clinical Assessment of Analgesic Effect Eight patients (3 male and 5 female) between 42 and 61 years of ages who visited the Department of Oral Surgery, Branch Hospital of University of Tokyo, and who experienced pain in the oral mucous membrane were included in this study. All patients gave informed written consent.

First, the oral surgeon made the diagnosis and evaluated the severity of pain according to a 5-grade scale of severe pain, moderate pain, slight pain, tenderness, and no pain. Two percent diflunisal oral ointment containing 20% CMC–Na was applied to the oral cavity in an amount of 0.1–0.3 g per treatment according to the symptoms. The oral surgeon then interviewed the 8 subjects to assess the analgesic effect. Total assessments of ointments were also carried out according to the Standard of Assessment of Analgesic Efficacy proposed by Saito et al. (Table 1), in which scores for the degree of pain relief, time of onset and duration were pooled to evaluate the total efficacy of the ointment.[12]

RESULTS AND DISCUSSION

1. Evaluation of the Spreadability of 2% Diflunisal Oral Ointments Figure 1 shows the spreadability of each oral ointment after storage at 20°C for 7 days. The Y-intercept (at $\log X=0$) is a parameter of viscosity, and lower values indicate higher viscosity. The Y-intercept was 2.524 cm for 2% diflunisal oral ointment containing 20% CMC–Na and 2.357 cm for 2% diflunisal oral ointment containing 40% CMC–Na. For comparison, we also measured these parameters in commercially available oral steroid ointments, Kenalog® and Dexaltin®. The Y-intercepts were 2.385 cm for Kenalog® oral ointment and 2.591 cm for Dexaltin® oral ointment. These values were in the range reported to be clinically appropriate for skin ointments (about 2.0–3.5 cm), confirming the appropriate viscosity of each ointment.[13–15] The slope a parameter of ointment spreadability, was 0.114 for 2% diflunisal oral ointment containing 20% CMC–Na, 0.126 for Kenalog® oral ointment, and 0.127 for Dexaltin® oral ointment, showing good spreadability, but 0.048 for 2% diflunisal oral ointment containing 40% CMC–Na, indicating poor spreadability.

None of the ointment showed changes in spreadability under each storage condition (at 5°C, 20°C or 30°C) for 28 days from immediately after preparation (data not shown). Therefore, in the experiments below, 2% diflunisal oral ointment containing 20% CMC–Na was used.

2. Stability of Diflunisal in 2% Diflunisal Oral Ointments Chart 1 shows the chromatogram of

Table 1. Standard of Clinical Evaluation on Analgesic Effect

5-grade scale for pain relief assessment (A) (measured at 30 min after application)		
severe pain	→no pain	=4
severe pain	→tenderness	=3
moderate pain	→no pain	=3
severe pain	→slight pain	=2
moderate pain	→tenderness	=2
slight pain	→no pain	=2
severe pain	→moderate pain	=1
moderate pain	→slight pain	=1
slight pain	→tenderness	=1
tenderness	→no pain	=1
no change		=0
increase of pain		=0

Rank-ordered grade for appearance time (B)	
within 10 min after application	=3
within 20 min after application	=2
over 20 min after application	=1
not effiecacious forever	=0

Rank-ordered grade for duration of action (C)	
over 5 hrs	=3
over 3 hrs	=3
less than 3 hrs	=1
less than 1 hrs	=0

Grades of global analgesic effect (value of A+B+C)	
8–10	=remarkable
5–7	=moderate
3, 4	=slight
0–2	=not effective

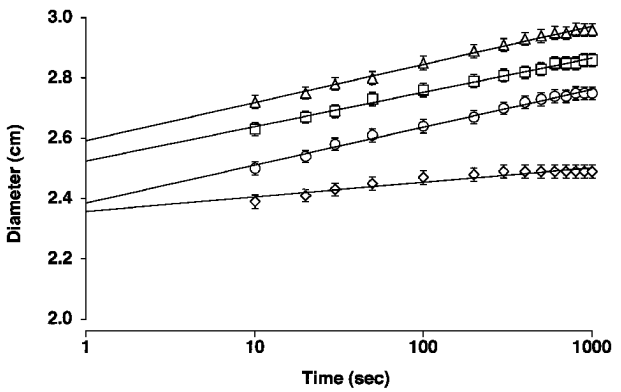

Fig. 1. Spreadability of 2% Diflunisal Oral Ointment Stored at 20°C for 7 Days

The diameter (Y, cm) of the spread was measured (X, sec) from 10 sec to 1000 sec using a spreadmeter. Y-intercepts indicate viscosity, while slopes indicate spreadability. Each point represents mean ± S.D. ($n=3$).
□—□: 2% diflunisal oral ointment containing 20% CMC-Na
Y = 0.114 log X + 2.524, r = 0.997
◇—◇: 2% diflunisal oral ointment containing 40% CMC-Na
Y = 0.048 log X + 2.357, r = 0.978
○—○: Kenalog® oral ointment
Y = 0.126 log X + 2.385, r = 0.996
△—△: Dexaltin® oral ointment
Y = 0.127 log X + 2.591, r = 0.987

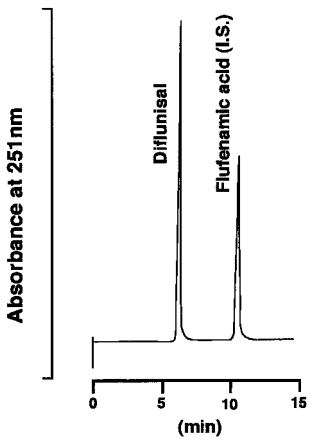

Chart 1. The Chromatogram of Diflunisal and Flufenamic Acid (I.S.) in the 2% Diflunisal Oral Ointment from Reverse-Phase HPLC

HPLC Conditions:
Column: TSK ODS-80TM (4.6 mm × 150 mm I.D.)
Mobile Phase: 0.1% Trifluoroacetic Acid/CH_3CN–H_2O (45 : 55)
Flow Rate: 1.0 ml/min
Detection: UV 251 nm

diflunisal in 2% diflunisal oral ointment with flufenamic acid as an internal standard. The retention time was 6.0 min for diflunisal and 10.3 min for flufenamic acid, showing good separation. Figure 2 shows changes in the percentage of the residual amount of diflunisal to the amount immediately after ointment preparation under each condition until 100 days. In 2% aspirin oral ointment reported previously, the concentration of aspirin as an active ingredient decreased significantly after storage at 30°C for 50 days.[3] In 2% diflunisal oral ointment, there were no changes observed over time even at 30°C up to 100

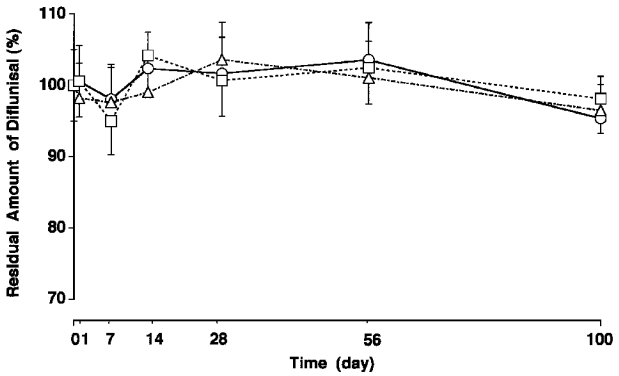

Fig. 2. Stability of Diflunisal in 2% Diflunisal Oral Ointment Stored at 5℃, 20℃ and 30℃

The values were normalized with the values at 1 day as 100%. Each point represents mean±S.D. ($n=4$).
□···□: 5℃, △·−·−·△: 20℃, ○—○: 30℃.

days, confirming adequate stability of ointment at room temperature.

3. Measurement of Absorption of Diflunisal from Oral Mucous Membrane Test patches to which 2% diflunisal oral ointment was applied were attached to the oral mucous membrane and immediately detached. The mean recovery of diflunisal among 6 subjects calculated from the residual amount in the patches was 91.0±0.06% (S.D.). The recovery rate in each subject was used for correction and the residual amounts of diflunisal in the ointment after designated periods were expressed as the percentage of values immediately after application. In the patch tests of ointment containing gentian violet, the absence of any leakage of ointment from the patches was confirmed.

From the residual rate of diflunisal shown in Fig. 3, 3% and 17% of diflunisal on the test patch was suggested to have been absorbed through the oral mucosa 10 and 60 minutes after application, respectively. Diflunisal that reached the lesion through the oral mucosa was considered to produce an analgesic effect.

4. Antinociceptive Test in Experimental Hyperalgesia As shown in Fig. 4, the nociceptive threshold force in rats did not increase in the control group to which only basal oral ointment was applied, but was increased in the 2% aspirin oral ointment group and 2% diflunisal oral ointment group. The thresholds after 1 h returned almost to those before the cutaneous injection of 20% brewer's yeast, indicating adequate alleviation of pain. In the 0.5% diflunisal oral ointment group, the increase in nociceptive threshold was inadequate. This experiment

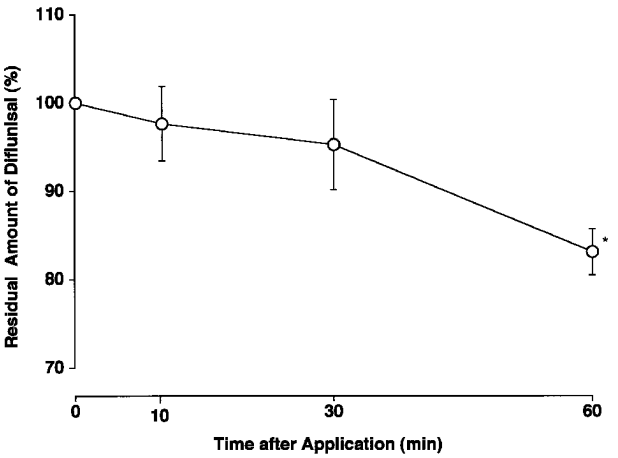

Fig. 3. Residual Amount of Diflunisal in Oral Ointment Applied on Test Patches and Attached to Oral Mucous Membrane

Fifty mg of ointment were applied to test patches, and attached to the oral mucous membrane. Then the residual amounts of diflunisal in test patches were measured at designated intervals after attachment. The values were normalized with the value immediately after attachment as 100%. Each point represents mean±S.D. ($n=6$).
$^{*}p<0.05$ compared to the value at the immediately after application (0 h) (Bonfferroni's test).

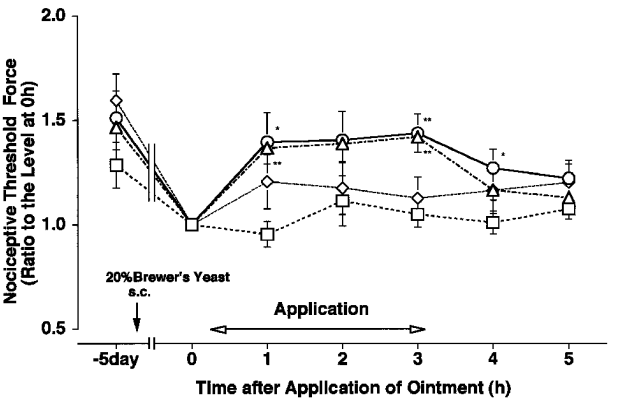

Fig. 4. Antinociceptive Effects of Diflunisal Oral Ointments and Aspirin Oral Ointment in Rat

○—○: 2% diflunisal oral ointment, ◇···◇: 0.5% diflunisal oral ointment, △·−·−·△: 2% aspirin oral ointment, □——□: Control basal oral ointment, Each point represents mean±S.D. ($n=6$). $^{**}p<0.01$, $^{*}p<0.05$ (Student's t-test)

suggested that the appropriate concentration of diflunisal in oral ointment is 2%.

5. Clinical Assessment of Analgesic Effect

Table 2 shows the results of pain relief assessment (A). Of the 3 patients who complained of severe pain before treatment, 2 reported no pain (grade 4), and 1 reported tenderness (grade 3), after treatment. The patient who had complained of moderate pain reported no pain (grade 3), and the 4 patients who had

Table 2. Effect of 2% Diflunisal Oral Ointment on Pain Associated with Oral Mucous Diseases

Pt (age sex)	Disease	Pain relief assessment			Appearance time (B)	Duration of action (C)	Global analgesic effect
		Before application	After application	Grade (A)			(A+B+C) (Grade)
A.S. (56 M)	aphthous ulcer	severe pain	tenderness	3	3	3	9 (remarkable)
N.M. (48 F)	aphthous ulcer	slight pain	no pain	2	3	2	7 (moderate)
K.D. (61 F)	aphthous ulcer	moderate pain	no pain	3	2	3	8 (remarkable)
S.T. (50 M)	decubital ulcer	slight pain	no pain	2	2	3	7 (moderate)
O.U. (48 F)	aphthous ulcer	severe pain	no pain	4	2	3	9 (remarkable)
J.T. (57 F)	aphthous ulcer	severe pain	no pain	4	3	2	9 (remarkable)
E.B. (48 F)	aphthous ulcer	slight pain	no pain	2	2	3	7 (moderate)
I.H. (42 M)	aphthous ulcer	slight pain	no pain	2	3	3	8 (remarkable)

complained of slight pain all reported no pain (grade 2) after treatment. The interval until onset of the analgesic effect (B) was within 10 minutes (grade 3) in 4 patients and within 20 minutes (grade 2) in 4 patients. The duration of the analgesic effect (C) was 5 hours or longer (grade 3) in 6 and 3 hours or longer (grade 2) in 2. Global analgesic efficacy (A+B+C) was evaluated as remarkable (grade 8–10) in 5 and moderate (grade 5–7) in 3. These results were significantly better ($p=0.014$) than the previously reported clinical effects of 2% aspirin oral ointment[4] (Mann-Whitney test).

The pain-relieving effect of diflunisal in the ointment might be ascribed to the inhibitory activity on cyclo-oxygenase in the oral mucous membranes or to the direct analgesic action of diflunisal at the peripheral sensory nerve endings, as discussed previously for the application of aspirin ointment for postherpetic neuralgia and rheumatalgia.[16,17].

From these results, the 2% diflunisal oral ointment prepared in this study was suggested to be stable at room temperature for 100 days or longer and to have significantly greater clinical efficacy than 2% aspirin oral ointment.

In patients with pain associated with lesions of the oral mucosa, it is important not only to identify its cause but also to reduce pain as soon as possible in terms of the patient's quality of life. There are only a few commercially available oral analgesic ointments, all of which contain steroids. Therefore, the development of diflunisal oral ointment may increase treatment options for patients with painful oral lesions.

We will carry out further pharmaceutical evaluations of this ointment, including consistency, adhesiveness, disintegration, and bleeding[18] as well as evaluation of the base to develop a more useful diflunisal ointment. Moreover, we also intend to evaluate non-steroidal anti-inflammatory analgesic agents other than salicylic acid.

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