[S3-5] *[4/21/2000 (Fri) 16:50~17:30/Byulkwan Auditorium, Bldg 26]*

Preclinical Study of DA-5018, a Non-narcotic Analgesic Agent

Soonhoe Kim

Research Laboratories, Dong-A Pharm. Co., Ltd.
47-5 Sanggal-Ri, Kiheung-Up, Yongin-Si, Kyunggi-Do, KOREA, 449-900

Abstract---DA-5018 is a synthetic capsaicin derivative under development as a non-narcotic analgesic agent. DA-5018 showed a potent analgesic activity against acute and chronic pain model(Table1,2.), but it had a narrow margin of safety. DA-5018 did not bind to opioid(κ ,δ , μ), NK1, CGRP receptors *in vitro* and its analgesic effect was not antagonized by naloxone, and it did not develop analgesic tolerance. In addition DA-5018 had no inhibitory effects against cyclooxygenase and 5-lipooxygenase activities. DA-5018 significantly increased the release of substance P from the slices of the rat spinal cord. These results suggest that DA-5018 is not a narcotic nor aspirin-like analgesic and the release of substance P is one of analgesic mechanism of action of DA-5018. We found that DA-5018 was almost ten times more potent and was at least 100-times less irritable compared to capsaicin. Accordingly, development of topical formula was adopted. Topical formula was desiged and screened by flux test of DA-5018 using hairless mouse skin and several formulas were selected. With these topical formulas we assessed the analgesic efficacy and carried out the toxicity, skin irritation and pharmacokinetic studies. In streptozotocin-induced hyperalgesic rat and 50 % galactose-fed hyperalgesic rat as diabetic pain models, DA-5018 cream increased the pain thresholds up to 77.0% and 24.4% respectively, while Zostrix-HP(capsaicin cream) increased by 65.9% and 21.0%. DA-5018 cream showed a good analgesic effect as well in FCA-induced arthritic rat. DA-5018 cream did not show any toxicological signs in acute and chronic toxicity test and had little skin irritation in car swelling and scratching test. Pharmacokinetics of DA-5018 were studied after topical application of ^{14}C-labelled or unlabelled DA-5018 cream. Plasma and skin concentrations except applied skin were below the detection limit and after 7-day cummulative application, plasma concentrations were also below detection limit DA-5018 may have an advantage over capsaicin and is now being developed as a topical agent for the treatment of pains. DA-5018 cream was approved for Korean IND and is now under a Phase II clinical study for arthritic pain after finising Phase I study. DA-5018 was also liscensed out to Stiefel Company in America in

1999.

Introduction

DA-5018, a synthetic capsaicin derivative, is an analgesic which acts in a different mode of action from nonsteroidal-antiinflammatory drugs(NSAIDS) and narcotic analgesics. Our research of DA-5018 has been performed by two ways, oral and topical administration routes. However, orally given DA-5018 showed very narrow safety margin in animal studies, development of topical formula of DA-5018 was adopted. The topical formula of capsaicin cream indicated for postherpetic neuralgic pain was developed in 1987 and subsequently approved for the treatment of diabetic neuropathic and rheumatoid arthritic pain. However, it has a major problem that burning sensation upon application decreases patient's compliance. Moreover, incomplete penetration of this formula limits its efficacy. DA-5018 is not less effective than capsaicin as an analgesic and, what is better, topical irritation is markedly reduced.

Formulation

The objective of formulation study was to find the cream formula which fulfill the following two prerequisites. Firstly, the penetration of DA-5018 through hairless mouse skin should be larger than that of capsaicin cream. Secondly, the cream formula should be stable at room temperature for a longer than 36 months. Considering the case of capsaicin 0.075% cream, we postulated that the level of DA-5018 in cream formula would be determined in the range of 0.05-0.5%. To verify these facts, we examined the physicochemical properties such as solubility in water and various organic vehicles, W/O distribution coefficient. The effects of pH in water phase, ratio of oil/water and the concentration of active ingredient in cream on skin penetration were investigated subsequently. The physical stabilities were also studied. Based upon these results, we found that about 0.3% was most acceptible. The penetration of DA-5018 was increased in a concentration-dependent manner in hairless mice skin. We found this formula having about six and fifteen times larger flux compared to capsaicin 0.075% cream at 0.1% and 0.3%, respectively. With the several formulas showing good scores in this study, we performed the subsequent studies.

Efficacy Study

With this formula we continued to assess the analgesic efficacy in various animal models. In conclusion, DA-5018 cream showed dose-dependent analgesic efficacy, which lead us to fix the concentration of DA-5018 at 0.3%.

1. Streptozotocin-induced diabetic neuropathy model

SD rats were rendered diabetic with intravenous injection of 60 mg/kg streptozotocin. Diabetes was confirmed by the elevation of blood glucose level within a week. When tested by modified Randall-Selitto paw pressure test, these streptozotocin-induced diabetic rats displayed a decrease of pain threshold to noxious stimuli, which is an evidence of hyperalgesia. Four weeks after diabetes induction when hyperalgesia is prevalent, each of 100 mg of DA-5018 (0.1%, 0.3%), capsaicin 0.075% cream, and ketoprofen 3% gel was topically applied on the right paw of rats for 5 hours a day during 10 days. On the repeated dosing, pain threshold was measured at the 5th and 10th day. The pain threshold of diabetic rats, when drug treatment was started, was decreased by 44% compared to that of before streptozotocin injection. Up to day 10, 0.1% DA-5018 cream showed no differences from vehicle control on the pain threshold. 0.3% DA-5018 cream brought about the increase of pain threshold by 41.4% at day 5 without statistical significance, but by 77.0% at day 10, significantly. 0.075% capsaicin cream increased pain threshold by 22.8% and 65.9% at day 5 and 10, respectively. Ketoprofen gel showed no relief of diabetes-associated pain (Figure 1).

2. Galactosaemic neuropathy model

From the beginning of this experiment, SD rats were maintained on 50%-galactose diet. Pain threshold was measured weekly by a modified Randall-Selitto test for 5 weeks. The following procedures including drug treatment and pain threshold measurement were the same with those of streptozotocin-diabetic model. Galactose-fed rats showed a decrease of pain threshold to noxious stimuli(Fig 1). DA-5018 vehicle had no effect on pain threshold up to day 10. 0.3% DA-5018 cream increased pain threshold by 25.9% and 24% at day 5 and 10 respectively, while 0.075% capsaicin cream by 16.7% and 21%. Ketoprofen gel was not effective (Figure 2).

3. Freund's complete adjuvant(FCA)-induced arthritis

The arthritic rats were made by intradermal injection of 0.6 mg Mycobacterium butyricum to the left hind paw. Arthritic pain was confirmed by measurement of pain threshold to Randall-Selitto test. Drug treatment began 30th day after FCA injection and experiment was performed following the same procedures as above. At day 5 and 10, 0.3% DA-5018 cream significantly increased pain threshold compared with vehicle control. 0.075% capsaicin cream increased the pain threshold at day 5 and significantly at day 10 (Figure 3).

4. *Croton oil*-induced inflammation

One hour before the topical application of 2% croton oil on the right ear of ICR mouse, 20 mg of each cream was topically applied to the same site. Five hours after topical applications, tissue samples were obtained from the same sites of right and left ear of mouse using a punch. Differences between the weight of the left and the right ear were regarded as the extent of

edema. Compared with untreated control, 0.1% and 0.3% DA-5018 cream and 0.075% capsaicin cream inhibited croton oil-induced ear swelling by 30.3%, 36.9% and 32.0% respectively. The inhibitory effects of 0.3% DA-5018 and 0.075% capsaicin cream exhibited statistically significant differences compared to vehicle control. Ketoprofen gel inhibited ear swelling by 20.4% without significance.

Safety Study

We assessed the skin irritability and toxicity of DA-5018 by the following studies.

1. General Pharmacology

The general pharmacological properties of DA-5018 on central nervous, cardiovascular, gastrointestinal and other organ systems were studied in experimental animals. 0.3% DA-5018 cream had no effects on behavior, hexobarbital-induced sleeping time, body temperature, spontaneous activity, blood pressure, heart rate, intestinal movement, urine volume and electrolytes excretion even at a highest dose of 2 g/kg in rats.

2. Skin irritation

1) Ear swelling test

DA-5018 cream was applied topically to the mouse and rat ear. Neither ear volume nor scratching behavior was affected by DA-5018 cream. When using both mice and rats, 0.075% capsaicin cream caused significant increase of ear volume (Figure 4).

2) Thermal hyperalgesia in rats

Thermal hyperalgesia was assessed in normal and carrageenan-induced inflamed paw by means of plantar test apparatus. In this study, we measured the paw withdrawal latency to noxious radiant heat application in unrestrained rats after 3 h application of the test creams. In the 0.3% DA-5018 cream treated rats, there was no significant latency change either in normal or in inflamed paw. On the other hand, 0.075% capsaicin cream significantly decreased the withdrawal latency in the normal paw throughout the 6-day testing period and potentiated the latency decrease in the inflamed paw.

3) Local irritant effects on human beings

In a blind study, 37 healthy men and women volunteers were randomly treated with either of DA-5018, capsaicin cream or cream vehicle on the back of the hand. Skin irritation was evaluated by visual analogue scales of burning sensation and itching. 0.3% DA-5018 cream exerted no sign of irritation. On the contrary, capsaicin cream significantly increased burning sensation and itching from 15 min after application to 5 min after washing out the cream. The incidence of burning sensation was over 90%.

In conclusion, these results indicate that DA-5018 cream has little local skin irritation properties as a decisive merit over capsaicin cream.

3. 13-week dermal toxicity study

This study was carried out to assess the potential dermal and systemic toxicity of DA-5018 administered topically to beagle dogs daily for 13 consecutive weeks. Each group was administered one of three creams containing DA-5018 in concentrations of 0.1%, 0.3% and 0.9%, respectively. All creams were administered at a dose level of 500 mg/kg/day. All animals were sacrificed after 13 weeks of treatment. All animals survived until termination of treatment. There were no dermal changes at the treatment sites that were not seen in dogs receiving the base cream only. Cardiovasular and ophthalmological assessments revealed no effect of DA-5018. Values obtained in the clinical pathology studies suggest that there was no effect of DA-5018 in this experiment and this judgement was borne out by the absence of meaningful variations in the organ weight data. Furthermore, there was no toxicologically significant tissue change, macroscopic or microscopic, in any treated animals. In conclusion, daily dermal administration of cream formulations of DA-5018 revealed no topical or systemic evidence of an adverse effect of this substance during 13 weeks administration to beagle dogs.

Mode of Action

In receptor binding assay, DA-5018 exhibited very low affinity against opiate receptor subtypes(Table 3). DA-5018 showed no inhibitory effects on cyclooxygenase and 5-lipoxygenase activities(Table 4). DA-5018 significantly increased the release of substance P from the slices of the rat spinal cord(Table 5). And DA-5018 reduced substance P contents in the paw skins of FCA-induced arthritic rats. These results suggest that the release of substance P is partially involved in the mechanism of analgesic action of DA-5018 (Figure 5).

Pharmacokinetics

Pharmacokinetics of DA-5018 were evaluated after a subcutaneous injection or topical application of 14C-labelled or unlabelled DA-5018 to rats and rabbits. After subcutaneous injection of 14C-labelled or unlabelled DA-5018 to rats, the plasma total activity peaked at 2 hr with the terminal half-life of 5.34 hr, whereas unlabelled DA-5018 peaked at 1 hr with the terminal half-life of 1.26 hr. Moreover, the AUC and MRT increased significantly based on total radioactivity compared with intact DA-5018. Above data indicated that DA-5018 is extensively metabolized in rats, and the terminal half- life of the metabolite(s) was longer than

that of DA-5018. The cumulative percentages of biliary excretion of dose after subcutaneous injection of [^{14}C]DA-5018 was 40.2%, whereas the value was only 2.14% when unlabelled DA-5018 was injected. After topical application of 0.1% or 0.3% ^{14}C-labelled or unlabelled DA-5018 cream, 500 mg/kg to rats, the plasma and tissue concentrations except applied skin were under the detection limit. After consecutive 7 days topical application of unlabelled 0.1% and 0.3% DA-5018 cream to rats, the plasma concentrations were also under the detection limit. But the urinary excretion of DA-5018 was significantly increased by repeated topical administration. After topical application of unlabelled 0.1% and 0.3% DA-5018 cream to rabbits the plasma and urine concentrations were under the detection limit. Above data indicated that the skin penetration of DA-5018 was lower and the metabolism of DA-5018 was higher in rabbits than that in rats.

Summary

From these results of preclinical studies, we concluded that DA-5018 cream as topical analgesic agent may have an advantage over capsaicin cream. DA-5018 cream is now being developed for the treatment of pain associated diabetic neuropathy, postherpetic neuralgia and other pains including the rheumatoid arthritis and sport trauma. A Korean IND of DA-5018 was approved and a Phase II clinical study is in progress now after finishing Phase I clinical study. DA-5018 was also linscensed out to Stiefel Company in America in November last year and was under development for postherpetic neuralgic pain and pruritis.

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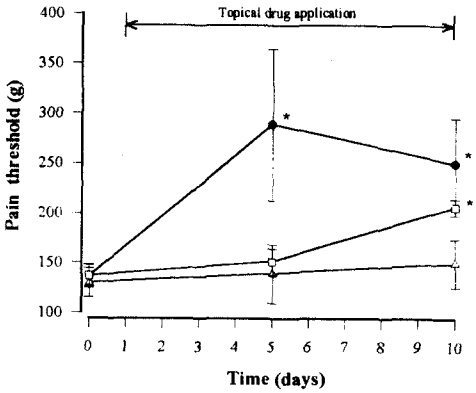

Fig. 3. Effects of DA-5018 cream(●, 0.3%), capsaicin 0.075% cream(□) and vehicle(△) applied topically on pain thresholds in FCA-induced arthritic rats. Drugs were applied for 10 days. An asterisk denotes a significant difference from day 0 at $p<0.05$(*).

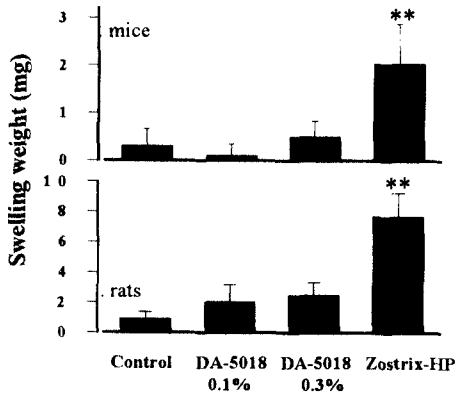

Fig. 4. Ear swelling induced by DA-5018 and capsaicin cream applied topically in mice(A) and rats(B). Each bar represents the mean of 10 to 13 animals. An asterisk denotes a significant difference from control at $p<0.05$(*) and $p<0.01$(**).

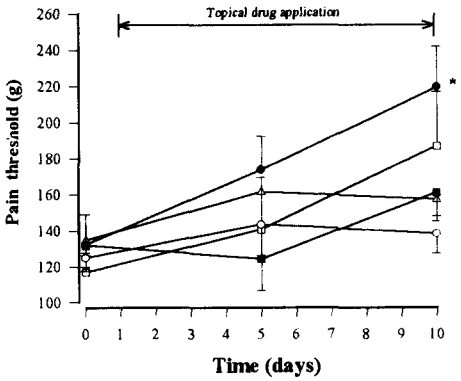

Fig.1. Effects of DA-5018 cream(○, 0.1%;●, 0.3%), capsaicin 0.075% cream(□) ketoprofen gel(■) and vehicle(△) on pain thresholds in streptozotocin-diabetic rats Drugs were applied topically for 10 days. An asterisk denotes a significan difference from pre-drug value at $p<0.05$(*).

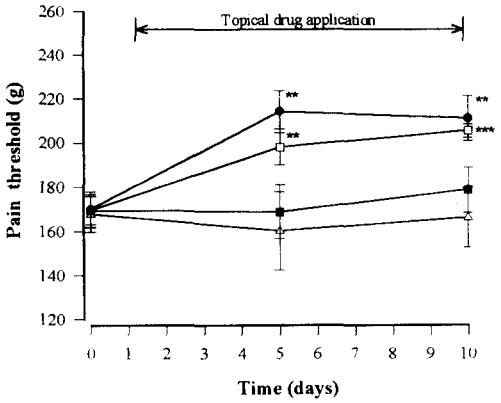

Fig. 2. Effects of DA-5018 cream(●, 0.3%), capsaicin 0.075% cream(□), ketoprofen gel(■) and vehicle(△) on pain thresholds in galactose-fed rats. Drugs were applied topically for 10 days. An asterisk denotes a significant difference from day 0 at $p<0.01$(**) and $p<0.001$(***).

Table 1. Effect of p.o. Administered DA-5018 on Acute Pain Models

Items	Species	ED_{50} (mg/kg)		
		DA-5018	MOR	APAP
Acetic acid-Writhing	Rat	2.4	2.5	18.2
PBQ-Writhing	Rat	1.3	1.8	--
Randall-Selitto	Rat	4.5	18.3	477
Tail Pinch	Rat	5.8	18.2	932
Tail Flick	Rat	20.5	39.9	--

Table 2. Effect of s.c. Administered DA-5018 on Acute & Chronic Pain Models

Items	Species	ED_{50} (mg/kg)				
		DA-5018	MOR	CAP	APAP	KPF
Acetic acid-Writhing	Rat	0.09	0.3	1.44	45.4	--
Randall-Selitto	Rat	0.66	3.9	42	643	--
Tail Flick	Rat	2.0	2.6	14.2	>1000	--
Hot Plate	Mouse	1.9	--	26.5	>2000	--
Formalin test / Phase I	Rat	0.2	3.5	--	--	--
/ Phase II		0.17	4.3	--	--	--
FCA-induced Arthritis	Rat	1.07	--	23.5	--	2.97
Croton oil-induced Inflammation	Rat	1.6	--	12.7	--	710
STZ-induced Diabetic Neuropathy	Rat	0.2 (*No tolerance for 7 consecutive days*)				

Table 3. Opiate Receptor Binding Assay

Drugs	Ki (nM)			
	Non-selective	μ-opioid	κ-opioid	δ-opioid
DA-5018	299±8.88	735±215	2930±163	1550
Capsaicin	> 10000	> 10000	> 10000	> 10000
Morphine	0.94±0.76	4.95±4.45	44.8±5.08	11.5
Naloxone	-	-	1.27±0.635	22

(Mean ± SD)

Table 4. Effects of COX-1, 2 and 5-LO

Assay	IC_{50}
Cyclooxygenase-1	> 30 μM
Cyclooxygenase-2	> 10 μM
5-Lipooxygenase	> 30 μM

Table 5. Release of SP from Spinal Cord

Drugs	Conc. (μM)	Immunoreactive SP (pg/mg wet protein)
Control	0	0.225±0.387
DA-5018	1	1.180±0.158
	10	2.099±0.818
Capsaicin	1	2.351±0.144
	10	2.379±0.789

(Mean ± SD)