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Effect of Treatment with Transdermal Ketoprofen on Adjuvant-Induced Arthritis

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Abstract : This study demonstrates the anti-arthritic effect of topical application of ketoprofen gel containing *N*-methyl-2-pyrrolidone (NMP) in adjuvant arthritis therapy. Adjuvant arthritis was induced by a single injection of Freund's complete adjuvant (FCA). Mature female Sprague-Dawley rats were designated to 3 groups such as control group, K10 group (ketoprofen 10 mg/rat), and NK10 group (ketoprofen 10 mg/rat containing NMP). The anti-arthritic activity of ketoprofen containing NMP was tested not only as to its capability to suppress the inflammatory edema, but also bone damage (X-ray score and regional bone uptake) of the hind paw in arthritis-induced rats. These results showed a higher efficacy of ketoprofen containing NMP than ketoprofen treatment in the adjuvant-induced arthritis. Ketoprofen containing NMP has good intrinsic characteristics for formulation in an efficacious anti-inflammatory topical application.

Key words **:** Adjuvant arthritis, Ketoprofen, *N*-methyl-2-pyrrolidone, rat

Introduction

The administration of non-steroidal anti-inflammatory drugs (NSAIDs) began over 100 years ago with the introduction of salicylic acid for the treatment of rheumatic diseases (3,13). They are now the most commonly used drug due to their analgesic, antipyretic, and anti-inflammatory properties (7). The term NSAID refers to anti-inflammatory agents that inhibit some component of the enzyme system that converts arachidonic acid into prostaglandins and thromboxanes (8). Ketoprofen is a potent NSAID that is used for the treatment of rheumatoid arthritis. Unfortunately, because the oral administration of ketoprofen can cause gastric irritation and renal adverse effects, ketoprofen is restricted to IV and IM administration. Considering that most inflammatory diseases occur locally and near the body surface, transdermal delivery of NSAIDs may turn out to be an interesting strategy to deliver these drugs directly to the diseased site and to increase local concentration (1,9). However, poor skin penetration limits the efficacy of topically applied ketoprofen.

N-methyl-2-pyrrolidone (NMP), a powerful and widely used organic solvent, has been increasingly used in a variety of industries, including petroleum refining, microelectronics, pesticide formulation, and veterinary medicine. NMP was investigated as a cosolvent for model drug compounds of widely varying polarity such digoxin, sulfamethoxazole, hydrocortisone acetate, theophylline, phenytoin, and reserpine (12). NMP reported as a potential enhancer is amphiphilic solvent, and reduces the size of droplets. It thus has same function in the emulsion system (4).

The aim of this study was to evaluate the anti-arthritic effect of a topical formulation of ketoprofen containing NMP on the basis of early detection of arthritis induced by intradermal injection of mycobacterial adjuvant in rat using radionuclide bone scan employing ^{99m}Tc-MDP.

Materials and Methods

Materials

Raw ketoprofen (30 mg/g) gel and ketoprofen (30 mg/g) gel containing 2% *N*-Methyl-2-pyrrolidone (NMP) were provided by Sanga Pharm Co., Ltd.

Animals

7 week old female Sprague-Dawley rats (Samtako, Korea) were used for this study. To reduce the stress associated with the experimental procedure, rats were handled daily for 1 week before experimentation. Rats were divided into two groups: one for measurement of paw volume and x-ray score (n=10), another for measurement of regional bone uptake (n=5). And each group was subdivided into three groups:

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control group, K10 group (ketoprofen 10 mg/rat), and NK10 group (ketoprofen 10 mg/rat containing NMP). Five rats were housed per cage (43×27×18 cm) in an air-conditioned environment (room temperature 23±2°C, humidity 55±5%) that was illuminated from 6:30 to 18:30. Animals were fed with a commercial diet (Samyang Feed Co., Korea), and had free access to tap water. The protocols were in accordance with guidelines of Chonnam National University of Animal Care and Use Committee.

Induction of arthritis

On day 0, each rat was injected in the plantar region of the right hind limb with Freund's complete adjuvant (Gibcobrl, USA, Lot No. 1020159) containing 0.6 mg of *Mycobacterium butyricum* (Difco, USA, Lot No. 138137LA) suspended in 0.1 ml of paraffin oil.

Treatment of topical ketoprofen

Animals were divided into three groups; positive control group, K10 group (ketoprofen 10 mg/rat), and NK10 group (ketoprofen 10 mg/rat containing NMP).

Adjuvant-arthritis rats were treated with ketoprofen 10 mg/rat (K10 group) and ketoprofen 10 mg/rat containing NMP (NK10 group) at a site on the back every day for 14 days (from day 7 to day 21 after the adjuvant injection). After administration of drugs, the treated site was dressed initially by applying gauze, and then covering with commercially available, adhesive tape for 3 hours.

Measurements of paw volume

Paw volumes of the injected (right) and non-injected (left) hind paws were measured up to a mark made on the tibiotarsal joint by using a messcylinder.

Radiographic assessment of bony changes

Radiographic changes were assessed under blind conditions using a previously described scoring system (10). Each limb was assessed for osteopenia and bone erosions and graded from 0 to 3 as follows: 0=no change; 1=slight change; 2=moderate change; and 3=severe change. All limbs were graded, and the scores were summed up to a maximum possible score of 3.

Measurement of regional ^{99m}Tc-MDP bone uptake

Bone scans were obtained using a large field of view gamma camera, equipped with a parallel-hole and low-energy collimator, 3 hours after intravenous injection of ^{99m}Tc-MDP (0.05 mCi/rat) to rats. Whole body images were acquired with a gamma camera (set at 140 KeV photoelectric peak, 20% symmetrical window) at 3 hrs. Regions of interest (ROIs) for regional skeletal uptake were measured in anterior view. Thereafter, regional skeletal uptakes of adjuvant-injected and non-injected paws were assessed by evaluating the rectangular ROIs.

Statistical analysis

All results were expressed as mean±standard deviation. The statistical significance of differences was assessed by the Student's *t-test;* P values of < 0.05 were considered significant.

Results

Inhibition of paw edema

In control group, the adjuvant-injected paw showed that primary inflammation began on day 3 after adjuvant injection. On day 7, the increase of paw edema reached 150%, which increased until the end of experiment. On the other hand, the non-injected paw showed a 110% increase in paw edema on day 21 after injection (Fig 1).

This edema was inhibited by ketoprofen (10 mg/rat) and NMP-ketoprofen (10 mg/rat) treatment from day 14. Transdermal administration of ketoprofen gel containing NMP suppressed paw edema in non-injected paw as well as injected paw. In the injected paw, NK10 group was significantly inhibited paw edema compared to K10 group from day 14 to 21 ($p < 0.05$), but did not show significant difference in non-injected paw (Fig 1).

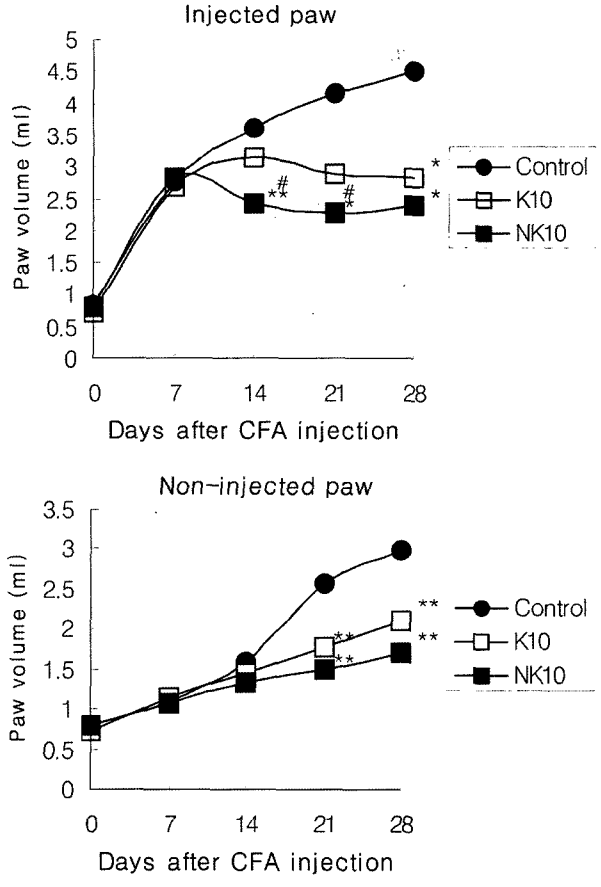

Fig 1. Inhibitory effect of ketoprofen containing *N*-methyl-2-pyrrolidone on paw edema in adjuvant-induced arthritis rats. Values represent means of 10 animals. $^{*}P<0.05$, $^{**}P<0.01$ compared to control group. $^{\#}P<0.05$ compared to K10 group.

Inhibitory effect on X-ray score increase

In control group, the main radiological legions were initially detected osteoporosis, erosion and periosteal reaction in hind paw after day 14, especially in the adjuvant-injected hind paw. Severe bone legions were detected in all regions of the femur and tibia of the adjuvant-non injected paw as well as the adjuvant-injected paw after day 28.

As shown in Fig 2, the bone legions of K10 and NK10 groups were significantly inhibited compared to control group on day 21 and 28. NK10 group was significantly inhibited bone destruction compared to K10 group on day 21 ($p<0.05$).

Inhibitory effect on regional bone uptake

^{99m}Tc-MDP scintigraphy was performed after intravenous injection of ^{99m}Tc-MDP (0.05 mCi/rat). Images of the entire body were acquired with a gamma camera at 3 hrs. ROIs for regional skeletal uptake were measured in anterior view. Thereafter, regional skeletal uptakes of adjuvant-injected and non-injected paws were assessed by evaluating the rectangular ROIs.

Bone legions in control group were initially detected by bone scintigraphy on day 7. Bone legions in adjuvant-injected and non-injected paws were significantly detected on day 21, which continued to increase until the end of experiment. But, the increase of bone uptake in adjuvant-injected paw was significantly inhibited as compared to control group by K10 and NK10 groups on day 14 and 28 (Table 1), and the increase of bone uptake in adjuvant-non injected paw was significantly inhibited on day 28 (Table 2).

In the adjuvant-injected paw, NK10 group was significantly inhibited the increase of bone uptake compared to K10 group on day 28 ($p<0.05$), but was not significantly inhibited in the non-injected paw (Table 2).

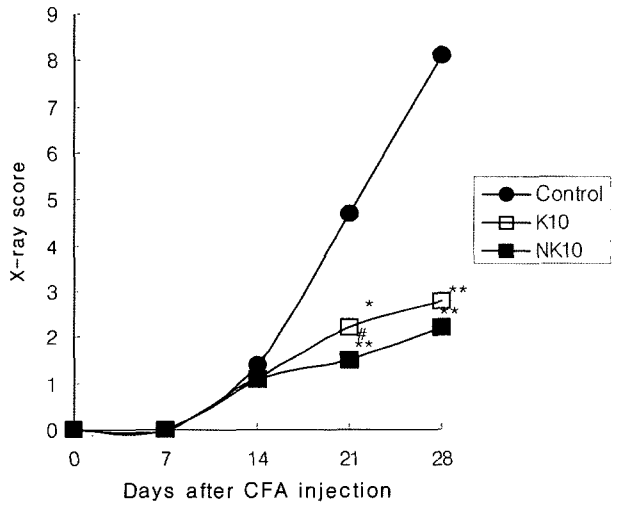

Fig 2. Inhibitory effect of ketoprofen containing *N*-methyl-2-pyrrolidone on X-ray changes in adjuvant-induced arthritis rats. Values represent means of 10 animals. $^{*}P<0.05$, $^{**}P<0.01$ compared to control group. $^{\#}P<0.05$ compared to K10 group (n=10).

Table 1. Inhibitory effect of ketoprofen containing *N*-methyl-2-pyrrolidone on regional ^{99m}Tc-MDP bone uptake in the adjuvant-injected paw (Units: Counts/464 Pixels)

Group	Time after adjuvant injection	
	Day 14	Day 28
Control	5,153±921	7,888±1784
K10	3,100±248**	3,110±249**
NK10	2,771±308**	2,452±332**#

Values represent means±S.D. of 5 animals.
$^{*}P<0.05$, $^{**}P<0.01$ compared to control group.
$^{\#}P<0.05$ compared to K10 group.

Table 2. Inhibitory effect of ketoprofen containing *N*-methyl-2-pyrrolidone on ^{99m}Tc-MDP bone uptake in the adjuvant-non injected paw (Units: Counts/464 Pixels)

Group	Time after adjuvant injection	
	Day 14	Day 28
Control	2,538±280	4,983±1048
K10	2,312±304	2,114±326**
NK10	2,148±515	2,064±432**

Values represent means±S.D. of 5 animals.
$^{*}P<0.05$, $^{**}P<0.01$ compared to control group.

Discussion

Drug delivery through the transdermal route is limited by low skin permeability. One of the major functions of skin is to prevent the body from losing water into the environment and to block the entry of exogenous agents. The skin composite structure is indicated by the 3 distinct layers; the stratum corneum, the viable epidermis and the papillary layer of the dermis (11). The stratum corneum, the outermost layer of the skin, acts as a major barrier and is often rate limiting (5). So, considerable research work has been focused on discovering methods to increase stratum corneum permeability (5). One approach was to employ chemical penetration enhancers, which may increase the permeability of stratum corneum by increasing drug diffusivity within the membrane and/or by increasing drug partition from the applied formulation into the skin and/or by increasing the effective concentration of drug in the vehicle (14).

The physicochemical property and percutaneous absorption behavior of enhancer can be considered as one of the most important factors controlling the pattern of enhancing effects and side effects. Transdermal formulations containing *N*-methyl-2-pyrrolidone (NMP) have been applied industrially and investigated in the delivery of numerous drugs. NMP, a powerful and widely used organic solvent, has been increasingly used in a variety of industries, including petroleum refining, microelectronics, pesticide formulation, and veterinary medicine. NMP enhances a penetration of transdermal drugs by increasing solubility and reducing size of droplets (4).

Anti-arthritic activity studies were carried out for verifying the effect of transdermal ketoprofen on adjuvant-induced arthritis. It was shown in the present study that anti-arthritic activity of ketoprofen gel using NMP was more potent than that of ketoprofen showed in paw edema and X-ray views. Additional evidence for disease-modifying activity observed in bone scintigraphy, in which bones destruction were significantly protected by administration of ketoprofen containing NMP.

From above all studies, we showed that daily topical application of ketoprofen containing NMP effectively inhibited inflammatory edema and bone destruction of hind paw in adjuvant-induced arthritic rats. The ability of skin permeable solvent molecules to influence cotransport of drugs has been previously reported with ethanol (2). It is also likely that NMP enhanced ketoprofen flux by disordering and/or solubilizing the lipid bilayers of the SC and by increasing the partitioning of drug into the skin, both of which have been previously observed in the presence of NMP (12).

Although the mechanisms of action of the anti-inflammatory and joint protective activities of ketoprofen containing NMP in the adjuvant-induced rats are not proved in the present studies, it is reasonable consider that ketoprofen administration immediately after detection of adjuvant-induced arthritis blocks pathogenic changes in bones by adjuvant injection.

This results demonstrate that early topical application of ketoprofen gel using NMP effectively inhibited the increase in edema of the hind paws and bone destruction of arthritic rats. The greater efficacy of ketoprofen contained NMP than ketoprofen was demonstrated in a clinical trial with adjuvant-induced arthritis. Ketoprofen contained NMP has good intrinsic characteristics for formulation in an efficacious anti-inflammatory topical application.

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Adjuvant-induced arthritis에 대한 경피용 ketoprofen 제제의 치료효과

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요 약 : 본 연구는 랫드의 우측 후지 발바닥에 Freund's complete adjuvant를 투여하여 관절염을 유발한 후에 *N*-methyl-2-pyrrolidone (NMP) 기제를 첨가한 경피용 ketoprofen 제제의 관절염 치료 효과를 확인하기 위해서 수행되었다. NMP를 함유한 ketoprofen 경피용 제제의 관절염 치료 효과를 알아보기 위해서 Sprague-Dawley 암컷 랫드를 대조군과 약물 투여군으로 분리하고, 약물 투여군은 ketoprofen 10mg/rat 투여군(K10군)과 NMP가 함유된 ketoprofen 10mg/rat 투여군(NK10군)으로 분류하였다. 실험적 관절염을 유발시킨 후 ^{99m}Tc-MDP을 이용한 bone scan에서 골병변이 나타난 7일 후부터 14일 동안 약물을 매일 한번씩 랫드의 등에 국소 도포하여 다음과 같은 결론을 얻었다. NMP의 기제를 첨가한 ketoprofen 경피용 제제가 ketoprofen 단독 경피용 제제 보다 후지 부종을 현저하게 감소시켰고, 단순 방사선 사진과 scintigraphy에서 관절염에 의한 골 파괴를 효과적으로 억제하였다. 이상의 연구 결과를 기초로 하여 관절염 치료에 있어 NMP를 함유한 ketoprofen 경피용 제제가 ketoprofen 단독 제제보다 효과가 있다는 결론을 얻을 수 있었다.

주요어 : Adjuvant arthritis, Ketoprofen, *N*-methyl-2-pyrrolidone, rat.