Journal of Oleo Science

doi : 10.5650/jos.ess16036
J. Oleo Sci. **65**, *(12) 1045-1053 (2016)*

JOURNAL OF OLEO SCIENCE
Jos

Pharmacokinetic Studies of Gel System Containing Ibuprofen Solid Nanoparticles

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Abstract: In the therapy of rheumatoid arthritis, ibuprofen (IBU) is widely used; however, it has been limited the clinical use by its systemic side effect, such as gastrointestinal lesions. Therefore, we prepared topical gel ointment used IBU solid nanoparticles (IBU_{nano}-gel formulation). In addition, we demonstrated their anti-inflammatory effect by using arthritis model rat (adjuvant-induced arthritis rat, AA rat). The gel formulations were prepared using additives (Carbopol 934, 2-hydroxypropyl-β-cyclodextrin and methylcellulose) and bead mill-method. The IBU particle size in the IBU_{nano}-gel formulation was 208 nm. The increase in inflammation of the hind feet of AA rats was attenuated by the treatment with the IBU_{nano}-gel formulation, and preventive effect was higher than that of a gel formulation containing IBU-microparticles (IBU_{micro}-gel formulation, mean particle size 85.4 μm); the accumulation and permeability through the skin of IBU from the IBU_{nano}-gel formulation were significantly larger in comparison with the IBU_{micro}-gel formulation. Further, no gastrointestinal lesions were observed in AA rats following the repetitive administration of the 5% IBU_{nano}-gel formulation (0.30 g) for 42 days (once a day). These results suggest that the dermal application of IBU-nanoparticles provide effective and efficient therapy that spares patients from unwanted side effects.

Key words: solid nanoparticle, ibuprofen, gel system, drug delivery, rheumatoid arthritis

1 INTRODUCTION

Ibuprofen[IBU, molecular mass 206.30]is a nonsteroidal anti-inflammatory drug(NSAID)whose racemic form is considered to be a non-selective cyclooxygenase(COX)inhibitor[1]. IBU is widely used as one of the best tolerated NSAIDs available for the therapy of postoperative pain and rheumatic and rheumatoid arthritis(RA)[2,3]. On the other hand, it also shows adverse effects that include ulcers/bleeding of the gastrointestine, dyspepsia, diarrhoea, nausea, increased hepatic enzymes, headache, rash, hypertension, constipation, epistaxis, priapism, salt and fluid retention, dizziness. Although incidence of adverse gastrointestinal reactions of IBU is the lowest in the all non-selective NSAIDs, this is only the case at low doses of IBU, and the usually advisable maximum daily dose is 600 mg. In addition, IBU shows low solubility or dissolution rate to water[4-6]. From these reason, the drug delivery systems (DDS)need to be precise in their control of drug distribution to reduce the systemic side effect.

In the chronic use of the drug, the topical and transdermal dosage forms are desirable, although the delivery through the skin is limited by its barrier properties. Therefore, it is important to design enhancement techniques to assist the dermal delivery of IBU, and several research has been done to develop the topical and transdermal IBU formulations[7-9]. These systems have been employed to improve the delivery of IBU through the skin, and it have been developed the different dermal formulations such as patches[7], gels[8] and liposomes[9] incorporating various permeation enhancers. In addition, strategies using nanoparticles and nanocarriers have also been demonstrated[9]. A number of groups have been studied the lipid nanocarriers for dermal drug delivery. The various systems tried include lipid nanocapsules(LNC, a lipid core surrounded by a tensioactive shell), nanostructured lipid carriers(NLC, a mixture of liquid and solid lipids)and solid lipid nanoparticles(SLN, they are made up of lipids that solidify at room temperature stabilized by a surfactant shell)[10]. We have reported that dermal applications using nanoparticles enhance drug permeability through the skin[11-13]. Therefore, it is expected that its drug system lead to an alternative strategy for increase in drug permeation[14-17]. More-

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Accepted August 3, 2016 (received for review February 15, 2016)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

over, the topical DDS may provide an expansion in the therapeutic use of IBU. Drug systems using nanoparticles are useful for therapy *via* the skin, although the characteristics needed for high skin penetration and diffusion within the skin differ for different kinds of drugs[11−13]. Therefore, it is important to determine the pharmacokinetics for more drugs. In this study, we have designed topical formulations containing IBU solid nanoparticles, and demonstrated their pharmacokinetics, stability and the anti-inflammatory effect by using arthritis model rat (adjuvant-induced arthritis rat, AA rat). In addition, we discussed the mechanism of the skin penetration in the formulations containing solid nanoparticles by using the IBU_{nano}-gel formulations and previously designed gel formulations containing tranilast (TL_{nano}), indomethacin (IMC_{nano}), ketoprofen (KET_{nano}) nanopariticles[11−13].

2 EXPERIMENTAL

2.1 Animals

Male 6-13-week-old Dark Agouti (DA) rats and 7-week-old Wistar rats were used in this study. All animal experiments were performed in accordance with the Kinki University School of Pharmacy Committee for the Care and Use of Laboratory Animals.

2.2 Preparation of a gel formulation containing IBU-nanoparticles

The gel formulation was prepared according to our previous reports[11−13]. Briefly, IBU-nanoparticles were prepared using Bead Smash 12 and zirconia beads (Wakenyaku Co. Ltd, Kyoto, Japan)[11−13, 18−21]. Conventional IBU powder (solid, IBU-microparticles, 85.4 ± 0.23 μm, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and methylcellulose METOLOSE SM-4 (MC, Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) frozen in liquid nitrogen were milled by the Bead Smash 12 for 30 sec at 4℃ (3,000 rpm). The mixtures were dispersed in 0.5% 2-hydroxypropyl-β-cyclodextrin solution (HPβCD, Nihon Shokuhin Kako Co., Ltd., Tokyo, Japan), and milled again at 4℃ (30 sec × 15 times, 5,500 rpm). The particle size of milled IBU (IBU_{nano}) was 0.208 ± 0.081 μm. After milling, Carbopol® 934 (Carbopol, Serva, Heidelberg, Germany) was added to prepare a gel ointment (IBU_{nano}-gel formulation). A preparation of gel formulation containing IBU-microparticles was done by adding IBU-microparticles, MC and HPβCD (IBU_{micro}) into Carbopol gel (IBU_{micro}-gel formulation). The formulation of the gels containing IBU was as follows: 5% IBU, 0.5% MC, 0.5% HPβCD, 3% Carbopol, w/w%. The particle size and IBU concentration were measured using a SALD-7100 (Shimadzu Corp., Kyoto, Japan, refractive index 1.45-0.010i) and HPLC methods. The dispersity in the formulation base was determined as follows: the 5% IBU_{micro}- and IBU_{nano}-gel formulations were divided into 10 parts, and kept for 1 month (22℃ in the dark). The solubility of IBU in purified water with and without 0.5% HPβCD was 0.009% and 0.007%, respectively (the inclusion complex by 0.5% HPβCD was 0.002%).

2.3 Release of drug from a IBU_{nano}-gel formulation

An experiment was carried out using a Franz diffusion cell (reservoir volume 12.2 mL, 1.6 cm i.d. *O*-ring flange) and MF™-MEMBRANE FILTER of 25 and 450 nm pore size (25 nm-membrane, 450 nm-membrane, Merck Millipore, Tokyo, Japan) according to our previous reports[11, 12]. The IBU-gel formulation (0.30 g) was spread uniformly over the membrane, and the diffusion cells were incubated at 37 ℃, and 100 μL aliquots of sample solution were withdrawn from the reservoir chamber filled buffer (0.85% NaCl-10 mM phosphate buffer, pH 7.4). The IBU concentration in the sample solution was measured by a Shimadzu LC-20AT system equipped with an GL Science Inertsil® ODS-3 column (Tokyo, Japan). The wavelength for detection and column temperature was 210 nm, 35℃, respectively. A propyl *p*-hydroxybenzoate was used as internal standard; the mobile phase consisted of 0.1% phosphoric acid/acetonitrile (60/40, v/v) at a flow rate of 0.25 mL/min.

2.4 Application of gel formulations containing IBU-nanoparticles to AA rats

The experiment was done following to our previous reports[11, 12]. Arthritis was induced by the injection of a heat-killed *Mycobacterium butyricum* (10 mg/mL, Difco, Detroit, MI) in Bayol F oil (adjuvant) into DA rats. The rats injected Bayol F oil alone was use as control group. The application (0.30 g) of IBU-gel formulations and a commercially available formulation (VESICUM® formulation 5%) was started after adjuvant injection, and treated with the right foot daily (9:00). Inflammation is determined by measuring paw edema, and was quantified using the according to Eq. 1:

$$\text{Paw edema}\,(\Delta\text{mL}) = \text{Paw volume of arthritis rat} - \text{Paw volume of normal rat} \quad \text{Eq. 1}$$

AUC_{edema} (AUC during 0-42d) was analyzed from the following Eq. 2:

$$AU_{Cedema} = \int_{0d}^{42d} V_{edema}\,dt \quad \text{Eq. 2}$$

The t, V are the days after adjuvant injection and the volume of paw edema, respectively. In this study, the AUC_{edema} show the inflammatory scores.

2.5 *In vitro* skin penetration of gel formulations containing IBU-nanoparticles

The *in vitro* skin penetration experiment was carried

out using the Franz diffusion cell connected to the abdominal skin of 7 week-old Wistar rats according to our previous reports[11, 12]. The IBU-gel formulation (0.30 g) was uniformly spread over the stratum corneum of the skin, which was then mounted in a Franz diffusion cell at 37 ℃ (reservoir chamber was filled 0.85% NaCl-10 mM phosphate buffer, pH 7.4). The IBU concentrations were measured by the HPLC method described above, and were analyzed according to the following Eqs. 3-5[11, 12]:

$$t_{lag} = \frac{\delta^2}{6D} \quad \text{Eq. 3}$$

$$J_c = \frac{K_m \cdot D \cdot C_{IBU}}{\delta} = K_p \cdot C_{IBU} \quad \text{Eq. 4}$$

$$Q_t = J_c \cdot A \cdot (t - t_{lag}) \quad \text{Eq. 5}$$

where t_{lag} is the lag time, J_c is the IBU penetration rate, δ is thickness of the skin (0.071 cm, average for 5 rats), K_m is the skin/preparation partition coefficient, Q_t is the total amount of IBU appearing in the reservoir solution at time t, A is the effective area of skin (2.0 cm^2) and D is the diffusion constant within the skin. In the calculation, a nonlinear least-squares computer program (MULTI) was used[11, 12, 22].

2.6 *In vivo* percutaneous absorption of gel formulations containing IBU-nanoparticles

The *in vivo* percutaneous absorption experiment was carried out according to our previous reports[11, 12]. An IBU-gel formulation (0.30 g) was fixed on the shaved abdominal skin, and 200 μL of venous blood was collected from the jugular vein to measure the IBU concentration at intervals 0-24 h after the application of the IBU-gel formulation. The blood was centrifuged (3,000 rpm, 20 min, 4℃), and the IBU concentrations were measured by the HPLC method described above. The IBU concentration in the plasma after a single injection of 300 μL of IBU solution containing 1% dimethyl sulfoxide (100 μg/kg) into the femoral vein was analyzed according to Eq. 6[23]:

$$C_{IBU} = C_0 \cdot e^{-k_e \cdot t} \quad \text{Eq. 6}$$

where k_e (2.24 ± 0.21 h^{-1}, n = 7) is the elimination rate constant for IBU from the plasma. C_0 is the initial concentration of IBU in the plasma (1.83 ± 0.18 nmol/mL), C_{IBU} is the IBU concentration in the plasma. The distribution volume (V_d) were 69.0 ± 1.16 mL/kg (n = 7).

The absorption of IBU after the administration of IBU-gel formulation was calculated as the apparent absorption rate constant (k_a, h^{-1}) according to Eq. 7[23]:

$$C_{IBU} = \frac{k_a \cdot F \cdot D}{V_d (k_a - k_e)} (e^{-k_e \cdot (t - t_{lag})} + e^{-k_e \cdot (t - t_{lag})}) \quad \text{Eq. 7}$$

where t_{lag} is lag time (h), t is time (0-24 h) after IBU administration. C_{IBU}, k_a is the IBU concentration in the plasma and the absorption rate constant, respectively. In the calculation, a nonlinear least-squares computer program (MULTI) was used.

The AUC (area under the IBU concentration-time curve), $AUMC$ (area under the first moment curve) and MRT (mean residence time) were analyzed as follows (Eqs. 8-10):

$$AUC = \int_{0h}^{24h} C_{IBU} dt + \frac{C_{IBU\ at\ 24h}}{k_e} \quad \text{Eq. 8}$$

$$AUMC = \int_{0h}^{\infty h} C_{IBU} \cdot t dt \quad \text{Eq. 9}$$

$$MRT = \frac{AUMC}{AUC} \quad \text{Eq. 10}$$

2.7 Drug accumulation in the skin from gel formulations containing IBU-nanoparticles

The accumulation of IBU in skin tissue was determined following to our previous reports[11, 12]. IBU-gel formulation (0.30 g) was treated with the shaved abdominal skin, and the pieces (2.0 cm^2) of abdominal skin were applied were excised at 6-24 h after the start of the experiment. The samples were homogenized in methanol by a homogenizer (Physcotron, MICROTEC CO., LTD., Chiba, Japan), and were centrifuged (15,000 rpm, 20 min, 4℃). The supernatants was analyzed by the HPLC method described above.

2.8 Statistical analysis

Statistical differences were evaluated by unpaired Student's, Aspin-Welch's t-tests and ANOVA followed by Dunnett's multiple comparison; P values less than 0.05 were considered significant. The particle size represent the means ± S.D., and the other data represent the means ± S.E. in this study.

3 RESULTS AND DISCUSSION

3.1 Design of Gel Formulations containing IBU-nanoparticles and It's Anti-Inflammatory Effect

We previously reported that the MC permits the formulation containing of drug nanoparticles by mill-methods using tranilast (TL), indomethacin (IMC) and ketoprofen (KET)[11–13, 18–20]. Similarly, in this study, IBU-nanoparticles could not be prepared by the bead mill-method in the absence of MC, and so IBU solid nanoparticles were prepared by the bead mill-method in the presence of MC (IBU reaches a meringue state by the bead mill-method without MC). In addition, we previously reported that Carbopol is suitable for the preparation of dermal formulations with nanoparticles[11, 12]. From these previously study, a gel formulation with IBU_{nano} was prepared by using the Carbopol. Figure 1 shows the particle size distributions of gel formulation containing 5% IBU_{nano}. The particle sizes in the IBU_{micro}- and IBU_{nano}-gel formulations were 85.4 ± 0.23 μm and 0.208 ± 0.081 μm, respectively (Fig. 1). Moreover, for 1 month after preparation, the IBU_{micro}- and IBU_{nano}-gel formulations were stable (particle size: IBU_{micro}, 85.9 ± 0.26 μm; IBU_{nano}, 0.221 ± 0.082 μm) with no decreases in IBU content in the IBU_{micro}- or

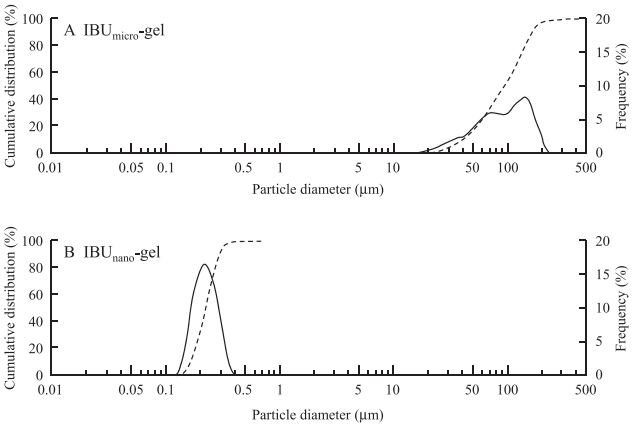

Fig. 1 Particle size distribution of 5% IBU_{micro}- and IBU_{nano}- gel formulations.
Dashed line, cumulative distribution; solid line, frequency. Mean particle size: IBU_{micro}-gel formulation, 85.4±0.23 μm; IBU_{nano}-gel formulation, 0.208±0.081 μm.

IBU_{nano}-gel formulations observed during 1 month at 22℃. These data show that the formulations in this study are suitable for the preparation of gel ointment with IBU_{nano}.

Therefore, we used the gel ointment with IBU_{nano}, and investigated IBU release and skin penetration from IBU_{nano}-gel formulations. Figure 2 shows the IBU penetration through a membrane filter after the treatment with IBU_{micro}- and IBU_{nano}-gel formulations. The amount of IBU released from the IBU_{nano}-gel formulation through a 450 nm-membrane was significantly greater than that from the IBU_{micro}-gel formulation. In experiments using 25 nm- and 450 nm-membranes, the amounts of IBU released from the IBU_{micro}-gel formulation were similar, however, the IBU penetration profile of the IBU_{nano}-gel formulation through a 25 nm-membrane was significantly lower than that through the 450 nm-membranes. The profiles of IBU penetration in the IBU_{micro}- and IBU_{nano}-gel formulations were similar in experiments using 25 nm-membranes. This result shows that the solubility of IBU was similar between the IBU_{micro}- and IBU_{nano}-gel formulations, and the IBU emitted from the IBU_{nano}-gel formulation remains in its nanoparticle state.

It is important to demonstrate the therapeutic effects of the IBU_{nano}-gel formulation on RA. It was known that the biological characteristics of AA rats correspond to those that occur in human RA[24-27]. Therefore, the AA rat is used in studies to develop novel topical formulations for the treatment of RA in this study. Figure 3 and Table 1 show the preventive effects of the IBU_{micro}- and IBU_{nano}-gel formulations on paw edema in AA rats. Although paw edema in the right side of AA rats to which the IBU_{micro}-gel formulation was applied tended to be low than that of AA rats receiving the gel formulation containing no IBU (control-gel formulation), no significant difference was found in the

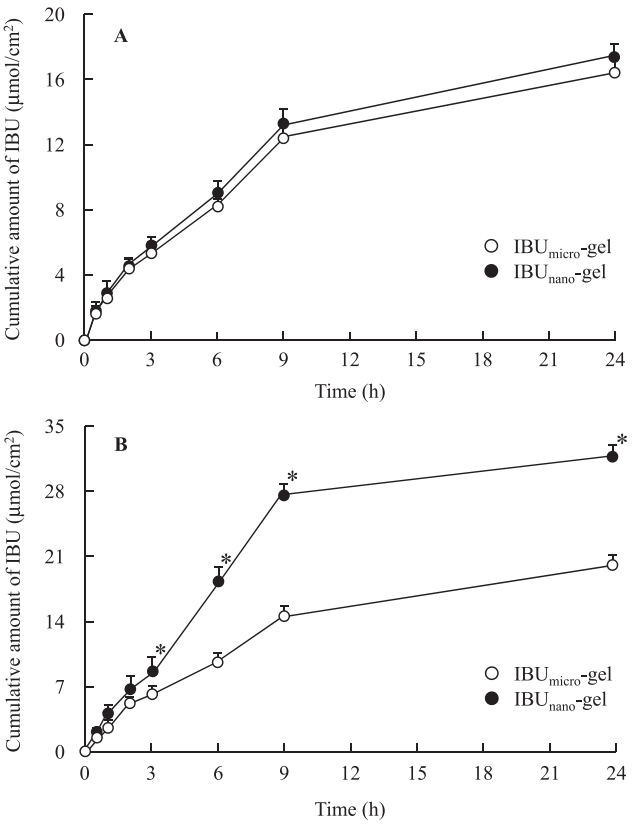

Fig. 2 Release of drug from IBU-gel formulations through 25 nm- and 450 nm- Membranes.
5% IBU-gel formulation (0.30 g) containing IBU-microparticles (IBU_{micro}-gel, open circles) or IBU-nanoparticles (IBU_{nano}-gel, closed circles) was applied to 25 nm-(A) or 450 nm-membranes (B). Means±S.E., n=7. *$p<0.05$ vs. IBU_{micro}-gel formulation for each category.

AUC_{edema} values. Paw edema in the right side of AA rats receiving the IBU_{nano}-gel formulation was significantly lower than that of AA rats receiving the control-gel formulation in the days following adjuvant injection. In addition, the AUC_{edema} values of the right side of AA rats receiving the IBU_{nano}-gel formulation were significantly less in comparison with those of AA rats receiving the control- or IBU_{micro}-gel formulations. The paw edema in the left side of AA rats to which the IBU_{micro}- or IBU_{nano}-gel formulations was treated did not differ significantly from that of rats applied the control-gel formulation. Moreover, no hemorrhagic lesions of the gastric or small intestinal mucosa were found in AA rats following the repetitive administration of the 5% IBU_{nano}-gel formulation (0.30 g) for 42 days (IBU-gel formulation was treated to the right foot at 9:00). These data show that the enhanced plasma IBU concentration in AA rats receiving the IBU_{nano}-gel formulation do not reach concentrations needed cause systemic side effects (gastrointestinal lesion). In addition, the preventive effect on paw

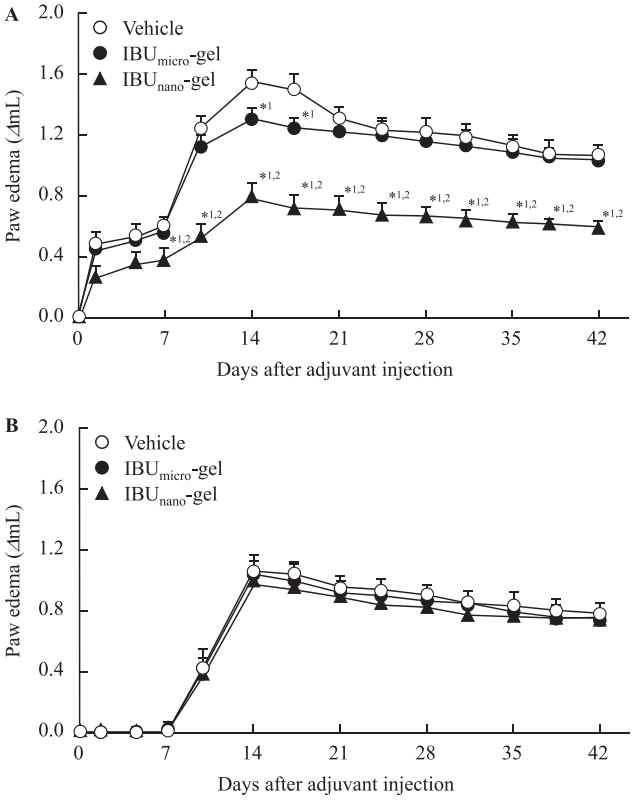

Fig. 3 Paw edema of the right and left hind feet of AA rats treated with 5% IBU-gel formulations.
Gel formulations (0.30 g) containing no IBU (vehicle, open circles), IBU-microparticles (IBU$_{micro}$-gel, closed circles) or IBU-nanoparticles (IBU$_{nano}$-gel, closed triangles) were treated to the right foot at 9:00. Means ± S.E., n = 7. *[1]$p < 0.05$ vs. Vehicle for each category. *[2]$p < 0.05$ vs. IBU$_{micro}$-gel formulation for each category.

Table 1 Preventive effects of 5% IBU-gel formulations on inflammation in AA rats.

Formulation	AUC_{edema} (mL · day)	
	Right	Left
Non-treatment	51.5 ± 2.3	33.6 ± 1.6
Vehicle	51.8 ± 2.5	33.9 ± 1.5
IBU$_{micro}$-gel	49.3 ± 2.4	33.2 ± 1.6
IBU$_{nano}$-gel	34.1 ± 2.2[*1,2,3]	32.5 ± 1.7

The AUC_{edema} values were determined according to Eqs. 1 and 2. Non-treatment: non-treated AA rat. Vehicle: AA rat treated with gel formulation without IBU. IBU$_{micro}$-gel: IBU$_{micro}$-gel treated AA rat. IBU$_{nano}$-gel: IBU$_{nano}$-gel treated AA rat. Means ± S.E., n = 7. *[1]$p < 0.05$ vs. each non-treatment group. *[2]$p < 0.05$ vs. each vehicle group. *[3]$p < 0.05$ vs. each IBU$_{micro}$-gel group.

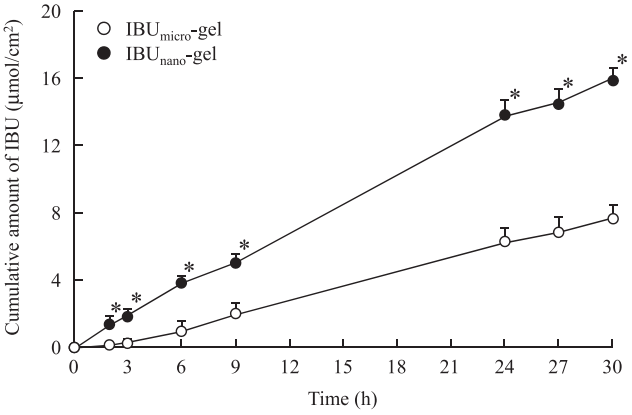

Fig. 4 *In vitro* skin penetration of IBU released from IBU-gel formulations.
5% IBU-gel formulations (0.30 g) containing IBU-microparticles (IBU$_{micro}$-gel, open circles) or IBU-nanoparticles (IBU$_{nano}$-gel, closed circles) were treated to abdominal skin pieces. Means ± S.E., n = 7. *$p < 0.05$ vs. IBU$_{micro}$-gel formulation.

edema pain of the IBU$_{nano}$-gel formulation was higher than that of a commercially available formulation (VESICUM® formulation 5%, AUC_{edema} in right side 40.6 ± 1.9, mL·day, n = 7 rats). These results show that IBU$_{nano}$-gel formulation provide the effective therapy without systemic side effect for RA, since the application of IBU$_{nano}$-gel formulation lead to the achievement of relatively high local IBU concentrations.

3.2 Pharmacokinetic Analysis of Gel System containing Drug Solid Nanoparticles

First, we analyzed the pharmacokinetic parameters of IBU-gel formulations in the percutaneous penetration. Figure 4 shows the profiles of IBU penetration through rat skin after treatment with the IBU$_{micro}$- and IBU$_{nano}$-gel formulations, and Table 2 shows the pharmacokinetic parameters analyzed from the data of Fig. 4. The amounts of IBU penetrating increased linearly after the treatment of either IBU-gel formulation, but the J_c, K_p and D values for the IBU$_{nano}$-gel formulation were significantly higher than those for the IBU$_{micro}$-gel formulation. Moreover, the t_{lag} for the IBU$_{nano}$-gel formulation was significantly lower than that for the IBU$_{micro}$-gel formulation. The K_m for the IBU$_{micro}$- and IBU$_{nano}$-gel formulations showed no significant difference. Figure 5 shows the profiles of IBU absorption through rat skin receiving the treatment of the IBU$_{micro}$- and IBU$_{nano}$-gel formulations, and Table 3 shows the pharmacokinetic parameters analyzed from the data of Fig. 5. The plasma amount of IBU increased following the treatment of both the IBU$_{micro}$- and IBU$_{nano}$-gel formulations, but the k_a and AUC values in the skin of rats receiving the IBU$_{nano}$-gel formulation were significantly higher than those of the IBU$_{micro}$-gel formulation. In addition, the t_{lag} for the IBU$_{nano}$-gel for-

Table 2 Pharmacokinetic analysis of 5% IBU-gel formulations in the skin penetration study.

Formulation	J_c (nmol/cm^2/h)	K_p ($\times 10^{-3}$ cm/h)	K_m	t_{lag} (h)	D ($\times 10^{-3}$ cm^2/h)
IBU_{micro}-gel	287 ± 33	4.1 ± 0.48	0.33 ± 0.11	0.90 ± 0.37	1.17 ± 0.40
IBU_{nano}-gel	584 ± 21*	8.0 ± 0.60*	0.10 ± 0.02	0.17 ± 0.02*	5.29 ± 0.78*

A Franz diffusion cell was used in the experiments, and the parameters were evaluated by using Eqs. 3-5. IBU_{micro}-gel: rat skin treated with IBU_{micro}-gel formulation; IBU_{nano}-gel: rat skin treated with IBU_{nano}-gel formulation. Means ± S.E., n = 7. *$p < 0.05$ vs. IBU_{micro}-gel formulation for each category.

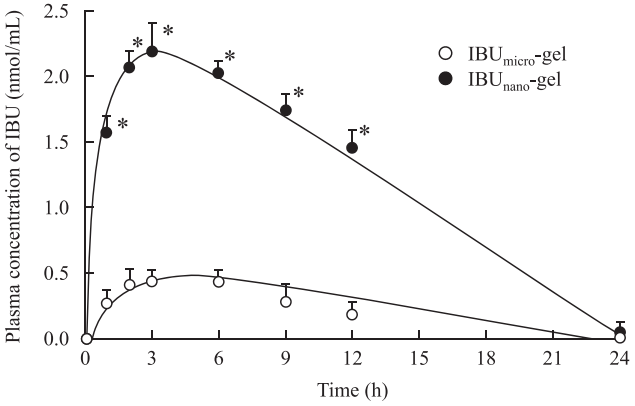

Fig. 5 Plasma IBU concentration after the application of IBU-gel formulations.
Solid lines represent the fitted curves for multiple applications of 5% IBU-gel formulation. 5% IBU-gel formulations (0.30 g) containing IBU-microparticles (IBU_{micro}-gel, open circles) or IBU-nanoparticles (IBU_{nano}-gel, closed circles) were treated to the rat abdominal skin. Means ± S.E., n = 7. *$p < 0.05$ vs. IBU_{micro}-gel formulation.

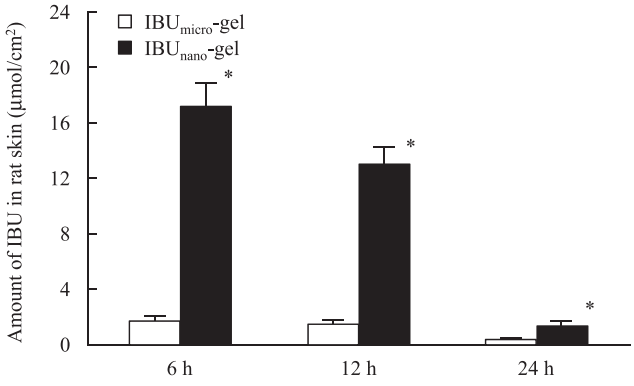

Fig. 6 IBU content in rat skin after the application of IBU-gel formulations.
5% IBU-gel formulations (0.30 g) containing IBU-microparticles (IBU_{micro}-gel, open columns) or IBU-nanoparticles (IBU_{nano}-gel, closed column) were treated to the rat abdominal skins. Means ± S.E., n = 7. *$p < 0.05$ vs. IBU_{micro}-gel for each category.

mulation was lower in comparison with that for the IBU_{micro}-gel formulation. On the other hand, the *MRT* values for the IBU_{micro}- and IBU_{nano}-gel formulations showed no significant difference. Figure 6 shows the content of IBU in rat skin tissue treated with IBU_{micro}- and IBU_{nano}-gel formulations. The contents of IBU in the rat skin tissues receiving the IBU_{nano}-gel formulation were significantly higher than those of the IBU_{micro}-gel formulation over 6-24 h. We previously reported that the skin penetration of solid drug nanoparticles is higher than that of a liquid drug[11, 12]. Taken together, we hypothesize that solid nanoparticles have the ability to supply high amounts of IBU from the IBU_{nano}-gel formulation, with high penetration through the skin and diffusion within the skin.

Next, we discussed the mechanism of the skin penetration in the formulations containing solid nanoparticles by using the IBU_{nano}-gel formulations and previously designed TL_{nano}-(71 ± 25 nm), IMC_{nano}-(186 ± 101 nm), KET_{nano}-(83 ± 71 nm) gel formulations (Table 4)[11-13]. Table 4 shows the comparison of particle size, *in vitro* skin penetration, *in vivo* skin accumulation and *in vivo* percutaneous absorption in the IBU-, IMC-, KET-, TL-gel formulations (the data were cited with those in refs. 11-13 for discussion of the skin penetration mechanism in the nano gel formulations). The skin penetration profile and drug accumulation

Table 3 Pharmacokinetic analysis of 5% IBU-gel formulations in the percutaneous absorption study.

Formulation	k_a ($\times 10^{-2}$ h^{-1})	t_{lag} (h)	*AUC* (nmol·h/mL)	*MRT* (h)
IBU_{micro}-gel	5.2 ± 0.5	0.45 ± 0.09	6.1 ± 1.8	6.1 ± 0.4
IBU_{nano}-gel	8.7 ± 0.6*	0.11 ± 0.04*	27.3 ± 2.8*	7.3 ± 0.6

The parameters were evaluated by using Eqs. 6-10. k_e, 2.24 ± 0.21 h^{-1}. IBU_{micro}-gel: rat treated with IBU_{micro}-gel formulation; IBU_{nano}-gel: rat treated with IBU_{nano}-gel formulation. Means ± S.E., n = 7. *$p < 0.05$ vs. IBU_{micro}-gel formulation for each category.

Table 4 Comparison of skin penetration, skin accumulation and percutaneous absorption in the IBU-, IMC-, KET-, TL-gel formulations.

		IBU-gel formulation	IMC-gel formulation	KET-gel formulation	TL-gel formulation
Particle size (μm)	Microparticles	85.4 ± 0.23	17.5 ± 12.000	7.7 ± 0.30	50.5 ± 26.3
	Nanoparticles	0.208 ± 0.081	0.186 ± 0.101	0.083 ± 0.071	0.071 ± 0.025
In vitro skin penetration		$IBU_{micro} < IBU_{nano}$	$IMC_{micro} < IMC_{nano}$	$KET_{micro} < KET_{nano}$	$TL_{micro} < TL_{nano}$
In vivo accumulation in skin tissue		$IBU_{micro} < IBU_{nano}$	$IM_{micro} < IMC_{nano}$	$KET_{micro} < KET_{nano}$	$TL_{micro} < TL_{nano}$
In vivo percutaneous absorption		$IBU_{micro} < IBU_{nano}$	$IMC_{micro} = IMC_{nano}$	$KET_{micro} < KET_{nano}$	$TL_{micro} < TL_{nano}$

The data were combined with those in refs. 11-13 for discussion of the skin penetration in the nano gel formulations. Means ± S.D.

in skin tissue were similar for the TL_{nano}-, IMC_{nano}- and KET_{nano}-gel formulations, and higher than for the micro gel formulations containing TL, IMC and KET (TL_{micro}-, IMC_{micro}- and KET_{micro}-gel formulations)[11-13]. These results were the same as the current results for the IBU-gel formulations. In addition, in the *in vitro* skin penetration experiments, the penetration rate of the IMC_{nano}-(186 nm), KET_{nano}-(83 nm) gel formulations (mean particle size) was higher than that of an ointment containing dissolved IMC, KET (commercially available IMC-gel formulations (IDOMETHINE$_{KOWA}$ gel 1%), KET-gel formulations (SECTOR gel® 3%)[12, 13], Taken together, we hypothesize that drug infiltration into the skin tissue is enhanced for particles in the size range of approximately 80-200 nm in comparison with drugs in the liquid state, and this increase in drug infiltration may cause the high skin penetration and drug accumulation in the skin tissue for the nano gel formulations. In contrast to the results in skin penetration and drug accumulation in skin tissue, the plasma concentration behavior of the TL_{nano}-, KET_{nano}-gel formulations differed from that of the IMC_{nano}-gel formulation in *in vivo* percutaneous absorption experiments[11-13]. No difference in *AUC* was observed between the IMC_{micro}- and IMC_{nano}-gel formulations. However, the plasma concentration following the administration of the TL_{nano}- and KET_{nano}-gel formulations was higher than that following the administration of the TL_{micro} and KET_{micro}-gel formulations. In addition, the plasma concentration following the administration of the TL_{nano}- and KET_{nano}-gel formulations was lower than that following the administration of the ointments containing the dissolved drugs (commercially available ointments)[11, 13]. It has been reported that drug solubility can be expected to be enhanced at particle sizes less than 100 nm[28]. From these results and reports, it is possible that the solubilities of the drugs in the TL_{nano}- and KET_{nano}-gel formulations are higher than in the micro gel formulations in skin tissue, while the IMC solubility in the IMC_{nano}-gel formulation may not be enhanced since the particle size (186 nm) is over 100 nm. It is hypothesized that solid drugs that infiltrate into the skin tissue are dissolved, and that the liquid drugs can then shift into the blood. On the other hand, the mean particle size of IBU_{nano}-gel formulation (208 nm) is similar to that of IMC_{nano}-gel formulation (186 nm). Although the drug particle size of IBU_{nano}-gel formulation is over 100 nm, the plasma concentration behavior of the IBU_{nano}-gel formulation was higher than that of IBU_{micro}-gel formulation in *in vivo* percutaneous absorption experiments (Fig. 5). It was known that the lipid solubility of drug was related to skin penetration, and the oil-water partition coefficients (LogP) of IBU (9.92) was clearly higher than that of IMC (0.91). It was suggested that the drug nanoparticles with high LogP may be enhanced the solubility in the approximately 200 nm of particle size, and the liquid IBU shift into the blood. From these findings, the LogP also may affect the skin penetration and drug accumulation in the skin tissue for the nano gel formulations. Further studies are needed to confirm the characteristics needed for high skin penetration and diffusion within the skin of nano gel formulations. Therefore, we are now preparing gel formulations containing particles of various sizes in the drugs, which have different LogP, and investigating their characteristics including skin penetration, drug accumulation in skin tissue and percutaneous absorption.

4 CONCLUSIONS

We have prepared IBU-nanoparticles by using a mill-method and several additives, and designed a topical DDS used IBU-nanoparticles. In the IBU_{nano}-gel formulation, the IBU concentration in skin tissue and its therapeutic effect on local inflammation were clearly higher than for our IBU_{micro}-gel formulation or a commercially available IBU-gel formulation. In addition, we reported that the particle size and LogP may affect the skin penetration and diffusion within the skin of nano gel formulations: (I) the drug with particle sizes less than 100 nm shows the systemic delivery; (II) the drug with particle sizes over 100 nm (100-200 nm) shows the local delivery; (III) the drug with high LogP tend to shift into the blood in the particle sizes of approximately 200 nm. The regulation of drug particle size and LogP in the

nano gel formulation may be able to lead to design the DDS for systemic or local delivery.

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