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 solid-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 solid-gel formulation was 208 nm. The increase in inflammation of the hind feet of AA rats was attenuated by the treatment with the IBU solid-gel formulation, and preventive effect was higher than that of a gel formulation containing IBU-microparticles (IBU microparticles-gel formulation, mean particle size 85.4 μm); the accumulation and permeability through the skin of IBU from the IBU solid-gel formulation were significantly larger in comparison with the IBU microparticles-gel formulation. Further, no gastrointestinal lesions were observed in AA rats following the repetitive administration of the 5% IBU solid-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) inhibitor1. 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 gastrointestinal, 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 water4-6. 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 formulations7-9. 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 patches7, gels8 and liposomes9 incorporating various permeation enhancers. In addition, strategies using nanoparticles and nanocarriers have also been demonstrated9. A number of groups have been studied the lipid nanocarriers for dermal drug delivery. The various systems tried include lipid nanoparticles (LNC, a lipid core surrounded by a ten-sioactive 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 skin11-13. Therefore, it is expected that its drug system lead to an alternative strategy for increase in drug permeation14-17. More-
over, the topical DDSs 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. 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 (TLnano), indomethacin (IMCnano), ketoprofen (KETnano) nanoparticles.

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. Briefly, IBU-nanoparticles were prepared using Bead Smash 12 and zirconia beads (Wakenyaku Co. Ltd, Kyoto, Japan). 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) were frozen in liquid nitrogen and milled by the Bead Smash 12 for 30 sec at 4°C (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°C (30 sec × 15 times, 5,500 rpm). The particle size of milled IBU (IBUnano) was 0.208 ± 0.081 μm. After milling, Carbopol® 9934 (Carbopol, Serva, Heidelberg, Germany) was added to prepare a gel ointment (IBU nano-gel formulation). A preparation of gel formulation containing IBU-nanoparticles was done by adding IBU-microparticles, MC and HPβCD (IBU nano) into Carbopol gel (IBU nano-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 1.45-0.010) and HPLC methods. The dispersity in the formulation base was determined as follows: the 5% IBU nano- and IBU nano-gel formulations were divided into 10 parts, and kept for 1 month (22°C 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 25/450-MEMBRANE FILTER of 25 and 450 nm pore size (25 nm-membrane, 450 nm-membrane, Merck Millipore, Tokyo, Japan) according to our previous reports. The IBU-gel formulation (0.3 g) was spread uniformly over the membrane and the diffusion cells were incubated at 37°C, 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°C, 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. 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:

\[
\text{Paw edema (AmL)} = V_{\text{r}} - V_{\text{f}} \]

where \( V_{\text{r}} \) is the paw volume of arthritis rat and \( V_{\text{f}} \) is the paw volume of normal rat.

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

\[
AUC_{\text{edema}} = \int_{0}^{42} V_{\text{edema}} \cdot \text{dt}
\]

The \( t_{\text{d}} \) are the days after adjuvant injection and the volume of paw edema, respectively. In this study, the \( AUC_{\text{edema}} \) show the inflammatory scores.

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

The \( \text{in vitro } \) skin penetration experiment was carried out...
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AUMC
δ
K
C

from the jugular vein to measure the IBU concentration at carried out according to our previous reports 2.6
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 cm²) and D is the diffusion constant within the skin. In the calculation, a nonlinear least-squares computer program (MULTI) was used:

\[ t_{lag} = \frac{\delta^2}{6D} \]  \hspace{1cm} \text{Eq. 3}  
\[ J_c = \frac{K_w \cdot D \cdot C_{BU}}{\delta} = K_p \cdot C_{BU} \]  \hspace{1cm} \text{Eq. 4}  
\[ Q_t = J_c \cdot A \cdot (t - t_{lag}) \]  \hspace{1cm} \text{Eq. 5}  

where \( t_{lag} \) is the lag time, \( J_c \) is the IBU penetration rate, \( \delta \) is thickness of the skin (0.071 cm, average for 5 rats), \( K_w \) 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 cm²) and D is the diffusion constant within the skin. In the calculation, a nonlinear least-squares computer program (MULTI) was used:

\[ C_{BU} = C_0 \cdot e^{-k_e \cdot t} \]  \hspace{1cm} \text{Eq. 6}  

where \( k_e (2.24 \pm 0.21 \text{ h}^{-1}, n = 7) \) 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), \( C_{BU} \) 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 (\( k_{abs} \), h⁻¹) according to Eq. 7:

\[ C_{BU} = \frac{k_e \cdot F \cdot D}{V_d (k_e - k_p)} \left( e^{-k_e \cdot t} - e^{-k_p \cdot t} \right) \]  \hspace{1cm} \text{Eq. 7} 

where \( t_{lag} \) is lag time (h), \( t \) is time (0-24 h) after IBU administration, \( C_{BU}, k_e \) 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):

\[ AUC = \frac{\int_0^{24h} C_{BU} \cdot dt}{K_e} \]  \hspace{1cm} \text{Eq. 8}  
\[ AUMC = \frac{\int_0^{24h} C_{BU} \cdot t \cdot dt}{K_e} \]  \hspace{1cm} \text{Eq. 9}  
\[ MRT = \frac{AUMC}{AUC} \]  \hspace{1cm} \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 cm²) 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 (Physcoltron, MICROTEC CO., LTD., Chiba, Japan), and were centrifuged (15,000 rpm, 20 min, 4°C). 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 transist-(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 nano-gel formulation were 85.4 ± 0.23 μm and 0.208 ± 0.081 μm, respectively (Fig. 1). Moreover, for 1 month after preparation, the IBU nano- and IBU nano-gel formulations were stable (particle size: IBU nano: 85.9 ± 0.26 μm; IBU nano, 0.221 ± 0.082 μm) with no decreases in IBU content in the IBU nano- or
IBU nano-gel formulations observed during 1 month at 22°C. 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. 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 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 IBU nano-gel formulation for 42 days. 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...
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edema pain of the IBU nano-gel formulation was higher than that of a commercially available formulation (VESICUM® formulation). 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 Jc, Kp and D values for the IBU nano-gel formulation were significantly higher than those for the IBU micro-gel formulation. Moreover, the tlag for the IBU nano-gel formulation was significantly lower than that for the IBU micro-gel formulation. The Km 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 ka 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 tlag for the IBU nano-gel for-
mulation was lower in comparison with that for the IBUmicro-gel formulation. On the other hand, the MRT values for the IBUmicro- and IBUnano-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 IBUnano-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. 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 using the IBUnano-gel formulations and previously designed TLnano- (71±25 nm), IMC nano- (186±101 nm), KET nano- (83±71 nm) gel formulations (Table 4). The skin penetration profile and drug accumulation supply high amounts of IBU from the IBU nano-gel formulation, with high penetration through the skin and diffusion within the skin.

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

<table>
<thead>
<tr>
<th>Formulation</th>
<th>(J_c) (nmol/cm²/h)</th>
<th>(K_p) ((\times 10^{-3})\text{cm/h})</th>
<th>(K_m)</th>
<th>(t_{lag}) (h)</th>
<th>(D) ((\times 10^{-3})\text{cm/h})</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBU micro-gel</td>
<td>287 ± 33</td>
<td>4.1 ± 0.48</td>
<td>0.33 ± 0.11</td>
<td>0.90 ± 0.37</td>
<td>1.17 ± 0.40</td>
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<tr>
<td>IBU nano-gel</td>
<td>584 ± 21*</td>
<td>8.0 ± 0.60*</td>
<td>0.10 ± 0.02</td>
<td>0.17 ± 0.02*</td>
<td>5.29 ± 0.78*</td>
</tr>
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</table>

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.

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

<table>
<thead>
<tr>
<th>Formulation</th>
<th>(k_e) ((10^{-2} \text{h}^{-1}))</th>
<th>(t_{lag}) (h)</th>
<th>(AUC) (nmol·h/mL)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBU micro-gel</td>
<td>5.2 ± 0.5</td>
<td>0.45 ± 0.09</td>
<td>6.1 ± 1.8</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td>IBU nano-gel</td>
<td>8.7 ± 0.6*</td>
<td>0.11 ± 0.04*</td>
<td>27.3 ± 2.8*</td>
<td>7.3 ± 0.6</td>
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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 nano-gel formulation for each category.
in skin tissue were similar for the TLnano-, IMC nano- and KET nano-gel formulations, and higher than for the micro gel formulations containing TL, IMC and KET (TLmicro-, 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 IMCnano- (186 nm), KETnano- (83 nm) gel formulations (mean particle size) was higher than that of an ointment containing dissolved IMC, KET (commercially available IMC-gel formulations (DOMETHINE® KOVA gel 1%), KET-gel formulations (SECTOR gel® 3%)).11-13 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 TLnano-, KETnano-gel formulations differed from that of the IMCnano- gel formulation in in vivo percutaneous absorption experiments.11-13 No difference in AUC was observed between the IMCmicro- and IMC nano-gel formulations. However, the plasma concentration following the administration of the TLnano- and KETnano-gel formulations was higher than that following the administration of the TLmicro and KETmicro-gel formulations. In addition, the plasma concentration following the administration of the TLnano- and KETnano-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 TLnano- and KETnano-gel formulations are higher than in the micro gel formulations in skin tissue, while the IMC solubility in the IMCnano-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 IBUnano-gel formulation was higher than that of IBU micro-gel formulation in in vivo percutaneous absorption experiments.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 IBUnano-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

<table>
<thead>
<tr>
<th></th>
<th>IBU-gel formulation</th>
<th>IMC-gel formulation</th>
<th>KET-gel formulation</th>
<th>TL-gel formulation</th>
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<tr>
<td><strong>Particle size</strong></td>
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<tr>
<td>(μm) Microparticles</td>
<td>85.4 ± 0.23</td>
<td>17.5 ± 12.000</td>
<td>7.7 ± 0.30</td>
<td>50.5 ± 26.3</td>
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<tr>
<td>Nanoparticles</td>
<td>0.208 ± 0.081</td>
<td>0.186 ± 0.101</td>
<td>0.083 ± 0.071</td>
<td>0.071 ± 0.025</td>
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<td><strong>In vitro skin penetration</strong></td>
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<td>IBU micro &lt; IBU nano</td>
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<td>IMC micro &lt; IMC nano</td>
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<td>KET micro &lt; KET nano</td>
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<td>TL micro &lt; TL nano</td>
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<td><strong>In vivo accumulation in skin tissue</strong></td>
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<td>IBU micro &lt; IBU nano</td>
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<td><strong>In vivo percutaneous absorption</strong></td>
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<td>IBU micro &lt; IBU nano</td>
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<td>TL micro &lt; TL nano</td>
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The data were combined with those in refs. 11-13 for discussion of the skin penetration in the nano gel formulations. Means ± S.D.
nano gel formulation may be able to lead to design the DDS for systemic or local delivery.

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