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α-Monoisostearyl glyceryl ether enhances percutaneous penetration of indometacin in-vivo

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Abstract

Molecules that reversibly remove the barrier resistance of skin enhance penetration. α-Monoisostearyl glyceryl ether (GE-IS) is a novel compound that can be used as a non-ionic surfactant and increases percutaneous penetration of indometacin in rat abdominal skin in-vitro. The present study investigated GE-IS-induced enhancement of indometacin penetration in-vivo. When 1% GE-IS in propylene glycol was applied to rat abdominal skin, serum and muscle concentrations of indometacin increased markedly. Anti-inflammatory activities of test solutions containing both indometacin and GE-IS were investigated in experimental models of acute and chronic inflammation. Application of indometacin with GE-IS to the skin produced greater inhibitory effects on carrageenan-induced rat paw oedema, UV-induced erythema in guinea-pigs, and adjuvant arthritis in rats, compared with application of indometacin alone. The results suggest that GE-IS enhances penetration in-vivo and improves the anti-inflammatory effects of indometacin in animal models. Thus, GE-IS might contribute to the development of cosmetic or medical formulations to improve transfer of bioactive substances to hypodermal sites.

Introduction

Skin forms an effective barrier to the permeation of external substances. Therefore, transdermal administration of chemicals or drugs is remarkably restricted by the physicochemical characteristics of skin. There are few drugs that have a sufficient permeability rate through skin, thus both topical and systemic transdermal therapeutic systems are necessary. In recent years, investigators have intensified their efforts to develop controlled delivery of drugs through human skin, and several approaches have been proposed, including chemical and physical enhancing methods (Barry 1987; Murakami et al 1998; Ogiso & Tanino 2000; Curdy et al 2001; Fujii et al 2001; Sugibayashi et al 2001). One representative chemical approach is the use of skin penetration enhancers. Various substances that act as enhancers of drug permeation have been investigated.

Generally, the stratum corneum (SC) is the main barrier limiting passive transdermal diffusion of most drugs. Therefore, formulations to reduce the barrier function of the SC have been studied extensively. On the other hand, the dynamics of drug delivery in tissues under the SC, for example epidermis, dermis, or muscle, are important when these tissues require the drugs (Sugibayashi & Morimoto 1998).

Although non-ionic surfactants are considered to be the most acceptable penetration enhancers because of their low levels of irritation, they have been reported to have only weak effects (Ashton et al 1986).

α-Monoisostearyl glyceryl ether (GE-IS) is an artificial compound that can be used as a non-ionic surfactant for emulsification of formulations such as creams. GE-IS functions as a penetration enhancer of indometacin in rat abdominal skin in-vitro (Suzuki et al 2001). Our previous study suggested that GE-IS acts directly on the SC to alter permeability, however the dynamics of drug delivery in tissues under the SC are not well understood. The aim of the present study was to elucidate the potency of GE-IS on indometacin penetration enhancement in-vivo.

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Experimental models of inflammation are widely used for investigating anti-inflammatory drugs. Carrageenan-induced paw oedema and UV-irradiated skin erythema are models of acute inflammation in which the inflammation occurs mainly in the epidermis and dermis under the SC. Adjuvant arthritis is a model of chronic inflammation in which articular tissues or the surrounding tissues are the sites of inflammation. In the present study, we selected indometacin, a typical non-steroidal anti-inflammatory drug, as a model drug to evaluate the influence of GE-IS on the percutaneous penetration of indometacin in these experimental models.

Materials and Methods

Materials

Indometacin, propylene glycol (PG), and reagents for high-performance liquid chromatography (HPLC) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Sumisho Pharmaceutical International Co., Ltd (Tokyo, Japan) kindly supplied the penetration enhancer 1-dodecylazacycloheptane-2-one (Azone). GE-IS was supplied from Kao Co. (Tokyo, Japan).

Test solutions

Four test solutions of PG alone, PG containing indometacin (1%), PG containing indometacin (1%) with Azone (1%), and PG containing indometacin (1%) with GE-IS (1%) were used. The test solutions were prepared according to Kaiho et al (1989) as follows: indometacin (1%) was dissolved in PG with heating (approx. 50°C). PG containing indometacin was mixed with enhancers (1%).

Animals

Male hairless rats (WBN/ILA-Ht, 7 weeks old) were purchased from Saitama Experimental Animals Supply Co., Ltd (Saitama, Japan). Male Wistar rats (8 weeks old), male Sprague–Dawley rats (7 weeks old), and male Hartley guinea-pigs were purchased from Charles River Japan, Inc. (Tokyo, Japan). All animals were maintained under the following conditions: 25±1°C, 55±10% humidity, and 12-h light/dark cycle (lights on: 0700–1900 h). Animals had free access to a standard diet (MF; Oriental Bio-Service Kanto Inc., Tsukuba, Japan) and drinking water. All animal experiments were conducted at the Experimental Animal Facility of Kao Tochigi Institute. The present study was approved by the Animal Care and Use Committee of the Kao Tochigi Institute. All experiments strictly followed the guidelines of the committee, which adheres to the governmental legislation in Japan.

In-vivo percutaneous absorption experiment

Serum concentration of indometacin

Three test solutions of PG containing indometacin (1%), PG containing indometacin (1%) with Azone (1%), and PG containing indometacin (1%) with GE-IS (1%) were used. Test solutions (0.1 mL) in occlusive cotton patches (1 cm in diameter) were applied to the abdominal skin of hairless rats for 8 h. Blood was collected from the jugular vein under anaesthesia 1, 2, 4, 6 and 8 h after application. For HPLC, serum samples were prepared according to previously reported methods (Kim et al 1988). Briefly, an appropriate volume (0.2 mL) of serum was extracted with dichloromethane (10 mL). After centrifugation at 2800 rev min^{-1} for 10 min at 4°C, the lower phase (8 mL) was placed into a vial and dried by N_2 gas purging. The vial was rinsed with 1 mL of 0.01 M NaOH, and the appropriate volume (0.05 mL) was injected onto the HPLC system.

Gastrocnemial concentration of indometacin

Additional rats were used for determinations of gastrocnemial concentrations of indometacin; at 2 h after application of test solutions the rats were killed by exposure to ether and the gastrocnemial muscle was collected. For HPLC, muscle samples were prepared according to previously reported methods (Yonemitsu et al 1988). Muscle (0.13 cm^2) was homogenized in methanol (0.5 mL) using a glass homogenizer for 1 min, and sonicated for an additional 20 min on ice. After centrifugation at 2800 rev min^{-1} for 10 min at 4°C, the supernatant (1.6 mL) was placed into a test tube and dried by N_2 gas purging. The dried sample was rinsed with 1 mL of 0.01 M NaOH, and an appropriate volume (0.05 mL) was injected onto the HPLC system.

Indometacin analysis

The indometacin concentration in the sample was determined by HPLC using a modification of a previously reported technique (Kim et al 1988). Briefly, indometacin was extracted with dichloromethane and deacylated to its fluorescent product, deschlorobenzoyl indometacin in 0.01 M NaOH. Deschlorobenzoyl indometacin was analysed by HPLC (Hitachi Intelligent Pump and Hitachi F-1050 fluorescence spectrophotometer) with a 4.6×15 mm i.d. column (Waters L-column) containing ODS (5 μm). The column temperature was maintained at 40°C, the flow rate was 1 mL min^{-1}, and the excitation and emission wavelengths were set at 278 and 358 nm, respectively. A mixture of acetonitrile and acetate buffer (25:75; pH 4) was used as the mobile phase.

Stock solutions of indometacin (50, 100, 500, 1000, 2000 and 5000 ng mL^{-1}) were prepared in distilled, deionized water. Appropriate volumes (0.1 mL) of stock solutions were added to blank serum (0.2 mL), and extracted with dichloromethane (10 mL). After centrifugation at 2800 rev min^{-1} for 10 min, the lower phase (8 mL) was placed into a vial and dried by N_2 gas purging. The vial was rinsed with 1 mL of 0.01 M NaOH, and an appropriate volume (0.05 mL) was injected onto the HPLC system. Calibration curves were constructed by plotting peak areas.

Carrageenan-induced rat paw oedema

Foot volume

This test was performed as described in a previous report

(Boughton-Smith et al 1993) with some modifications. Carrageenan (Zushikagaku Co., Kanagawa, Japan), 0.1 mL of 1% suspension in 0.9% saline, was injected into the right hind paw of male Wistar rats (8 weeks old) that had been fasted overnight (16 h). The footpad volume was measured using a plethysmometer before, and 2, 4, 6 and 8 h after carrageenan injection in conscious animals. Four test solutions of PG alone, PG containing indometacin (1%), PG containing indometacin (1%) with Azone (1%), and PG containing indometacin (1%) with GE-IS (1%) were used. Test solutions were applied to the footpad for 10 s every 2 h (2, 4 and 6 h after the oedema induction). The test solutions were not removed after the application.

Pain threshold (*Randall-Selitto method*)
The test was performed as described in a previous report (Hashimoto et al 1979). Conscious Wistar rats (8 weeks old) that had been fasted overnight (16 h) were gently covered with a cloth. The mechanical nociceptive threshold was quantified using an Analgesy-meter (Ugo Basile, Como, Italy). A pointed weight was pressed into the hind footpads, which exerts a force that increases at a constant rate. The pain threshold was expressed as the weight (g) at which the rats retracted their hind legs. The injection of carrageenan, the application of test solutions, and the time points of measurements are described above.

UV-irradiated skin erythema. Male Hartley guinea-pigs (380–420 g) were irradiated with 1 mW cm^{-2} UVB for 10 min on a shaved back that was wrapped with aluminium foil containing four holes (1 cm^2) (Wada et al 1982). After 2 h of irradiation, skin erythema was measured using a spectrocolorimeter (OFC-300A; Nippon Denshoku Ind. Co. Ltd, Tokyo, Japan), and 20 μL cm^{-2} of test solutions were applied for 1 h and then removed. Four test solutions of PG alone, PG containing indometacin (1%), PG containing indometacin (1%) with Azone (1%), and PG containing indometacin (1%) with GE-IS (1%) were used. Additional erythema measurements were made at 4, 6 and 8 h after UVB irradiation. Data are represented by the increase in erythema values measured by the spectrocolorimeter.

Adjuvant arthritis. The method of Wada et al (1982) was employed with some modifications. Briefly, adjuvant arthritis was induced by an injection of 0.1 mL paraffin oil containing killed and dried *Mycobacterium butyricum* (100 mg in 20 mL; Difco Lab., NJ, USA) into the right hind paw of male Sprague–Dawley rats. Establishment of adjuvant arthritis required 24 days after the injection of adjuvant. Four test solutions of PG alone, PG containing indometacin (1%), PG containing indometacin (1%) with Azone (1%), and PG containing indometacin (1%) with GE-IS (1%) were used. Test solutions were applied to the right hind paw for 10 s twice a day for 4 days. The test solutions were not removed after application. The footpad volume was measured using a plethysmometer 24, 26 and 28 days after injection of the adjuvant.

Statistical analysis

All values were expressed as means ± s.d. Statistical analysis of the data was performed using StatView (SAS Institute, Cary, NC, USA). Data were initially analysed using analysis of variance for each group. When a significant F value ($P < 0.05$) was obtained, a Fisher's PLSD multiple test was performed for post hoc analysis (SAS User's Guide 1998, SAS Institute).

Results

In-vivo percutaneous absorption experiment

Figure 1 shows the concentration curves of indometacin in rat serum after the application of test solutions with or without enhancers. The serum concentration (μg mL^{-1}) of indometacin at 1, 2, 4, 6 and 8 h after the application of the control solution was 0.06 ± 0.02, 0.36 ± 0.12, 3.11 ± 1.07, 7.67 ± 0.89, and 8.89 ± 0.99, respectively. On the other hand, the serum concentration of the GE-IS solution was 3.16 ± 2.20, 12.76 ± 5.51, 22.64 ± 4.21, 27.23 ± 2.77, and 26.66 ± 2.38 ($P < 0.01$ vs control), respectively. The serum concentration of the Azone solution was 0.20 ± 0.06, 4.73 ± 2.48, 15.83 ± 6.82, 25.81 ± 2.57, and 26.08 ± 0.99 ($P < 0.01$ vs control), respectively. There was no significant difference in serum concentrations of indometacin between the Azone-treated group and the GE-IS-treated group; however, the transfer rate of indometacin in PG solution with GE-IS into the circulation tended to be higher than that of indometacin with Azone at 1 or 2 h after application ($P < 0.05$).

The muscle concentration of indometacin (ng cm^{-2}) 2 h after the application of control, GE-IS, or Azone solution

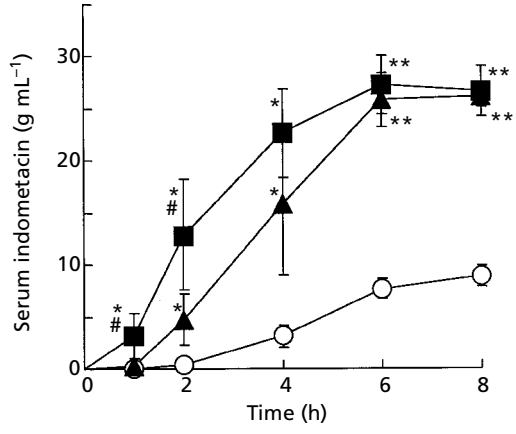

Figure 1 Effect of α-monoisostearyl glyceryl ether (GE-IS) on the percutaneous absorption of indometacin in rats: ○, propylene glycol (PG) solution with 1% indometacin; ▲, 1% Azone in PG solution with 1% indometacin; ■, 1% GE-IS in PG solution with 1% indometacin. Each value represents the mean ± s.d. of five rats. $*P < 0.05$, $**P < 0.01$, significantly different compared with PG solution with 1% indometacin. $\#P < 0.05$, significantly different compared with 1% Azone in PG solution with 1% indometacin.

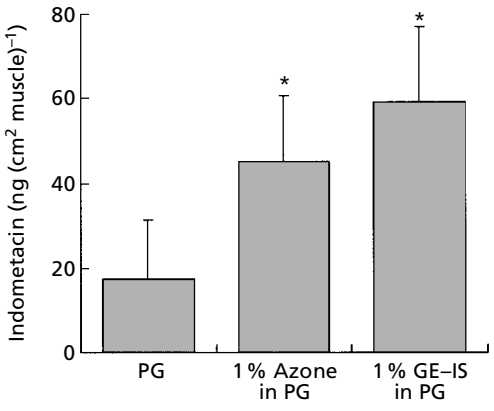

Figure 2 Indometacin concentration 2 h after application of samples in gastrocnemial muscle. The content of indometacin was fixed at 1% (w/w) in each solution. Each value represents the mean±s.d. of six rats. $*P < 0.05$, significantly different compared with propylene glycol (PG) solution with 1% indometacin. GE-IS, α-monoisostearyl glyceryl ether.

was 17.6±13.8, 59.1±17.9 ($P < 0.01$ vs control), or 45.3±15.5 ($P < 0.05$ vs control; Figure 2), respectively. These results demonstrate that GE-IS enhances the permeation of indometacin through hairless rat abdominal skin in-vivo. There was no significant difference in muscle concentrations of indometacin between the Azone-treated group and the GE-IS-treated group.

Carrageenan-induced rat paw oedema

Foot volume

The increase in foot volume was 0.30 to 0.34 mL at 2 h after the injection of 1% carrageenan. Following PG application, foot volume increased over time and peaked 6 h after the administration of carrageenan. The increase in foot volume (mL) at 4, 6 and 8 h after the induction of oedema was 0.71±0.15, 0.87±0.09, and 0.87±0.22, respectively. Topical treatment with indometacin solution without enhancers slightly inhibited the increase in foot volume as compared with PG application, whereas GE-IS or Azone solution significantly reduced paw oedema ($P < 0.05$ vs PG alone; Figure 3A). The increase in foot volume after topical treatment with indometacin solution at 4, 6 and 8 h was 0.62±0.13, 0.69±0.12, and 0.75±0.10, and after treatment with GE-IS solution was 0.49±0.13, 0.62±0.10, and 0.64±0.06, respectively. There was no significant difference in foot volume between the Azone-treated group and the GE-IS-treated group.

Pain threshold

The pain threshold over time is shown in Figure 3B. The pain threshold before the induction of paw oedema was approximately 130 g. Associated with the increase in foot volume, the pain threshold decreased in PG-treated rats and reached a minimum 6 h after the administration of carrageenan. The values of the pain threshold (g) in PG-treated animals at 4, 6 and 8 h were 84.5±24.9, 70.1±19.4, and 85.3±14.1, respectively. When indometacin with GE-IS was administered, the pain threshold values were 91.6±14.8, 108.6±34.7, and 127.3±32.9 ($P < 0.01$ vs PG alone), respectively. There was no significant difference in values of the pain threshold between the Azone-treated group and the GE-IS-treated group.

These data indicate that indometacin with GE-IS significantly increased the pain threshold and enhanced the anti-inflammatory effects of indometacin on carrageenan-induced rat paw oedema.

UVB-irradiated skin erythema

We examined the anti-inflammatory effects of indometacin with GE-IS on UVB-irradiated skin erythema in guinea-

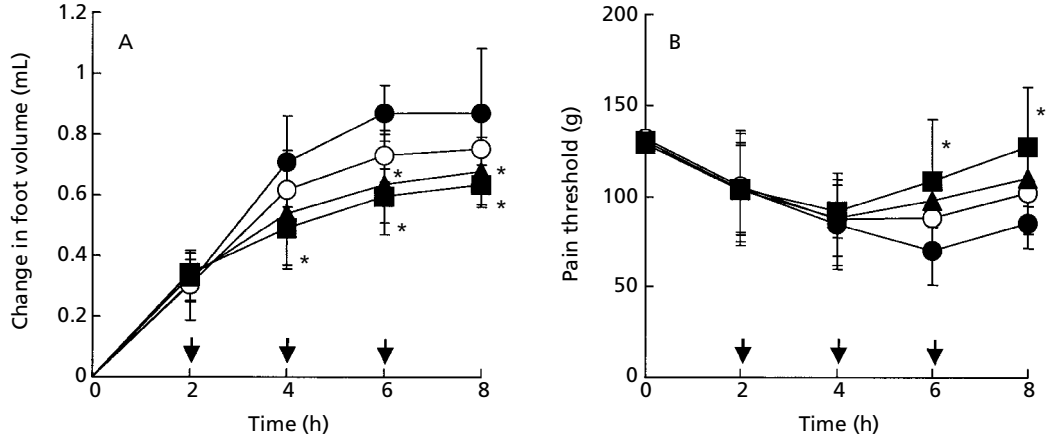

Figure 3 Effect of indometacin in propylene glycol (PG) solution with or without enhancers on (A) carrageenan-induced paw oedema and (B) pain threshold in rats: ●, PG solution without indometacin; ○, PG solution with 1% indometacin; ▲, 1% Azone in PG solution with 1% indometacin; ■, 1% α-monoisostearyl glyceryl ether in PG solution with 1% indometacin. The sample was applied for 10 s at each time point (arrows). Carrageenan was injected 2 h before the first application of sample. $*P < 0.05$, significantly different compared with PG solution without indometacin.

Table 1 Inhibitory effect of indometacin in propylene glycol solution with or without enhancers on UV-irradiated erythema.

Treatment		Change in erythema value after UV irradiation[a]			
Indometacin (1%)	Enhancer (1%)	2 h	4 h	6 h	8 h
−	–	0.77±0.90	1.46±0.52	1.68±1.01	1.30±0.86
+	–	0.62±0.58	1.27±1.05	1.44±0.72	1.01±0.83
+	Azone	0.72±0.80	0.98±0.59	1.09±0.97	0.83±0.84
+	GE-IS	0.60±0.76	0.53±0.92*	0.84±1.08	0.58±0.96

Data are mean±s.d. of six guinea-pigs. [a]Data represent the increase in erythema value as measured by spectrocolorimeter. Samples were applied on UV-irradiated area at the given time points for 1 h. *$P < 0.05$, significantly different compared with propylene glycol solution without indometacin and enhancers. GE-IS, α-monoisostearyl glyceryl ether.

pigs (Table 1). The differences in the increase in the erythema value in each group were not significant 2 h after irradiation. Test solutions were applied for 1 h from 2 h after irradiation, and changes in erythema were observed. Increases in the erythema value were significantly suppressed by treatment with indometacin solutions containing enhancers ($P < 0.05$) compared with PG alone. In contrast, the reduction in the erythema value after treatment with indometacin solution (no enhancers) was slight (no significant difference), indicating that indometacin alone barely permeates the SC under these experimental conditions. There was no significant difference in erythema values between the Azone-treated group and the GE-IS-treated group. These findings suggested that GE-IS augmented the penetration of indometacin through guinea-pig skin.

Adjuvant arthritis

Severe arthritis developed 24 days after immunization with mycobacteria (Table 2). The foot volume (mL) increased from 1.47±0.05 to 2.45±0.21 in the PG-treated group. Treatment with indometacin with GE-IS significantly decreased foot volume by 46% after a 4-day treatment ($P < 0.05$ vs PG alone). Indometacin alone did not induce a significant reduction in foot volume. There was a significant difference in foot volume between the Azone-treated and GE-IS-treated groups ($P < 0.05$). The present experiment indicated that GE-IS-enhanced penetration of indometacin into tissues under the foot skin.

Discussion

In the present study, GE-IS in PG applied to the abdominal skin of rats increased serum and muscle indometacin concentrations. Furthermore, GE-IS enhanced the anti-inflammatory effect of indometacin in acute and chronic experimental models. These data suggest that GE-IS acts to enhance penetration of indometacin in-vivo and enhances indometacin penetration to the inflammatory site.

Indometacin transfer into the circulation from closed

Table 2 Therapeutic effect of indometacin in propylene glycol solution with or without enhancers on adjuvant arthritis.

Treatment		Change in foot volume (mL)			Inhibition (%)[a]
Indometacin (1%)	Enhancer (1%)	24 days	26 days	28 days	
−	–	0.98±0.20	0.95±0.23	1.02±0.23	–
+	–	1.00±0.21	0.90±0.22	0.97±0.16	4.9
+	Azone	1.00±0.07	0.97±0.13	0.89±0.10	12.8
+	GE-IS	0.95±0.11	0.75±0.16	0.55±0.12*	46.4

Data are mean±s.d. of seven rats. Establishment of adjuvant arthritis required 24 days after injection of the adjuvant. Samples were applied for 10 s twice a day for 4 days. [a]Inhibitory effect of indometacin at 28 days. *$P < 0.05$, significantly different compared with propylene glycol solution without indometacin and enhancers. GE-IS, α-monoisostearyl glyceryl ether.

patches containing GE-IS was markedly increased, suggesting that GE-IS enhanced the penetration of indometacin. We previously demonstrated that GE-IS enhanced the percutaneous penetration of indometacin through excised rat skin to a similar extent as Azone (Suzuki et al 2001). Ogiso et al (1995) examined the enhancing capacity of various chemicals using full-thickness rat skins. They reported a 10-fold rate of indometacin enhancement in isoproylmyristate, cineol and D-limonen, and a 5-fold enhancement in *n*-octanol, Azone, oleic acid and sodium oleate. We hypothesized that the enhancement effect of GE-IS on percutaneous penetration of indometacin might be equivalent to those chemicals.

In the present study, when 1% GE-IS in PG was applied to the abdominal skin using a closed patch, no skin irritation was observed. The result suggested that GE-IS is less irritable to rat skin at this concentration.

The loss of penetration improvement in stripped rat skin indicated that Azone acts on the SC (Morimoto et al 1986). Further, differential scanning calorimetry experiments and the non-polar nature of Azone suggest that it partitions directly into the lipid bilayer structure and disrupts it (Barry 1987). Our previous report indicated that GE-IS also acts directly on the SC and alters the permeability of the skin (Suzuki et al 2001).

The transfer rate of indometacin in PG solution with GE-IS into the circulation tended to be higher than that of indometacin with Azone at 1 or 2 h after application (Figure 1). The difference in the transfer rate between GE-IS and Azone is thought to be owing to the mode of action on the SC. Although it might be that GE-IS has a greater affinity for SC than Azone, the mechanism of action is not known and is currently being investigated in our laboratory.

The concentration of indometacin in rat muscle was significantly higher in the GE-IS group than in the indometacin group alone, suggesting that GE-IS induced an increase in indometacin transfer into muscle as well as into the circulation. The data also suggest that there is enhanced indometacin accumulation at the inflammatory site.

Generally, in-vitro studies with excised skin in diffusion cells are used for investigation of penetration enhancers. It is commonly thought that bioactive substances, which have high permeability into the SC, transfer into the circulation or hypodermal tissue, and consequently have high efficacy on target organs (Sugibayashi & Morimoto 1998). There have been few attempts, however, to clarify whether penetration enhancement of drugs improves anti-inflammatory effects. In the present study, we investigated the enhancing effects of GE-IS on translocation of indometacin into the circulation or muscle, leading to greater anti-inflammatory effects. GE-IS markedly enhanced anti-inflammatory effects of indometacin on acute inflammatory models, carrageenan-induced rat paw oedema and UVB-irradiated erythema in guinea-pigs, and on a chronic inflammatory model, mycobacteria-induced adjuvant arthritis in rats. Our data clearly demonstrate that the enhancing effect of GE-IS on the skin penetration of indometacin promotes the anti-inflammatory effects of the drug at the sites of inflammation (e.g. oedema, erythema, arthritis). This study provides evidence that promotion of percutaneous penetration of biologically active substances is reflected in the efficacy of these substances in target organs. The dynamics of drug delivery in tissues under the SC (e.g. epidermis, dermis or muscle) might be important when drugs need to have an effect on the local tissues under the SC (Sugibayashi & Morimoto 1998). Thus, the use of GE-IS may contribute to the development of cosmetic or medical formulations of skin penetration enhancers for improving transfer of bioactive substances to hypodermal sites.

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