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Microemulsion-based hydrogel formulation of penciclovir for topical delivery

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ABSTRACT

The purpose of this study was to investigate microemulsion-based hydrogel (MBH) as a topical delivery system for penciclovir. Topical delivery of penciclovir in the forms of microemulsion, MBH and the commercial cream was evaluated in vitro and in vivo. The results of permeation test in vivo in mice showed that compared with the commercial cream, MBH and microemulsion could significantly increase the permeation of penciclovir into both epidermis and dermis. Stability test showed that MBH stored at 4 °C for 3 months had no significant change in physicochemical properties. Skin irritation test in rabbit demonstrated that single application or multiple applications of MBH did not cause any erythema or edema, slight skin irritation for microemulsion. Microstructure changes of skins after administration observed under light microscope and scanning electron microscope (SEM) might result from the interaction of the ingredients of microemulsion with skins, which was related with the permeation enhancement of penciclovir. It can be concluded that the MBH could be a promising vehicle for topical delivery of penciclovir.

1. Introduction

Microemulsion is a colloidal dispersion composed of oil phase, aqueous phase, surfactant and co-surfactant at appropriate ratios, which is a single optically isotropic and thermodynamically stable liquid solution with a droplet diameter usually within the range of 10–100 nm (Changez and Varshney, 2000; Tenjarla, 1999). There are several advantages of microemulsion for the topical delivery of drugs. First, the concentration of drugs in skin is increased because large amount of drug can be incorporated in the formulation. Second, the increased thermodynamic activity of the drug may favor its partitioning into the skin. In addition, the ingredients of microemulsion may reduce the diffusional barrier of the stratum corneum (SC) and increase the permeation rate of drug via skin by acting as permeation enhancers (Peltola et al., 2003). So it is promising for both transdermal and dermal delivery of drugs as an efficient route of drug administration (Kreilgaard, 2002; Rhee et al., 2001; Kreilgaard et al., 2000; Baboota et al., 2007; Kamal et al., 2007; Chen et al., 2007). However, most of the microemulsions possess a very low viscosity and therefore their application, especially in pharmaceutical industry may be restricted due to inconvenient application (Lawrence and Rees, 2000). In order to overcome this disadvantage, some gelling agents such as Carbomer 940, xanthan gum and carrageenan have been used to increase the viscosity of microemulsion and form MBH which are more suitable for topical application when compared with microemulsion as a vehicle for drug delivery (Lapasin et al., 2001; Peltola et al., 2003; Spiclin et al., 2003; Valenta and Schultz, 2004; Gulsen and Chauhan, 2005; Chen et al., 2006).

Penciclovir, 9-[4-hydroxy-3-(hydroxymethyl) butyl] guanine, is very effective for the treatment of herpes simplex virus, varicella zoster virus, Epstein-Barr virus, hepatitis virus and cytomegalovirus (Smith et al., 2001; Abdel-Hag et al., 2006; Schmid-Wendtner and Korting, 2004; Andrei et al., 2004). Various dermal formulations of penciclovir have been prepared to overcome the disadvantage of poor bioavailability (5–10% through oral administration) and reduce adverse side effects. In order to enhance the permeation of penciclovir in skin, microemulsion and liposome have been explored (Yang and Wang, 2005; Zhu et al., 2008).

In this study, the MBH formulation containing 0.5% penciclvir was prepared with Carbomer 940 as the gelling matrix, and its physicochemical properties were characterized and the stability was investigated. In vitro drug permeation through excised mouse skin and in vivo drug distribution in epidermis and dermis of mice were evaluated. Skin irritation was observed after single or multiple administrations, and the effect of microemulsion and MBH on the skin surface was discussed through the observation under light microscope and SEM.

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2. Materials and methods

2.1. Materials

Penciclovir (purity 99%) was procured from Lizhu Co. Ltd. (Changzhou, China) and oleic acid (OA) from Kemiou Chemical Reagent Co. Ltd. (Tianjin, China). Cremorphor EL was purchased from Sigma Chemical Co. (St. Louis, USA). Ethanol and dimethyl benzene were purchased from Guangcheng Chemical Reagent Co. Ltd. (Tianjin, China). Poloxamer 188 was obtained from Shunqiang Biotechnology Co. (Shanghai, China) and Carbomer 940 from Shenxing Pharmaceutical Manufactory (Shanghai, China). Hematoxylin and eosin were supplied by Saichi Biotechnology Co. Ltd. (Beijing, China). Sodium carboxymethylcellulose (CMC-Na) was supplied by Zhonghaihua Commerce Co. Ltd. (Shenzhen, China). All other chemicals and solvents were of analytical reagent grade.

2.2. Preparation of microemulsion and MBH

2.2.1. Preparation of microemulsion

Formulation of penciclovir loaded microemulsion obtained by a simplex lattic experiment design in our previous study was comprised of OA (5%, w/w), Cremorphor EL (20%, w/w), ethanol (30%, w/w) and water (45%, w/w) (Zhu et al., 2008). The preparation technology was briefly described as follows: OA, ethanol and cremorphor EL were firstly mixed together, and then water was precisely added into the above mixture drop by drop with magnetic stirring at ambient temperature. After the resulting system was equilibrated with gently magnetic stirring for 30 min, appropriate amount of penciclovir was dissolved in above solution under ultrasonication, and then microemulsion containing penciclovir was obtained.

2.2.2. Preparation of MBH

Carbomer 940 was selected as hydrogel matrix based on previous reports (Chen et al., 2007; Mou et al., 2008). MBH containing penciclovir was prepared according to the established method (Chen et al., 2006). Carbomer 940 was swelled in a little water for 24 h and a high viscous solution was obtained, and then the penciclovir loaded microemulsion was slowly added to the viscous solution of Carbomer 940 under magnetic stirring. The pH values were subsequently regulated to 6–9, and MBH was obtained. The concentration of Carbomer 940 in MBH was 0.6% (w/w).

2.3. Characterization of MBH

The pH values were evaluated at 25 °C by using a pHS-25 digital acidimeter (Shanghai Rex Instrument Factory, Shanghai, China).

The viscosity was determined at 25 °C by using a NDJ-1 viscometer (Shanghai Balance Instrument Factory, Shanghai, China).

Transmission electron microscopy (TEM, JEM-1200EX, JEOL, Tokyo, Japan) was employed to observe the microstructure of microemulsion or hydrogel containing microemulsion. Samples were placed on a carbon-coated copper grid and then a drop of 1% phosphotungstic acid was covered on the sample, ultimately the superfluous phosphotungstic acid on the samples was wiped off by filter paper.

The droplet size/distribution of the prepared microemulsion or MBH (diluted with distilled water) was determined with N5 Submicron particle size Analyzer (Beckman Coulter, UK) which has a detection range from 2 to 5000 nm.

2.4. Stability of MBH

The stability of MBH containing penciclovir was investigated via clarity, particle size, phase separation observation and HPLC analysis of penciclovir at 4 °C up to 3 months. The centrifuge test was also performed to assess the physical stability with centrifuging rate at 13,000 rpm for 30 min (Chen et al., 2007).

2.5. In vitro permeation studies

2.5.1. Preparation of skins

Male Kunming mice weighing 20 ± 2 g were purchased from Experimental Animal Center of Shandong University (Shandong, China) for the permeation studies. The protocol of the study was approved by the Ethical Committee of Shandong University. Skins were obtained from the abdominal region of mice after removing hair carefully with a razor, and then the subcutaneous fat and connective tissue were trimmed. The excised skins were washed and examined for integrity and then stored in a refrigerator at 4 °C overnight for later use (Zhao et al., 2006).

2.5.2. In vitro permeation studies

This experiment was performed by using Franz diffusion cells with an effective diffusion area of 3.8 cm^2. The excised skin samples were clamped between the donor and the receptor chamber of Franz diffusion cells with the stratum corneum facing the donor chamber. 0.8 g of microemulsion or MBH (containing drug 4 mg) or cream (containing drug 8 mg) was administrated on stratum corneum, respectively. The receptor chamber was filled with 15 ml of physiological saline solution. The receptor medium was maintained at 37 ± 0.5 °C and stirred at 600 rpm throughout the experiment. For each experiment, 1 ml receptor medium was extracted at predetermined time intervals and then the same volume of pure medium was immediately added into the receptor chamber. All samples were filtered through a 0.45 μm pore size cellulose membrane filter and analyzed by HPLC.

2.5.3. Calculation of the in vitro data

Cumulative amount of drug (Q_n, μg cm^{-2}) in the three preparations in the receptor chamber was plotted as a function of time (t, h). The cumulative amount of penciclovir permeated through excised mouse skins was determined based on the following equation (Zhu et al., 2008):

$$Q_n = \frac{C_n \times V_0 + \sum_{i=1}^{n-1} C_i \times V_i}{S}$$

Where C_n stands for the drug concentration of the receptor medium at each sampling time, C_i for the drug concentration of the ith sample, and V_0 and V_i stand for the volumes of the receiver solution and the sample, respectively, S for the effective diffusion area.

2.6. In vivo permeation study in mice

All the experimental mice were housed in cages, with access to food and water ad libitum until use. 24 h prior to the experiment, the dorsal skins of mice were washed with physiological saline solution after their hair was removed with a razor. 0.8 g of microemulsion (drug content 0.5%, w/w), MBH (drug content 0.5%,w/w) or commercial cream (drug content 1%, w/w) was applied on the dorsal surface (3.14 cm^2), respectively. At 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after dorsal administration, the mice were put to death by dislocating neck after administration at predetermined interval points, and subsequently, the administrated skins were stripped. The skins were thoroughly washed using physiological saline solution after cleaned and polished with ethanol, and then placed in aqueous bath (60 °C) for 60 s to separate epidermis and dermis according to the established method (Puglia et al., 2001; Puglia et al., 2008). Epidermis and dermis were cut into pieces, and then added into the solution comprised of 1 ml of water and 200 μl of 10% perchloric

acid, respectively. The mixture obtained was ultrasonicated for 1 h after homogenizing, and then centrifuged at 4000 rpm for 15 min. The supernatant was filtered through a 0.45 μm pore size cellulose membrane filter and the filtrate was analyzed by HPLC.

2.7. Evaluation of skin irritation

Skin irritation tests were conducted in rabbits to determine whether microemulsion or MBH containing penciclovir could produce irritation after single and multiple applications. The rabbits (weighing 2.0–2.5 kg) purchased from Experimental Animal Center of Shandong University (Shandong, China) with removal of hair on the back 24 h prior to administration were used in this experiment, and they were randomly divided into two groups, including intact skin group and the skin injury group (which were obtained by scarifying intact skin until capillary hemorrhage). Both were subdivided into two subgroups again, single application subgroup and multiple applications subgroup. Dorsal skin for irritation test of each rabbit was divided into three regions for commercial cream, microemulsion or MBH, respectively. For single application, the preparations above were applied on the corresponding region for 24 h. One hour after removal of the drug, each administration site was then inspected for the presence of erythema and edema (Dreher et al., 1996). For multiple applications, these preparations were applied on the same skin regions described above for 24 h, 1 h after removal the drug, administrated sites were assessed for signs of skin irritation, and this test procedure was repeated for another 6 days. After withdrawal, observation for single or multiple administration was continued for 3 days (Dreher et al., 1996). The irritation scores of the test area were obtained by judging the extent of erythema and edema according to the criteria proposed by Paolino et al. (2002). Erythema and edema were graded as follows: 0 for no visible reaction, 1 for just present reaction, 2 for slight reaction, 3 for moderate reaction, and 4 for severe reaction. Eventually, the total scores for irritation test in each condition were calculated using the following equation.

$$\text{Average irritation scores} = \frac{\text{erythema recation scores} + \text{dropsy reaction scores}}{\text{amount of animals}}$$

2.8. Effect of microemulsion and MBH on the surface of skin

2.8.1. Histological examination by light microscopy

Kunming mice were divided into three groups (normal group, microemulsion treated group and MBH treated group). According to Section 2.6, the corresponding skins of mice were stripped at 24 h after application. The specimens obtained were immediately placed into 4% paraformaldehyde (fixing solution) to fix for 18 h, and then dehydrated by ethanol solution in different concentrations. The specimens were subsequently placed into dimethyl benzene for transparent and then putted in colliquative mineral wax. After clotting, the specimens embedded were cut into slices. The slices were smoothed in warm water and then dried at 45 °C. Finally, the slices were observed using light microscope after hematoxylin and eosin stain.

2.8.2. Ultrastructural examination by SEM

Excised skin samples were cut into appropriate sized cubes and immediately fixed at 4 °C in 2% paraformaldehyde and 2.5% glutaldehye in 0.2 M cacodylate buffer (pH 7.4) overnight, then washed 3 times with 0.2 M cacodylate and 7% sucrose buffer for 15 min and post-fixed with 2% osmium tetroxide for 1 h, and immersed in 0.5% aqueous uranyl acetate for 30 min. Specimens were then dehydrated in graded concentrations of ethanol, transferred to isoamyl acetate, and critical-point dried using liquid CO_2. The dried specimens were affixed with gold palladium in an ion coater and examined under SEM.

2.9. HPLC analysis of penciclovir

The samples were analyzed using the HPLC system including a separations module (Waters 2695), a diode array detector (Waters 2996) and a reversed phase C_{18} column (5 μm, 4.6 mm × 250 mm, Dikma). The mobile phase was a mixture of 0.1% acetic acid solution/acetonitrile at a ratio 98:2 (v/v) with the flow rate at 1 ml/min and the detection wavelength was set at 253 nm. Aliquots of 20 μl of each sample were injected into the column, and all operations were carried out at ambient temperature.

The peak area correlated linearly with penciclovir concentration in the range of 1–50 μg/ml and the lowest detection limit at 0.5 μg/ml.

2.10. Statistical analysis

Data were shown as mean ± S.D. ($n = 5$). Statistical data were analyzed by the Student's t-test at the level of $p = 0.05$.

3. Results and discussion

3.1. Preparation of MBH

In our previous studies on microemulsion, an inverse relationship between the permeation ability and the drug solubility in microemulsion was constructed. In order to obtain both high solubility and high permeation ability, the appropriate ratio of the components was chosen for the optimized formulation, which consisting of oil (5%), surfactant (20%), co-surfactant (30%) and water (45%) (Zhu et al., 2008). The MBH studied at present was derived from the above optimal microemulsion composition. In the preliminary tests of matrix screening for MBH, compared with Poloxamer 188 and CMC-Na, Carbomer 940 at the concentrations ranged from 0.25 to 1.0% possessed more suitable viscosity for topical delivery, so it is reasonable that Carbomer 940 was chosen as the matrix for MBH.

When the obtained microemulsion was mixed with Carbomer 940 which had adequately swelled in water, the appearance of Carbomer 940 viscous solution became ivory white, which was in accordance with the results obtained by Chen et al (Chen et al., 2006). The possible reason is that the dehydration of some ingredients such as surfactant and co-surfactant in microemulsion makes Carbomer 940 dissociated from hydrated state. Besides, Carbomer 940 could be directly added into microemulsion solution or the aqueous phase of microemulsion, and the order of the addition of Carbomer 940 had no significant influence on the formation of MBH, but might influence the homogenization swelling of Carbomer 940.

3.2. Characterization and stability of MBH

The pH value of microemulsion and MBH was 5.33 and 6.82, respectively, the pH near to 7.0 indicated that MBH could result in more less stimulation to skin than microemulsion. The viscosity (23.86 Pa s) of MBH increased significantly compared to that (7.92 mPa s) of microemulsion due to the addition of 0.6% Carbomer 940, which made the preparation more suitable for topical administration(Chen et al., 2006).

The droplets of microemulsion presented uniformly spherical under TEM, and the droplet size of microemulsion ranged from 10 to 100 nm and the mean diameter of droplets was 36.5 nm. For the MBH, spherical droplets of microemulsion were located in the gel

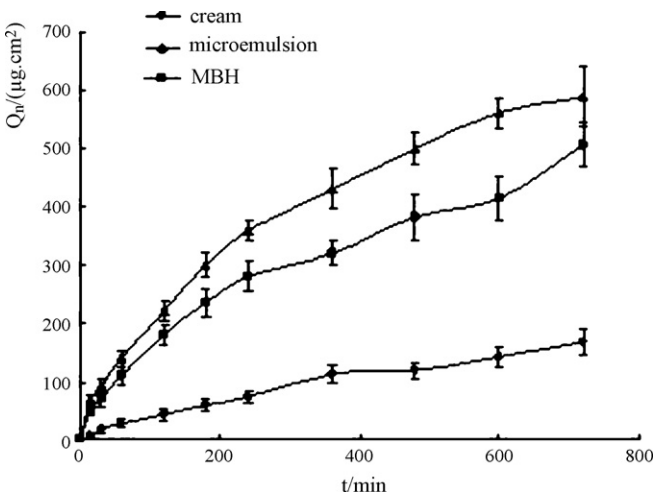

Fig. 1. Percutaneous permeation profiles of penciclovir from the microemulsion, MBH and the commercial cream (mean ± SD; $n = 5$).

network and the particle diameter of the droplets in MBH was not significantly changed compared with that of the microemulsion, which was in accordance with the study reported by Chen et al. (2007). It indicated that the gel network of Carbomer 940 could not markedly influence the morphology and diameter of microemulsion droplets.

Microemulsion and MBH were stable at 4 °C in the presence of penciclovir. There was no significant change of particle size, phase separation and degradation of penciclovir observed up to 3 months. The centrifuged tests indicated that microemulsion and MBH had good physical stability.

3.3. In vitro permeation studies

The permeation profiles in vitro of penciclovir through excised abdominal skins of mice were shown in Fig. 1. A steady increase of penciclovir in the receptor chambers with time was observed. The permeation profiles of microemulsion accorded with the Fick's diffusion equation. Statistical comparison of the flux throughout 12 h showed that the two microemulsion preparations of penciclovir provided fluxes higher than that of the control commercial cream which had only a low cumulative amount of penciclovir (168.98 μg cm^{-2}) at 12 h after application. Cumulative amount of penciclovir from microemulsion and MBH was respectively 2.5 times and 2 times that of the control commercial cream at 12 h post-application. The results were in accordance with previous studies in which the ingredients such as OA and ethanol in microemulsion as permeation enhancers could significantly reduce the barrier of stratum corneum and increase the diffusion coefficient of drug in skin, in addition to the contributions produced by microemulsion as the nanocarrier for drug, such as a very large surface area for drug transfer to skin due to the very small droplet size and high drug concentration within the upper layers of the skin which results in a higher concentration gradient as the driving force for transdermal drug delivery (Huang et al., 2008; El Maghraby, 2008).

The cumulative amount of penciclovir from microemulsion through the excised mouse skin was 589.50 μg cm^{-2} at 12 h after application. After microemulsion was mixed with Carbomer 940, the cumulative amount of penciclovir became 506.91 μg cm^{-2}. The result showed that addition of Carbomer 940 into microemulsion decreased markedly the permeability of penciclovir. It might attribute to the increased viscosity and transform from microemulsion to lamellar structure or a highly ordered microstructure (Trotta, 1999; Peltola et al., 2003). So a conclusion could be drawn

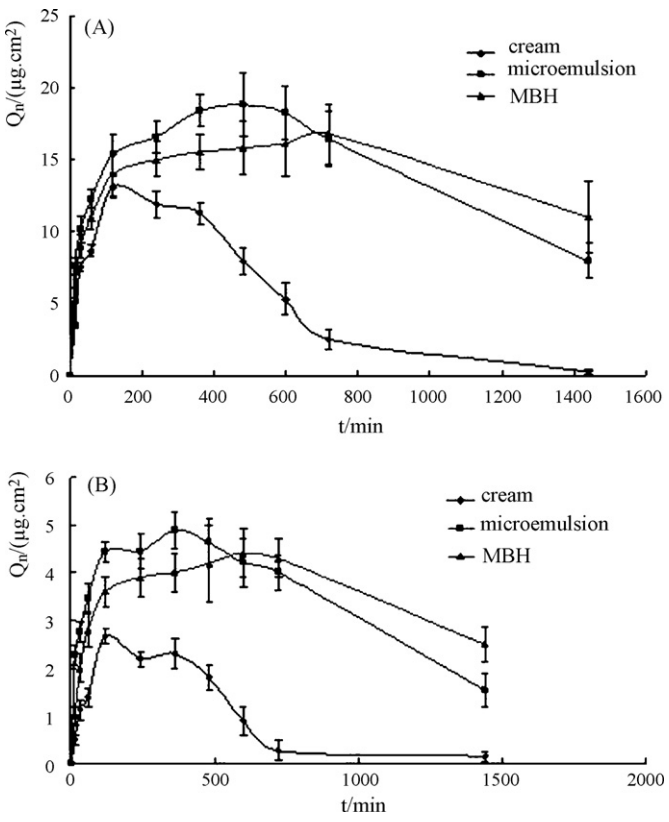

Fig. 2. In vivo penciclovir distribution profiles in epidermis (A) and dermis (B) from microemulsion, MBH and commercial cream (mean ± SD; $n = 5$).

that addition of Carbomer 940 in microemulsion would delay drug release.

3.4. In vivo permeation studies

The epidermis and dermis extracts obtained at every sampling point were analyzed by HPLC. The profiles of mean drug concentration in epidermis and dermis to time after application of the commercial cream, microemulsion and MBH were shown in Fig. 2. During initial 2 h, from above three preparations the cumulative amount of penciclovir permeated into epidermis had no significant difference, whereas in dermis, the cumulative amount of penciclovir from microemulsion was as much as 2 times comparing with the commercial cream, and MBH was almost 1.5 times, although the administrated dose of these two preparations were only half of the commercial cream. In subsequent time, for the commercial cream, the drug concentration in epidermis and dermis decreased obviously, and then lower than 0.5 μg cm^{-2} after 24 h, however, drug concentrations of the two other preparations were still maintained at a relative higher level at 12 h post-application and still higher than 0.5 μg cm^{-2} at 24 h after administration. The experimental results indicated that comparing with the commercial cream, microemulsion and MBH could enhance significantly permeation of penciclovir.

As shown in Fig. 2, the two microemulsion preparations presented the ability of slow-release. The difference between them was that during initial 8 h the cumulative drug amount distributed into epidermis and dermis from microemulsion was higher than that of MBH, however, at 24 h after application that from MBH was higher. It can be concluded that a part of drug was dissolved in the interfacial film composed of surfactant and co-surfactant molecules or oil phase in microemulsion, and drug molecules have to firstly permeate through the film before contact with the skin tissue, which led

Table 1
Average response scores of skin irritation for single application ($n = 5$).

Groups	Preparations	Average scores			
		1 h	24 h	48 h	72 h
Normal skins	Commercial cream	0.00	0.00	0.00	0.00
	Microemulsion	0.25	0.13	0.00	0.00
	MBH	0.00	0.00	0.00	0.00
Damaged skins	Commercial cream	0.13	0.00	0.00	0.00
	Microemulsion	0.63	0.13	0.00	0.00
	MBH	0.13	0.00	0.00	0.00

to the slow-release property of microemulsion. Once microemulsion droplets were packaged in the gel network of Carbomer 940, the movement of droplets would be limited, and then the diffusion of penciclovir dissolving in droplets would be also limited and this would slow down the releasing rates. Therefore, MBH showed the excellent ability of sustained release comparing with microemulsion.

In vivo experiment results indicated that the cumulative amount of penciclovir permeated into epidermis and dermis from MBH were all significantly higher than that of the commercial cream, though the administrated drug dose was only half of the latter. The possible reasons are that the good adhesiveness to skin due to the suitable viscosity of Carbomer 940 could enhance the skin permeation of drug because of the tight contact of drug preparation with skin and delayed release time. Additionally, the characteristics of microemulsion such as high drug concentration, the small droplet diameter and the reaction between OA, ethanol, Cremorphor EL and skin SC can also contribute to the permeation enhancement even though the droplets of microemulsion are located in gel network of Carbomer 940. Based on the above results, it could be inferred that MBH could not only intercept virus replication in superficial skins, but also inhibit the virus which was delitescent in cellula nervosa of dermis, thus the MBH could inhibit herpes virus in deep layer and decrease palindromia of herpes.

3.5. Evaluation of skin irritation

The results of skin irritation test of both single application and multiple applications were shown in Tables 1 and 2, respectively. The intensity criterion of skin irritation followed the protocol that scores of <0.5 meant no irritation, 0.5–3 for slight irritation, >6 showed severe irritation and others were moderate irritation.

As shown in Tables 1 and 2, for intact skins after single application or multiple applications, the three tested formulations had no irritation. However, to injured skins, after single application or multiple applications of microemulsion, slight skin irritation occurred. The possible reason was that the decrease of the metabolic capability for damaged skins induced accumulation of surfactant mixture and oil phase, which led to occurrence of irritation reaction. Therefore, it could be concluded that MBH had no irritation to skins under the conditions of the study, and this finding might be due to the change of microemulsion property after adding Carbomer 940. The network structure formed and the increase of the viscosity decreased the contact chances between skins and microemulsion. Thus the irritation of MBH was much weaker.

Table 2
Average response scores of skins irritation for multiple applications ($n = 5$).

Groups	Preparation	Average scores			
		1 h	24 h	48 h	72 h
Normal skins	Commercial cream	0.00	0.00	0.00	0.00
	Microemulsion	0.38	0.13	0.00	0.00
	MBH	0.00	0.00	0.00	0.00
Damaged skins	Commercial cream	0.25	0.00	0.00	0.00
	Microemulsion	0.88	0.25	0.13	0.00
	MBH	0.25	0.00	0.00	0.00

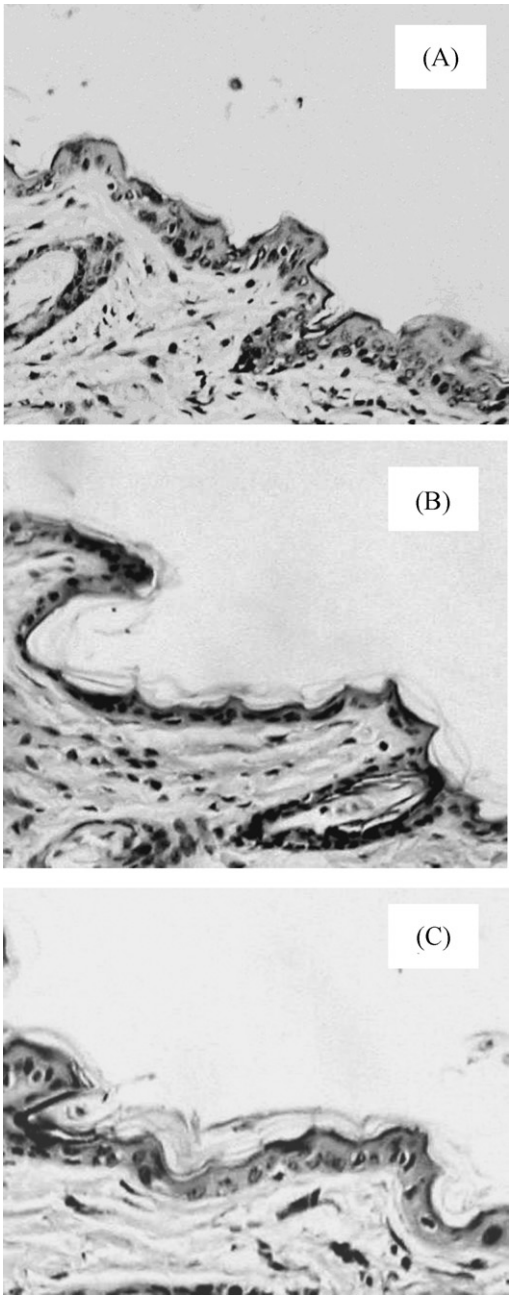

Fig. 3. Photomicrographs of sections of normal skin (A) and skin treated with MBH (B) or microemulsion (C) at 24 h after application.

3.6. Effect of microemulsion and MBH on skin

The skin was a multilayered organ and anatomically had many histological layers. It was generally described in terms of three tissue layers, the stratified, avascular, cellular epidermis, the underlying dermis of connective tissue, and the subcutaneous fat layer. Moreover, the highly vascularized dermis and the epidermis supported several skin appendages (Fang et al., 2003). Effect of microemulsion and MBH on skin was investigated by observing the section photomicrographs and SEM photographs of skins before and after treatment.

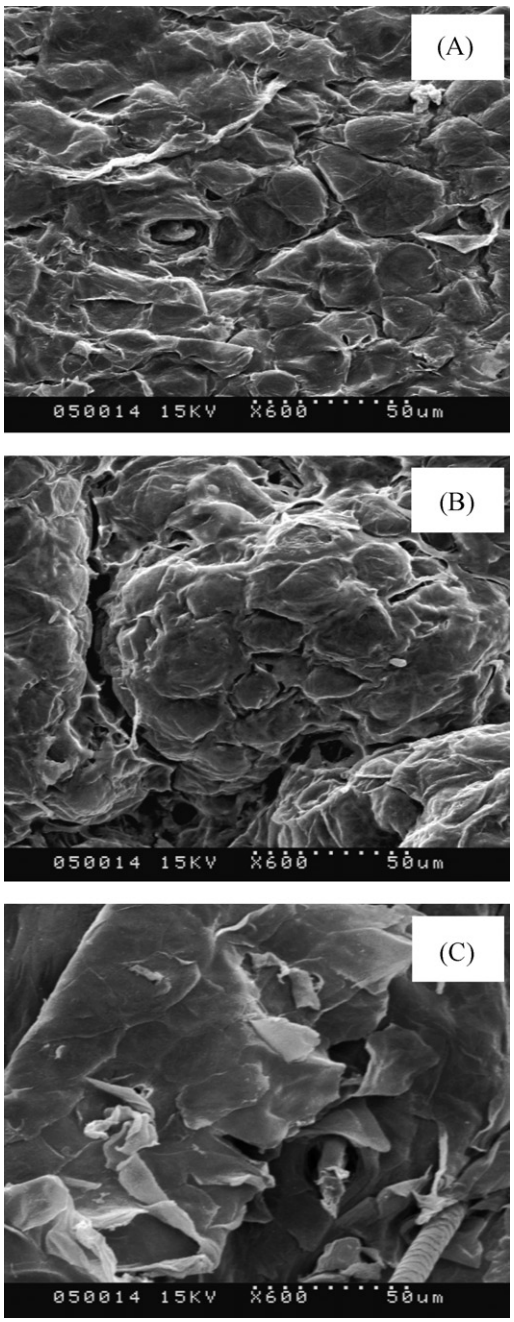

Fig. 4. SEM images of normal mouse skin (A) and skin treated with MBH (B) or microemulsion (C).

Photomicrographs of sections of normal skin (A), skin treated with MBH (B) or microemulsion (C) at 24 h after application were shown in Fig. 3. From Fig. 3A, microstructure of normal skin was observed to be a tight multilayer structure. The SEM images of the control microstructure of skin (Fig. 4A) showed tight cell junction and less keratin fragments. As compared to normal skins, the skin treated with MBH (Fig. 3B) showed scattered, loose SC and slight dermal edema. The SEM images also presented the increased cell gap and a flaky appearance of keratin, which may indicate the denaturation of keratinocytes in the SC layers (Fig. 4B). SC played an important part in preventing permeation of drugs, so it could be inferred that these disruptions of the SC morphology may have contributed to the enhancement effect on drug permeation. Fig. 3C illustrated the microscopic appearance of skin after application of microemulsion, SC layer became looser compared with Fig. 3B. The SEM images of skin treated with microemulsion showed significant modification of the skin surface (Fig. 4C). The surface appeared rougher than that of skin treated with MBH. Cell gaps further increased and normal cell junction was broken, furthermore, the phenomenon of the skin flake desquamating from the SC could be clearly observed. It could be inferred that compared with MBH, microemulsion could change the structure of SC to a greater extent, and this change could be beneficial to the permeation of drugs through skins. The conclusion was consistent with the results obtained from in vitro and vivo permeation experiments. It was possible that due to addition of Carbomer 940, three-dimensional structure forming in MBH decreased the chances of microemulsion contacting with skins, the effect of microemulsion in MBH on the appearance of skin was reduced. The components of microemulsion played an important role in changing the microstructure of skin. OA as the oil phase can disorder the highly packed SC intercellular domain lipids. When applied together with ethanol, OA is also believed to cause SC lipid extraction (Touitou et al., 2002). So the possible mechanism of permeation enhancement of microemulsion preparations for penciclovir was that when they were applied on skins, the intercellular lipid in skin was partly dissolved and then extracted, the corneocytes were separated from each other and desquamated from the intact SC, and then the barrier function of SC was weaken. As a result, drug molecules could permeate through the SC by passing the numerous cavities presented on the surface of SC after the treatment of microemulsion preparations.

4. Conclusion

In this work, MBH with suitable viscosity was constructed to deliver penciclovir for topical administration. The addition of Carbomer 940 into microemulsion resulted in the increase of the viscosity. Compared with the commercial cream, MBH could significantly enhance the permeation of penciclovir in vitro and in vivo. Furthermore, the MBH presented the excellent ability of slow-release and weaker irritation, which provided a probability for clinical application. Through light microscopy and SEM, the microstructure changes of SC could be clearly observed, and the percutaneous permeation enhancement might be related with the combined effects of the composition ingredients of microemulsion on the microstructure of skin. The MBH with powerful permeation ability and low irritation was expected as a promising drug delivery system.

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