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Preparation and *in vitro* evaluation of an ilomastat microemulsion gel by a self-microemulsifying system

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The purpose of this study was to construct a microemulsion gel formulation by a self-microemulsifying system for transdermal topical delivery of ilomastat. The optimum formulations were screened by penetration evaluation *in vitro*. Ilomastat microemulsion gel was prepared by drawing a ternary phase diagram and Pluronic F127 was added as gel matrix for the formulation. The optimal formulations had wide microemulsion existent field and good self-microemulsifying efficiency. The droplet size was within 100 nm. Statistical comparison of the permeation throughout 24 h showed that the two microemulsion gel preparations of ilomastat provided higher permeation than that of the normal gel which had only a low cumulative amount of ilomastat (6.03 $\mu g \cdot cm^{-2}$) 24 h after application. Cumulative amount of ilomastat from microemulsion gels A and B was 2.2 times and 1.8 times that of the normal gel at 24 h respectively. These results indicate that the microemulsion gel may be a promising vehicle for topical delivery of ilomastat.

1. Introduction

Ilomastat [HONHCOCH$_2$CH(i-Bu)CO-L-Trp-NHMe, GM 6001, galardin, Fig. 1] is a potent matrix metalloproteinase (MMP) inhibitor of the hydroxamate family which binds to the critical active-site zinc atom present in all members of this class of proteinases (Grobelny et al. 1992). The isobutyl group and tryptophan side chain are believed to bind to the subsites on the target enzymes which normally bind extracellular matrix proteins. In addition to its inhibition of MMPs, ilomastat inhibits bacterial metalloproteinases, such as thermolysin and *Pseudomonas aeruginosa* elastase (Barletta et al. 1996; Cotter et al. 1996; Grobelny et al. 1992; Hao et al. 1999). Ilomastat has been shown to inhibit angiogenesis in a chick chorioallantoic membrane model, to diminish neovascularization of the rat cornea stimulated by an implanted pellet containing a tumor extract, and to reduce the inflammation and proliferation resulting from application of phorbol esters to the skin of rats (Galardy et al. 1994). Human clinical trials for ophthalmic applications of ilomastat have been conducted without reported toxicities.

In recent years, self-microemulsifying drug delivery systems (SMEDDS) have attracted growing interest as promising means for the delivery of poorly water-soluble drugs. SMEDDS have gained this popularity largely due to their excellent efficiency in improving the drug solubility, increasing the dissolution rate, promoting oral absorption for poorly water-soluble drugs and simplicity of preparation (Holm et al. 2003). SMEDDS are isotropic mixtures of oil, surfactant, co-surfactant and drug substance. Oil, surfactant, and co-surfactant are essential components in view of solubilizing the poorly water-soluble drug and forming fine microemulsion droplets after being introduced into the aqueous media under gentle agitation. The properties of microemulsions, e.g. enhanced drug solubility, permeation enhancement ability, protection against enzymatic hydrolysis, and ease of manufacturing are often superior to those of conventional formulations and have been exploited in pharmaceutics, and they play particularly an important role in drug delivery systems (Kogan and Garti 2006; Lawrence and Rees 2000). However, most of the microemulsions possess a very low viscosity. To overcome this disadvantage, some gelling agents are added into the microemulsion to form microemulsion gels. The gels show great potential applications in the field of pharmaceutics, for instance, in transdermal delivery systems and in sustained release formulations (Vintiloiu and Leroux 2008).

Pluronics, also known as poloxamers, are a series of commercially available block co-polymers with pharmaceutical applications. Pluronic F127 is an ABA-type triblock copolymer consisting of polyoxyethylene (A) and polyoxypropylene units (B) with high molecular weight in pluronics. It is often used as gel matrix which displays low toxicity and does not increase serum triglycerides and cholesterol in animal models (Blonder et al. 1999). Medical uses of pluronic F127 have included the controlled delivery of drugs to the eye (El-Kamel 2002), nasal passage as well as parenteral and subcutaneous administration (Barichello et al. 1999).

In this study, a microemulsion gel containing 0.2% ilomastat was prepared with Pluronic F127 as the gel matrix by self-microemulsifying system. Self-microemulsion formulation was screened through drawing of a ternary phase diagram and microemulsion gel performance and physicochemical properties were characterized. The permeation behaviors of optimal microemulsion gel were evaluated and compared with normal gel through excised athymic mouse skin *in vitro*.

2. Investigations and results

2.1. Solubility and oil-water partition coefficient determination

The solubility of ilomastat in water, physiological saline, MCT, Cremophor EL, Transcutol, propylene glycol, ethanol and

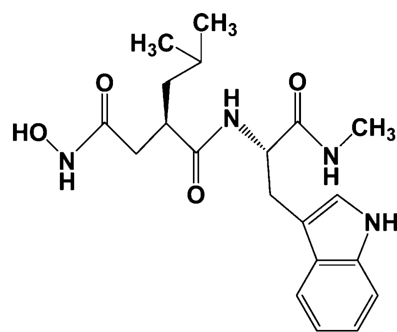

Fig. 1: Structure of Ilomastat

Table 1: Solubility of ilomastat in various solvents at 25 °C

Solvents	Solubility of ilomastat (mg·mL^{-1})
Water	0.14 ± 0.005
Physiological saline	0.13 ± 0.004
MCT	0.12 ± 0.009
Cremophor EL	1.54 ± 0.013
Transcutol	12.50 ± 0.22
Propylene glycol	9.88 ± 0.26
Ethanol	11.91 ± 0.12
PEG-400	8.20 ± 0.15

PEG-400 is shown in Table 1. The solubility of ilomastat in water or oil was found to be extremely low.
The partition coefficient (Log P) of ilomastat in 1-octanol-water and 1-octanol-physiological saline is presented in Table 2. The 1-octanol-water and 1-octanol-physiological saline partition coefficient was similar.

Table 2: Partition coefficient of ilomastat of 1-octanol-water and 1-octanol- physiological saline at 25 °C

Solvents	Partition coefficient of ilomastat (Log P)
1-Octanol-water	1.03 ± 0.012
1-Octanol-physiological saline	1.07 ± 0.017

2.2. Phase studies

The aim of the construction of ternary phase diagram was to find out the existence range of self-microemulsions. The ternary phase diagrams with various weight ratios of MCT-Cremophor EL-Transcutol and MCT-Cremophor EL-PG systems are shown in Fig. 2. The translucent microemulsion region is presented in phase diagrams in which the droplet size was within 100 nm. Each average droplet size of the rest region on the phase diagram was more than 100 nm.

2.3. Preparation of self-microemulsions and microemulsion gel

Various self-microemulsions were selected from the two phase diagram which had low concentrations of surfactant and cosurfactant. Ilomastat was dissolved into the surfactant, cosurfactant easily and the clear microemulsion gel could be quickly obtained by dispersing the self-microemulsions into the gel matrix. Pluronic F127 at concentrations of 17.8% in microemulsion gel exhibited the proper viscosity.

2.4. Droplet Size

Droplet size distribution is a critical factor to evaluate a microemulsion system. Droplet size is thought to have an effect on drug absorption as has been illustrated in several papers. The smaller the droplet size, the larger the interfacial surface area will be provided for drug absorption (Gershanik and Benita 2000; Kang et al. 2004; Pouton 2000). When the microemulsion gel A and B dispersed in distilled water, the resulted droplet sizes were 39.09 ± 0.66 nm and 24.31 ± 0.57 nm, respectively. The average droplet size of microemulsion gel was within 100 nm.

2.5. Zeta potential analysis

Generally, an increase of electrostatic repulsive forces between microemulsion droplets prevents the coalescence of microemulsion droplets. On the contrary, a decrease of electrostatic repulsive forces will cause phase separation. Ilomastat microemulsion gel A and B were diluted with distilled water, the resulted zeta potential was -8.3 ± 0.10 mV and -13.4 ± 0.31 mV, respectively. There is no marked difference in the absolute zeta potential value among the two preparations.

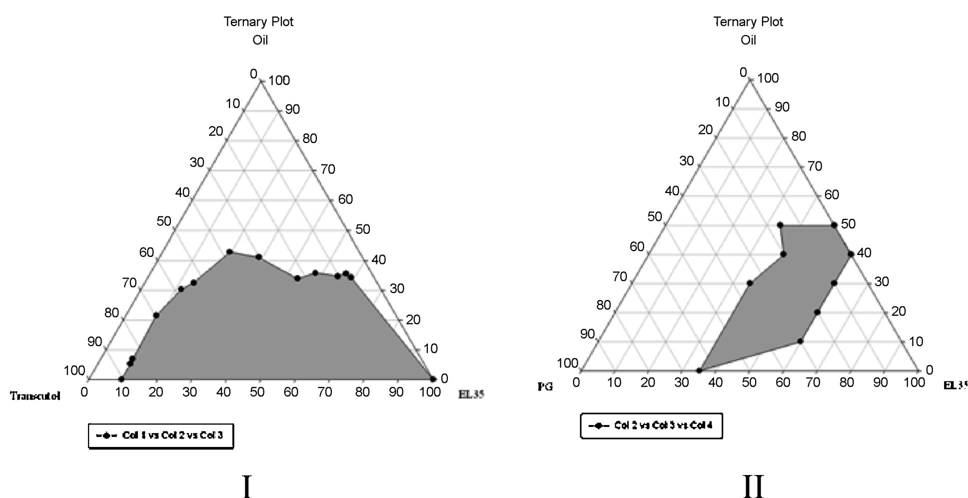

Fig. 2: I: Ternary Phase Diagram of Mixture Consisting of MCT-Cremophor EL-Transcutol system; II: Ternary Phase Diagram of Mixture Consisting of MCT-Cremophor EL-Propylene Glycol system. The gray domain indicates self-microemulsion region

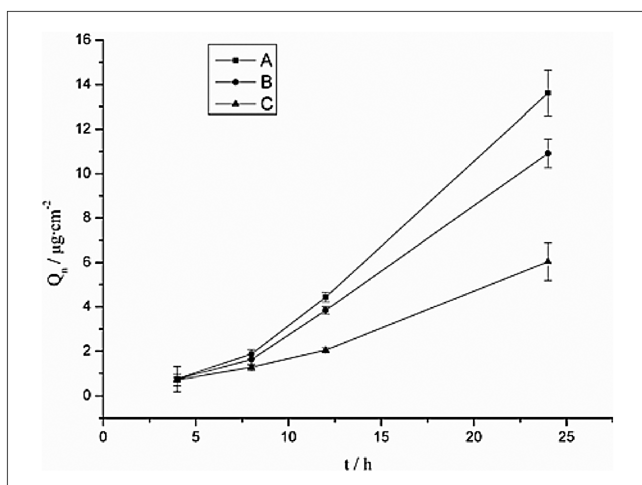

Fig. 3: Percutaneous permeation profiles of ilomastat from the microemulsion gel A, B and the controlled normal gel C (mean ± SD, n = 3)

2.6. *In vitro skin permeation studies*

The permeation profiles *in vitro* of ilomastat through excised abdominal skins of athymic mice are shown in Fig. 3. A steady increase of ilomastat 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 24 h showed that the two microemulsion gel preparations of ilomastat provided fluxes higher than that of the controlled normal gel which had only a low cumulative amount of ilomastat (6.03 μg·cm^{-2}) 24 h after application. Cumulative amount of ilomastat from microemulsion gels A and B was 2.2 times and 1.8 times that of the controlled normal gel respectively, 24 h post application. There was no significant difference in cumulative amount of ilomastat between microemulsion gels A and B at 24 h post application.

2.7. *HPLC analysis of ilomastat*

There is high adsorption of ilomastat in HPLC system metal tube which causes fairly broad peak and serious trail. After changing PEEK tube, the peaks become sharp and trail is avoided. Trifluoroacetic acid (0.02%) is used in mobile phase to obtain fine peak shape.

3. Discussion

In this study, a microemulsion gel based topical formulation was investigated which may be feasible for the transdermal delivery of ilomastat with enhanced the solubility and skin permeability. The poor aqueous solubility of ilomastat gives major drawbacks in its parenteral administration. In this study, attempt to provide parenteral formulation of ilomastat by loading in microemulsion was made to overcome its solubility limitation. Microemulsion is a superfine emulsion which consists of particle of less than 100 nm in diameter. Microemulsion as a potential drug delivery systems has been received attention in the recent years. The use of this approach may be application not only for improvement of the solubility of drugs but also for enhancement of percutaneous delivery.

MCT was chosen as an oil phase which was commonly used in available commercial products of microemulsion. The selection of the surfactant and cosurfactant were on the basis of compatibility with epidermis and/or microemulsification ability for MCT. Both Transcutol and propylene glycol have good epidermis acceptability and solubility of ilomastat. Hence, Transcutol and propylene glycol were selected as cosurfactants. The different microemulsion formulations were selected using the ternary phase diagrams.

In this paper, the application of microemulsion gel systems for percutaneous delivery of ilomastat was investigated and ternary phase diagrams were used to optimize the formulations. The results suggested that the microemulsion played a role in permeation enhancing effect. There is no topical commercial preparation of ilomastat, so we prepared normal gel as controlled formulation. Compared with the normal gel, the skin permeation ability of ilomastat was significantly increased by microemulsions, which might result from the special characteristics of microemulsions.

4. Experimental

4.1. *Materials and apparatus*

Ilomastat was supplied by Department of Drug Synthesis, Beijing Institute of Pharmacology and Toxicology. MIGLYOL 812 (medium chain triglycerides, MCT) was obtained from Sasol (Germany). Propylene glycol was purchased from Beijing Chemical Reagent Corporation (Beijing, China) and Transcutol was supplied by Gattefossé (France). Cremophor EL and Pluronic F127 were from BASF (Germany). Methanol (MeOH) and acetonitrile, both of HPLC grade were from Fisher Scientific (USA). Trifluoroacetic acid were from Acrōs Orgaics (New Jersey,USA,99%). Other chemicals are HPLC or analytical grade. All other chemicals were of reagent grade and were used without further purification. Athymic mice (Balb/C) were from animal center of Academy of Military Medical Sciences, 6–8 weeks, female. Zetasizer 2000 was made in Malvern Co., U.K., Hermle Z200A high speed centrifuger was made in German, Agilent 1200 HPLC was made in USA, TP-5 intelligent transdermal diffusion instrument was made in Tianjin Xinzhou Technology Limited Company, China.

4.2. *Solubility and oil-water partition coefficient determination*

The solubility of ilomastat in various oils, surfactants, and cosurfactants was measured, respectively. An excess amount of ilomastat was added into each selected vehicle, and the mixture was continuously shaken for 72 h in an isothermal shaker maintained at 37 ± 1 °C. Then the mixture was centrifuged at 4000 rpm for 15 min, followed by filtration through membrane filter (0.45 μm, 13 mm). The concentration of ilomastat in the supernatant was assayed by HPLC.

Weighed amounts of ilomastat were partitioned between water and 1-octanol for equilibration 72 h at 37 ± 1 °C. Ilomastat concentrations in the 1-octanol phase before (C_o) and concentrations in the aqueous after partitioning (C_{aq}) were measured using the same analytical techniques, from which the partition coefficients were calculated as $\log P = \log (C_o - C_{aq})/C_{aq}$.

4.3. *Construction of ternary phase diagrams*

Ternary phase diagrams were constructed with the concentrations of oil, surfactant and cosurfactant to form a clear solution in the system. In order to find out the concentration range of components for the existence range of microemulsions, ternary phase diagrams were constructed using a water titration method at ambient temperature (25 °C). MCT was used as oil phase, Cremophor EL was used as surfactant, propylene glycol and Transcutol were used as cosurfactants. For each phase diagram at specific surfactant/cosurfactant weight ratio, the ratios of surfactant and cosurfactant were varied as 1:9, 1:8,1:5, 1:4,1:2, 1:1, 2:1,3: 1, 5:1, 8:1, 9:1. The mixtures of oil, surfactant and cosurfactant at certain weight ratios were diluted with H_2O dropwise, under moderate magnetic stirring. The existence of self-emulsification fields was identified preliminarily by visual observation. The efficient self-emulsification region can be drawn according to the particle size distributions of the resultant emulsions which were determined using a laser diffraction sizer.

4.4. *Preparation of self-microemulsions, microemulsion gel by self-microemulsifying system and normal gel*

4.4.1. *Preparation of ilomastat self-microemulsions*

Ilomastat was added to the mixtures of surfactant and cosurfactant to be dissolved under stirring as described in Table 1, and then an appropriate amount of oil was added to the mixture and the self-microemulsion containing ilomastat was obtained by stirring the mixtures at ambient temperature.

Table 3: Composition of the three formulations (% *w/w*)

Formulations	MCT	Cremophor EL	Transcutol	Propylene Glycol	Ethanol	Pluronic F127	Distilled water
A)Microemulsion gel	22.2	11.1	22.2	/	/	17.8	26.7
B)Microemulsion gel	22.2	22.2	/	11.1	/	17.8	26.7
C)Normal gel	/	/	/	19.0	19.0	24.8	37.2

*Ilomastat: 0.2% *w/w*

4.4.2. Preparation of microemulsion gel by self-microemulsifying system

First, pluronic F127 was entirely dissolved in an appropriate amount of distilled water as the gel matrix. Then the self-microemulsion formulation was added to the gel matrix under stirring. After the self-microemulsion was entirely dispersed in the gel matrix, the clear microemulsion gel was obtained.

4.4.3. Preparation of normal gel

Ilomastat was added to the mixture of ethanol and propylene glycol to be dissolved under stirring as described in Table 3, and then the mixture was added to an appropriate amount of gel matrix under stirring. For pluronic F127 was dissolved in ethanol, the ratio of pluronic F127 was higher in gel matrix of normal gel than microemulsion gel. After the mixture was entirely dispersed in the gel matrix, the clear gel was obtained and used as controlled formulation.

4.5. Droplet size and zeta potential analysis

The average droplet size and zeta potential were measured by a Zetasizer 2000 (Malvern instruments Ltd., UK). The formulation (0.1 g) was diluted with distilled water at 37 °C under gentle stirring in a glass beaker. Then a 1.3 ml aliquot was withdrawn and added into a sample cell for droplet size measurement. Each size value reported was the average of at least three independent measurements. Zeta potential measurements were carried out on the same diluted sample using the same equipment and operating conditions, and the zeta potential values were calculated according to the Smoluchowski equation. Each sample was analyzed in triplicate. All measurements were performed at 25 °C.

4.6. In vitro permeation studies

Permeation of ilomastat from the formulations was determined using Franz diffusion cells fitted with excised athymic mouse (Balb/C) abdomina skin that represents the diffusional membrane. This study agrees with the protocol approved by the Ethics Committee of the Academy of Military Medical Sciences, this research also adheres to the Principles of Laboratory Animal Care. The experiment was performed by using Franz diffusion cells with an effective diffusion area of 1.77 cm^2. The excised abdomina skin samples were clamped between the donor and the receptor chamber of Franz diffusion cells with the stratum corneum facing the donor chamber 50 mg of different gels (containing drug 0.1 mg) was administrated on stratum corneum, respectively. The receptor chamber was filled with 16 ml of physiological saline solution containing ethanol (20%). The receptor medium was maintained at 37 ± 0.5 °C and stirred at 200 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 nylon membrane filter and analyzed by HPLC.
Cumulative amount of drug (Q_n, $\mu g \cdot cm^{-2}$) in the three preparations in the receptor chamber was plotted as a function of time (t, h). The cumulative amount of ilomastat permeated through excised athymic mouse skins was determined based on the following equation (Zhu et al. 2008):

$$Q_n = [C_n \times V + \sum_{i=1}^{n-1} C_i \times V_i]/S$$

where C_n stands for the drug concentration of the receptormedium at each sampling time, C_i for the drug concentration of the *i*th 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.

4.7. HPLC analysis of ilomastat

Ilomastat was analyzed by reversed phase HPLC using Agilent 1200 series (Agilent, USA). The HPLC system consisted of vacuum degasser, quaternary pump, an autosampler, a UV detector and workstation. The column was a Shiseido C_{18} column (5 μm, 4.6 mm × 15 cm, Japan). The mobile phase was an acetonitrile-water-trifluoroacetic acid (pH 2.5) (25:75:0.02) (v/v) mixture. The flow rate of the mobile phase was fixed at 1 ml/min and the detection wavelength was set at 220 nm. The retention time of ilomastat was about 6.9 min. The assay was linear in the concentration range of 0.5∼200.0 μg/ml with the lowest detection limit of 60 ng/ml of ilomastat. No interference from the formulation or skin tissue was observed. The method was validated with respect to accuracy and inter- and intra-day precision as per ICH guidelines and the relative standard deviation was less than 2% in both the cases. All samples should be filtered through an aqueous 0.45 μm pore size filter membrane in order to protect the column.

4.8. Statistical analysis

All skin permeation experiments were repeated three times and data were expressed as the mean value ± SD Statistical data were analyzed by one-way analysis of variance (ANOVA).
A multiple comparison test was used to compare different formulations, and a P value of 0.05 was considered to be significant.

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