Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(9):252-255

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The effect of total matrine nanoemulsion gel with confocal laser scanning microscopyon skin in mice

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ABSTRACT

To study transdermal absorption mechanism of matrinenanoemulsions gel on skin in mice. Coumarin 6 was used by embedding method as a fluorescent marker of total matrinenanoemulsion and nanoemulsion gel. Distribution of coumarin 6 on skin was analyzed by confocal laser scanning microscopy. The strongest fluorescence was found in epidermis of mice skin. And stronger fluorescence was found in the upper dermis. Fluorescence decreased significantly in deeperdermis. However,fluorescence in the hair follicle and its appendages was stronger than other areas. The fluorescence of total matrinenanoemulsion group in skin layers was stronger than total matrinenanoemulsion gel group and coumarin 6 solution control group. Nanoemulsion could increase permeation of coumarin 6 in each layer of skin. Fluorescence mainly distributed in epidermis, hair follicle and its appendages after administration.

Keywords: total matrine, nanoemulsions, nanoemulsion gels, confocal laser scanning microscopy

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INTRODUCTION

Matrine is the main active components of matrine. There is remarkable effect to treat eczemaand sore[1].Nanoemulsion, as a novel drug carrier, can promote the drug percutaneous absorption.Nanoemulsion-based gelis colloid thatthe nano emulsion added into the gel matrix which is composed with water soluble polymer materials[2,3].It not only owns the same advantages of NE, but also has the unique good points, such as high viscosity and having long time of drug action. And NE wasshort of these.

Coumarin 6 was used by embedding method as a fluorescent marker of total matrinenanoemulsion and nanoemulsion gel. Analyze distribution of coumarin 6 on skin by confocal laser microscopy.The transdermal mechanism of nanoemulsion gel was studied by comparing the fluorescence distribution of matrinenanoemulsion and matrinenanoemulsion gel.

Up to now, it is the first time that transdermal mechanism of matrinenanoemulsion gel was studied by using confocal laser scanning microscopy (CLSM)[4,6]. And there were not relevant reports in the domestic before.

EXPERIMENTAL SECTION

2.1 Equipment and Reagents

2.1.1 Equipment

BSI10S Analytical balance(Sartorius Instrument Systems, Inc.);HJ-6A magnetic stirrer(Changzhou Guohua Electric Co.,Ltd.); KH7200DB CNC ultrasonic cleaner(Kunshan Ultrasonic Instrument Co., Ltd.)

2.1.2 Reagent and material

SophoraFlavescens Alkaloids(Purchased from Ningxia Bauhinia Pharmaceutical Co., Ltd.); Sophora alkaloids nanoemulsion; Sophora alkaloids nanoemulsion gel(Tianjin Guangfu Science and Technology Development Co., Ltd.); Coumarin 6(Beijing J & K Technology Co.,Ltd.)Formalin reagent(10%,NBF)(Beijing GaoHua Albert Food Additive Co., Ltd.),IBM(Sinopharm Chemical Reagent Co.,Ltd); Polyoxyethylene Castor Oil(Beijing FengliJingqiu commerce limited liability company); Anhydrous ethanol(Beijing Chemical Plant)

2.1.3 Experimental animals

Kunming mice(male, 18~22g, purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd., animal license number:SCXK(BJ)2012-0001)

2.2 Preparation of matrinenanoemulsionand nanoemulsion gel

Preparation of matrinenanoemulsionwas made with HLB value law (hydrophilic-lipophilic balance method). By screeningnanoemulsion formulation, optimizing optimal nanoemulsion composition ratio and investigating optimal drug loading, Sophora alkaloids nanoemulsion formulation were consisting of isopropyl myristate (IPM),Cremophor(EL35), Ethanol, Sophora alkaloids and water.

According to the optimized formulation of Sophora alkaloids nanoemulsion, Sophora alkaloids were accurately weighed, dissolved in mixed oil, ethanol dissolved and mixed as internal medicated phase. Thendeionized waterwas added slowly to drug phase, constant stirred to clear and transparent. Sophora alkaloids nanoemulsion was made finally.

Took the mixed gel matrix (NP700: CP-934), added deionized water and glycerin grinding wettability. Then gradually added Sophora alkaloids nanoemulsion prepared, quickly stirred until gelatinous.It was nanoemulsion gel that formed.

2.3 Preparation of fluorescently labeled samples

Preparation of the control solutions: Weighed accurately reference standard amount coumarin 6, added IPM to dissolve by ultrasound, formulated as 0.5% solution of coumarin 6 as a control group.

Preparation of fluorescently labeled Sophora alkaloids nanoemulsion: To prepare Sophora alkaloids nanoemulsion in accordance with the early preparation ofnanoemulsion , dropped the 0.5% solution of coumarin 6 before adding water phase,constant stirring,then added water phase.

Preparation of fluorescently labeled Sophora alkaloids nanoemulsion gel: On the basis of the fluorescently labeled Sophora alkaloids nanoemulsion prepared, added gel matrix in accordance with the preparation of nanoemulsion gel, grinded evenly, and then made the fluorescently labeled Sophora alkaloids nanoemulsion gel.

2.4 Sample preparation and observation of CLSM

The mice were anesthetized with 0.25% urethane. The abdominal hair was removed with a depilatory cream. The fluorescent labeled Sophora alkaloids nanoemulsion, the nanoemulsion gels and control solution of coumarin 6 were administered to the abdominal skin. 6 hours later, killed the mice and peeled off the skin of administration, and washed it with saline, drained excess water by filter. Finally spread the skin on the slides and were mounted with glycerol. The slides were placed in CLSM, light Ar / He / Ne, 488nm of excitation wavelength, the pinhole size was fixed, then observed and captured images.

RESULTS AND DISCUSSION

The experimental results showed that different preparations of mouse skin after treatment, fluorescence of the epidermis is strong, followed with superficial dermal fluorescence, and fluorescence of the deep dermis significantly weakened. And the fluorescence intensity around the hair follicle and its subsidiaries was significantly stronger than in other regions. The three layers of skin fluorescence of Sophora alkaloids nanometer group was significantly stronger than other groups. It showed that nanoemulsion can increase penetration of coumarin 6 in three layers of skin. These results suggested that after nanoemulsion and nanoemulsion gel transdermal drug delivery, most of the drug were in the top layer of skin and hair follicle and its subsidiary and other parts. And which also suggest that hair follicles in the skin and its appendages transdermal drug also play a certain positive role.

The results are shown in Figure 1.

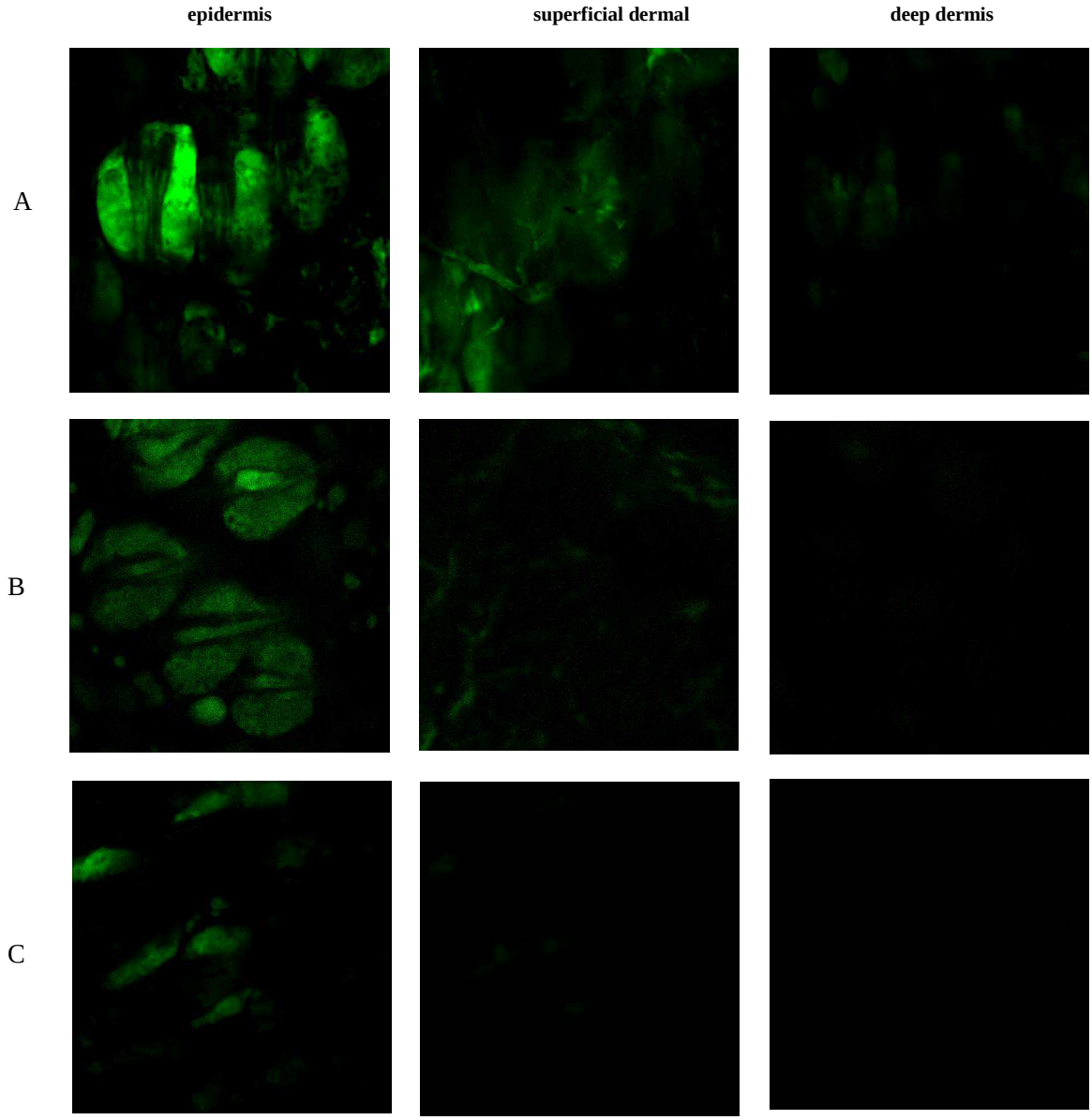

Fig. 1 The fluorescence distribution diagram of different preparations in each layer and subsidiaries (A:matrinenanoemulsion; B:matrinenanoemulsion gels; C:control solution of coumarin 6)

CONCLUSION

4.1 CLSM technology has the unique advantage in percutaneous penetration. On the one hand, CLSM can be used in transdermal drug research on living tissue, which better reflects the drug effect and impact on living tissue. On the other hand, CLSM enables transdermal drug process visualization and can clearly show the specific distribution of drugs and drug transdermal route in different parts of the skin.

4.2 Oil phase used in the preparation ofnanoemulsion was IPM. It has been reported that IPM is a fat-soluble high absorption enhancers, having a side chain structure. And thepromoting absorption mechanism of IPM may be a side chain structure of it owns. It can increase the stratum corneum lipid fluidity, so that the drug can seep the stratum corneumeasily. So the control group can also be observed the fluorescence phenomenon with the laser confocal microscope after the role of IPM using coumarin 6 solution prepared in mouse skin.

4.3 The experimental results showed that the fluorescence phenomenon of the matrine alkaloids nanoemulsion was significantly higher than nanoemulsion gel group.And the results are consistent with scanning electron microscopy and HE staining, indicating that the skeletal structure of the gel matrix to some extent limit the nanoemulsion

through skin absorption. While the fluorescent of nanoemulsion group was also significantly stronger than in the control group, indicating that transdermal nanoemulsion good performance is not due to have a penetration enhancement prescription ingredients exist, but because the various components of the prescription together constitute.The nanoemulsion system has a role in promoting transdermal drug absorption.

Acknowledgements
This project was supported by Beijing Natural Science Foundation (No: 7122093), Beijing Nova program(No: 2008A057), Beijing Excellent Talents Training Project(No: 2012D009999000002), National Key New Drug Creation of China (No: 2012ZX09103201-044), and the research and innovation team of Chinese herbal compound of Beijing University of Chinese medicine(No: 2011-CXTD-13).

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