DEVELOPMENT AND EVALUATION OF THE SINOMENINE TRANSDERMAL PATCH

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ABSTRACT

Sinomenine transdermal patch was prepared and its properties were studied. The patches were produced by salivation method. The releasing rate in vitro of the patch was determined by HPLC. Peel test was used to evaluate the adhesion. Acute skin irritation test was performed in comparison with formalin (0.8%) by using mouse model. The Sinomenine TDDS Patch was prepared. The releasing rate in vitro followed the Higuchi equation (r>0.99), the releasing amount was beyond 90% in 24h. The peel adhesion to steel (N/25 mm) is 10 or above. The skin irritation tests showed negligible erythema and edema. The Sinomenine transdermal patch was prepared successfully and it may be beneficial for topical use.

Keywords: Sinomenine; transdermal patch; peel test; skin irritation test.

INTRODUCTION

Sinomenine (7, 8-didehydro-4-hydroxy-3, 7-dimethoxy-17-methylmorphinan-6-one) is a pure alkaloid extracted from the Chinese medical plant *Sinomenium acutum*, which is distributed widely everywhere in China. Up until now, sinomenine has been widely used in the treatment of various rheumatic diseases (Liu *et al*., 1997). The topical application of sinomenine in the treatment of rheumatoid arthritis has potential advantages, such as minimal first-pass metabolism, few side effects and patient comfort/compliance (Liu *et al*., 1999).

Compared to the common formulations such as ointments, creams, gels and emulsions, patch types are more convenient for applications and can prevent active components from being washed out and can release them for a long period of time through a zero-order delivery. In this paper, the preparation and evaluation of the transdermal patch of Sinomenine was studied.

MATERIALS AND METHODS

Materials

Sinomenine reference substance (batch 0774-200105) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP), Beijing, China. Sinomenine, purity>98%, was obtained from Xian Sino-Herb Bio-technology Co. Ltd (Xian, China). Release liner, backing laminate and emulsion medical polyacrylic resin pressure-sensitive adhesives were kindly supplied by Tangshan health material plant (Hebei, China), PVA 1750±50 and PVP were purchased from China Medicine Group Shanghai Chemical Reagent Corporation (Shanghai, China), Azone was purchased from Chengdu Kedong Chemical Company Ltd (Sichuan, China), All other chemicals used were analytical-reagent grade

The transdermal patch preparation

PVA (1 g) and PVP (1g) were weighed in requisite ratios and mixed in 10 ml distilled water, stired the mixture over a hot water bath until dissolved. After the mixture was cooled down to 25℃, added Sinomenine (0.3 g), propylene glycol (0.5 ml), glycerol (0.5 ml), azone (0.3 ml) and the pressure-sensitive adhesives (2 ml), mixed together using a mechanical stirrer (IKA, RW16, Germany) at 800 rpm for 15 min under occluded condition (Paola *et al*., 2003). The mixture was then cast on the release liner with a micrometer adjustable casting knife (R. K. Coat Instruments, UK) set at 500 μm, and was dried at 80°C for 25 min. The total area of one formulation is about 300 cm^2. The patches were covered with backing laminate and cut into appropriate sizes. Drug loading for Sinomenine was about 1 mg per cm^2.

Studies of in vitro skin permeation

Release of Sinomenine from the patches

Modified diffusion cells according to Franz were used for the release and permeation study of Sinomenine from the patches of an area of 3.14 cm^2. The receiver compartment volume was 5 ml, a phosphate buffer saline solution (pH 6.75) tempered to 37±0.2°C was used as the acceptor medium. Full thickness skin from dorsal region of Kunming mouse (Animal's Experimental Center of Tongji Medical College), whose hair had been removed on the previous day with an electric clipper, was used as membrane. The mouse was sacrificed by cervical dislocation and dissected skin was used immediately. The transdermal patch was firmly pressed onto the center of

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the mouse skin and then the skin was mounted on the donor compartment. The donor compartment was then placed in position such that the surface of dermis side skin just touches the receptor fluid surface. The whole assembly was kept on a magnetic stirrer and the solution in the receiver compartment was continuously stirred using a magnetic bead.

At each predetermined time intervals, 400 µl of sample solution was taken from the receptor cells to determine the amount of Sinomenine permeated, and refilled with the same volume of the fresh receptor solution. The samples were kept at room temperature until analyzed by HPLC.

After a 24 h permeation, the samples was filtered through the micropore filter (0.45 µm). An aliquot of 20 µl was injected into the chromatograph. The concentration of Sinomenine released was determined by using reverse-phase high-performance liquid chromatograph as described below.

HPLC description

A Shimadzu Class VP series HPLC system with two LC-10AT pumps, a SPD-10A variable wavelength programmable UV/Vis detector, the stationary phase was Hypersil ODS-2 column (ThermoElectron Corporation, USA; 250 mm×4.6 mm, particle size 5 µm) was used. The mobile phase was methanol- phosphate buffer saline solution (pH9.0) (55∶45 v/v) (CPC, 2005). The temperature of column was 25 ℃. The detective wave was 262 nm. The flow rate was 1.0 ml/min. The system was equipped with Class VP series version 6.12 software.

Preparation of standard curve

Accurately weighed 10 mg Sinomenine reference substance into 10 ml volume bottle, dissolved by methanol to 10 ml. Then fetched solution of 0.1 ml, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml separately and diluted them by methanol to 1 ml. The injection volume was 20 µl.

The calibration curve was obtained by plotting peak area ratios of Sinomenine (y-axis) against its concentration (x-axis). The return equation is: Y=2785X+536 (r=0.9998). This shows that the coherency is obvious and there is better linear correlation between 0.1~1 mg ／ml.

Adhesion to steel (peel adhesion)

One week after preparation, the patches were cut into strips with a width of 25 mm and applied to a stainless steel plate, smoothed three times with a 2.0 kg roller, maintained for 20 minutes at 25°C, and pulled from the plate at a 180° angle at 300 mm/min rate (CPC, 2005). The test was performed with a tensile testing machine Acquati model AG/MC1 (Acquati, Arese, Italy). The force was expressed in N/25 mm width of the patch under test (CPC, 2005; Venkatraman *et al*., 1998). Peel adhesion values were the average of 5 replicates

Acute skin irritation test

The protocol for in vivo experiments of skin irritation was performed by a modified method of Draize *et al.* (1944) (Tardiff *et al.*, 2003 and Draize *et al*., 1944). Kunming male mice (Animal's Experimental Center of Tongji Medical College) were acclimatized to laboratory conditions for 1 week prior to experiments and were on standard animal chow and water ad libitum. The temperature of the room was maintained at 22±1°C and the humidity was maintained at 35-45% RH. The mice, whose hair was removed on the previous day, were marked as a circular area (~3 cm^2) with a felt tip marker on the dorsal surface. The animals were divided into 7 groups (n = 5) and treated as follows.

Group I	No treatment (normal);
Group II-VI	Control (applied with Sinomenine trans-dermal patch);
Group VII	Formalin (a standard irritant; 0.8% v/v).

The animals were treated daily with new patch/formalin solution up to 7 days and finally the treated skin was examined visually for erythema and edema. The skin irritation (erythema and edema) was evaluated by visual scoring by a modified method of Draize *et al*. (1944).

RESULTS AND DISCUSSION

Patch preparation

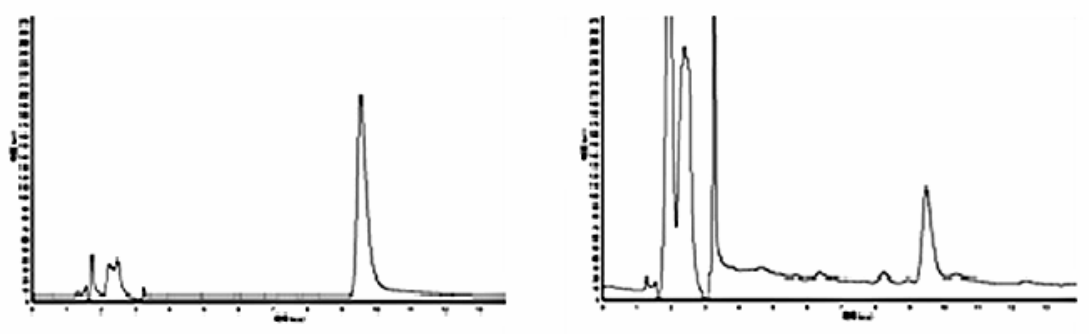
PVA, PVP were used as the skeletal material of preparation, Glycerol as humectant and plasticizer, Azone and propylene glycol as penetration enhancer.

Polyacrylic resin pressure-sensitive adhesives (PSAs) are materials that adhere to a substrate by application of light force and leave no residue when removed. Pressure-sensitive adhesives are also important components of transdermal drug delivery systems (TDDS), because they ensure intimate contact between the drug-releasing area of a TDDS and the skin surface, which is critical for controlled release of the drug (Yoshinori *et al.*, 2005).

Studies of in vitro skin permeation

The proposed method was applied to determine Sinomenine in the acceptor medium. The chromatogram of standard and sample solution was given in fig. 1. The results showed that the accuracy and sensitivity of the proposed method were suitable for the determination requirements.

The content of Sinomenine was calculated by extra-standardizing. Sinomenine permeated was calculated by summing the amount of Sinomenine in the receptor medium. Mean cumulative amounts were plotted versus



A. standard solution; B. sample solution

Fig. 1: HPLC of standard and sample solution.

Table 1: The permeation parameters for Sinomenine patch through the mouse skin.

T(h)	1	4	8	12	18	24
C (μg / ml)	118.2	213.0	308.3	389.4	482.3	573.7
Q (μg / cm^2)	188.3	339.2	491	620	768	913.5

square-root of time (Higuchi equations): $Q_t=K*T^{(1/2)}$, where, Q_t is the cumulative drug amount recovered in the receiving compartment (μg / cm^2), K is the kinetic constant indicative of the release rate (μg/$h^{(1/2)}$) and $t^{(1/2)}$ is the square-root of time ($h^{(1/2)}$).

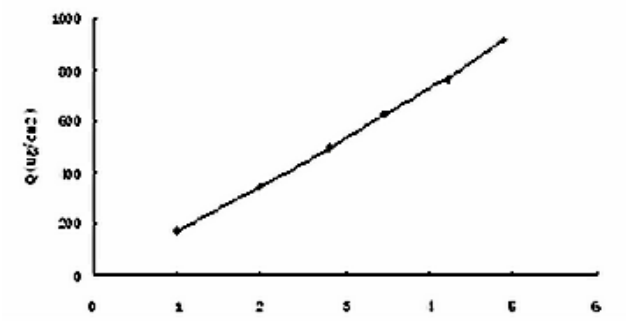


Fig. 2: The release curve of the cumulative drug amount.

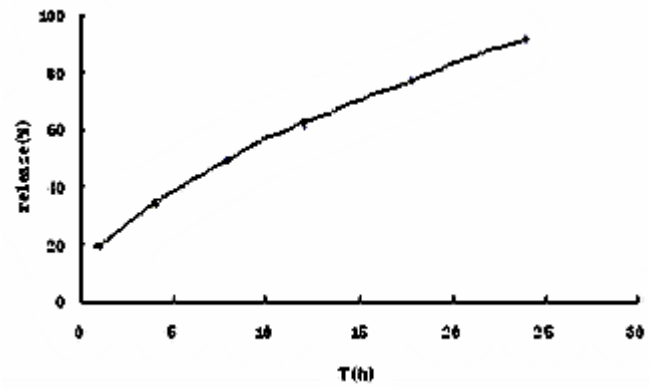


Fig. 3: The release curve of the cumulative drug percent.

According to the fig. 2, the Q/t curve is almost a straight line, that means the releasing process is a zero level one. The kinetic constant *K* (slope of the plot) and the release Lag-time (abscissa intercept) were calculated by linear regression: Q=186.8579 $t^{(1/2)}$-20.761, r=0.9981, which followed the Higuchi equation (r>0.99).

According to the fig. 3, the releasing amount in vitro was increasing by time, and the releasing amount of patch was beyond 90% in 24 h.

The high cumulative amount and release rate of drug permeated in the patch which may be due to the penetration enhancer and other matrix.

Adhesion to steel (peel adhesion)

The measure of patch strength between an adhesive and a substrate is defined as adhesion. The peel test is one of the standard tests used to evaluate the strength of adhesive patches. These properties are typically measured using the 180° peel adhesion test method. The force was 28.3N/25 mm under the test (n=5). According to ASTM (American Society for Testing Materials), the peel adhesion to steel (N/25 mm) is 10 or above, so the adhesive patches have satisfactory peel adhesion

Skin irritation test

The results of skin irritation tests of the Sinomenine transdermal patches in comparison with formalin (0.8%) showed that the transdermal systems induced negligible erythema and edema, but formalin induced severe erythema and edema. Formalin induced high grade of irritation, indicated by 'severe' inflammation and edema besides showing discontinuity in epidermis, thin epidermis, ulceration and hyperplasia.

CONCLUSION

A new Sinomenine transdermal patch was developed with a high *in vitro* permeation rate and excellent properties. It may be beneficial for topical use.

The next step should be the evaluation of this production *in vivo*. On the basis of the present results it may be concluded that the new pressure-sensitive adhesive matrix system is a potential delivery matrix.

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