Nano-liposomes of entrapment lidocaine hydrochloride on *in vitro* permeability of narcotic

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Abstract: In order to explore two kinds of nano-liposomes in lidocaine hydrochloride nano-liposomes on *in vitro* permeability of drug, and conduct comparison and analysis, this paper investigates cumulative infiltration situation of lidocaine hydrochloride flexible nano-liposomes and ordinary nano-liposomes by using modified Franz diffusion pool on mice vitro skin. Cumulative osmotic quantity of lidocaine hydrochloride flexible nano-liposomes for 9h was higher than ordinary nano-liposomes. ^{t}max (Maximum osmotic quantity time) of lidocaine hydrochloride flexible nano-liposomes and ordinary nano-liposomes in mice skin was 5 and 60min, the former ^{C}max (maximum dosage time) was 1.2 times of the latter. Drug was not found in mice plasma of ordinary nano-liposomes group, traces of drugs was detected in 0.5 and 1h in flexible nano-liposomes group, but the concentration was lower than the effective concentration. Compared with the classic skin transparent promoter and ordinary liposome, flexible nano-liposomes have more advantages, but its stability is less than ordinary nano-liposomes because of the addition of surface active substance. Flexible nano-liposomes have great development potential as a carrier of transdermal drug delivery field.

Keywords: lidocaine hydrochloride, flexible nano-liposomes, ordinary nano-liposomes, permeability.

INTRODUCTION

Embedding surfactant in traditional liposomes can change the deformability and the physicochemical properties of liposomes, form flexible liposomes, to make drug percutaneous transmittance increases greatly (Zhigang *et al*., 2010). Flexible nano-liposomehas the nature of effective permeability under large enough stress make drugs into the systemic circulation through the stratum corneum (Sevinc *et al*., 2013; Laxman *et al*., 2010). Lidocaine hydrochloride is mainly used for local anesthesia and antiarrhythmic, which works fast and with strong role. Lidocaine hydrochloride mucilage is used much in the internal checking of urethra, bladder, and gastrointestinal tract as a kind of hospital preparations, but there is no skin external preparation. Injection is inconvenience for anesthesia, and mucilage is only applicable to local anesthetic mucosa. Lidocaine hydrochloride gel and emplastrum have been reported abroad (Xiaoliang *et al*., 2010; Ruiwei *et al*., 2011; Chao and Shengwu, 2010). Indications are local anesthetics, transurethral enforcement examination and patients need the local anesthetic, but there is no research on lidocaine hydrochloride flexible nano-liposome (Xiangyang *et al*., 2012). This study prepared lidocaine hydrochloride flexible nano-liposome used on skin and compared infiltration situation of products and ordinary nano-liposomes in mice skin.

MATERIALS AND METHODS

General materials

Type P680A high-effective liquid phase color spectrometer (America Dionex Company); ultrasonic emulsification instrument (Fisher Scientific Company); Franz diffusion pool (Changsha Yue Ming Bo Equipment Trade CO., LTD.); Mastersizer type 2000 laser particle size analyzer (Shanghai Aijian Nanotechnology Development CO., LTD.). Lidocaine hydrochloride technical material (Shanxi Xin Baoyuan Pharmaceutical CO., LTD., 100.1% content, batch number 110905);lecithin, cholesterol, sodium cholate, perchloric acid, potassium chloride and sodium chloride (Chinese Medicine Group Chemical Reagent CO., LTD); other reagents are analytically pure, water is heavy steam water. Kunming mice (half male and half female, 18~22g, Xiangya Medical School Animal Center of Middle and Southern University, animal certificate Numbers WK0504-0048).

Method

Medicine system preparation

Weigh 2g lidocaine hydrochloride technical material by precision scale, add water to constant volume 10ml, and then get lidocaine hydrochloride reserve liquid. Put 300mg lecithin and 100mg cholesterol in 50ml eggplant shaped bottle, add 1ml lidocaine hydrochloride reserve liquid and 9ml chloroform methanol mixture to dissolve,

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put in 37└ water bottle for 3h to reduce pressure and evaporate, form a layer of lipid membrane. Add 10ml aqueous solution with 300mg sodium cholate after nitrogen injection, steam for 1h to remove organic solvent, form liposome suspension. Put this mixed suspension in the ice bath for 10min to explore continuous ultrasonic probe (30s/time, 10times in total), get milky white transparent liquid after 0.2μm filter head filtration which sealed and kept in 4└ refrigerator (Yuchen, *et al*., 2013). Same production and preparation for ordinary nano-liposomes excluding surfactant sodium cholic acid.

Measurement and inspection

Laser particle size analyzer measure grain size by 135º detection angle, scanning speed of 1000times/s, to get average grain size of lidocaine hydrochloride flexible nano-liposome (106±6.9) nm (n=15), average grain size of ordinary nano-liposom (110±5.5) nm (n=15).

Preparing pH 7.4 phosphate buffer solution (PBS): dissolve 8g sodium chloride, 0.2g potassium chloride, 2.89g disodium hydrogen phosphate, 0.2g potassium hydrogen phosphate into 1ml water. Ultra centrifugation separates unpacked free lidocaine hydrochloride. Take the above 1.5ml lidocaine hydrochloride flexible nano-liposome in the centrifuge tube, centrifuge in 4└ (18 000×g) for 5h, use FBS dissolve 1ml supernate to 100ml, measure free drug concentration. Add absolute ethyl alcohol into other1.5ml flexible nano-liposome lidocaine hydrochloride solution to 100ml, shake fully to dissolve, get a sample to measure after suspension clarified, finally get the total quantity (Xianxi *et al*., 2006). Encapsulation efficiency of lidocaine hydrochloride flexible nano-liposome after calculation is (80.1±1.02) % (n=5). According to the same calculation, encapsulation efficiency of ordinary nano-liposome is (78.8±1.25) % (n=5).

Chromatographic condition: BDS C18chromatographic column (4.6 mm×200mm, 5μm); moving phasemethyl alcohol-glacial acetic acid-triethylamine-water (50: 2: 1: 47); detection wave is 262mm; flow velocity is 0.8 ml/min; column temperature is 35└; sample size is 20μl. There is no other impurity peak in lidocaine hydrochloride peak position in blank receiving liquid chromatogram, which explains no interference for drugs.

Add appropriate lidocaine hydrochloride reserve liquid into blank receiving liquid to get a series of standard solution with the concentration of 5, 10, 20, 40, 60, 80, 100μg/ml, conduct sample measurement respectively, take peak areaA as the vertical coordinates, concentration C as horizontal axis for linear regression, get regression equation A= 0.0343 c + 0.014 9, r=0.999 8. It showed that linear relation of lidocaine hydrochloride concentration is good in the range of 5~100μg/ml. Standard solution recovery rate of low, medium and high concentrations (10, 40, and 100μg /ml) in lidocaine hydrochloride is respectively (100.07±0.30) %, (100.03±0.28) % and (100.04±0.20) % (n=3). RSD of within-day and daytime is 0.39% and 0.64%.

Penetrant test method

Divide six mice into two groups, each group of three mice, shave belly wool carefully using blade after put to death, take suitable size of abdomen skin and remove subcutaneous fat and tissue washed with normal saline. Fix mice skin of two groups respectively in two groups of the modified Franz diffusion pool when test, dermal layer to reception room, corneous layer to supply room. Add 1ml lidocaine hydrochloride flexible nano-liposome with the concentration of 20mg/ml into the first supply room; add 1ml lidocaine hydrochloride ordinary nano-liposome with the same concentration into the second supply room. Add normal saline into two groups' reception room with the volume of 22ml and 3.14cm2 effective diffusion areas. Open Franz diffusion pool device with the temperature of 37└ and stirring speed 300 r/min. Get sample 0.2ml in 2, 5, 10, 20, 30min and 1, 3, 5, 7, 9h (at the same time complement equivalent normal saline), filter by 0.2m filtration membrane, take subsequent filtrate to conduct sample measurement, calculate the accumulated osmotic quantity of lidocaine hydrochloride (Q).

Extraction and measurement method of drugs in the skin as followed:

The processing of skin sample: wipe skin (area 4cm2) after vivo study three times with 50% ethyl alcohol, and then wash 3times. Put skin into homogenizer after cut into pieces; add 10% 500 μl perchloric acid and 5ml distilled water move to 10ml centrifuge tube after homogenate, centrifugal (1000xg) for 15min, collect supernate, conduct sample measurement after filtration.

The linear scope and methodology verification: Take blank skin samples process according to the above methods, add appropriate lidocaine hydrochloride reserve solution to make a series of standard concentration solution, measure under the conditions of vitro penetration test. Results showed that the blank skin extract does not interfere with the measurement of lidocaine hydrochloride.

β Drug measurement in the skin: Take 60mice to shave belly wool carefully and scrape it using blade, wash surface skin by warm water, divide into two groups randomly after 24h convalesce, 30pieces each group. The skin medicine area is 2cm×2cm. One group was smeared 1ml lidocaine hydrochloride flexible nano-liposome with concentration of 20 mg/ml till evenly spreaded; the other group was smeared the equivalent lidocaine hydrochloride

ordinary nano-liposome till evenly spreaded and maintain a closed state. Take respectively three mice from two groups in 0, 2, 5, 10, 15, 20, 30, 60, 180 and 300min, put to death after taking away eyeballs and whole blood (300μl). Take skin and measure drug concentration in skin according to the method after extracting the same as above.

Drug extraction and determination in the blood is as follows:
Blood sample processing: Take 300μl whole blood to add 150 μ l 10% perchloric acid, vortex 5min, centrifuge (1000xg) 15min, collect supernate, take sample to measure.

α The linear scope and methodology verification: Compared with blank whole blood, the results showed that endogenous substances in plasma do not interfere with the measurement. Linear regression equation is A=20.019 8, c-0.0941, r=0.9997. Linear scope is 10~100 μ g/ml. Add appropriate lidocaine hydrochloride reserve solution into mice blank whole blood to make the final concentration of lidocaine hydrochloride is 10, 40 and 100 μ g/ml. Measure sample respectively after conducting the same as above.

β Blood sample measurement: take skin sample to measure after conducting the same as above; calculate pharmic content in mice blood.

RESULTS

The results of vitro permeation test
Make a graph with Q to time t (as shown in fig. 1). With each point before steady state to time regression, get steady transdermal rate Js and osmotic coefficient Pof two formulations of lidocaine hydrochloride (as shown in table 1), calculate the ratio of P value of flexible nano-liposome group and ordinary nano-liposome group is 1.25 (namely, increased permeation times ER). The accumulation of 9h osmotic quantity of lidocaine hydrochloride flexible nano-liposome and lidocaine hydrochloride ordinary nano-liposome is respectively (12.1±0.4) and (8.0±0.3) μg (n=3).

Results of mice skin penetration test
Linear scope and the methodology validation results: the linear regression equation is A=0.023c+6x10-5 (r= 0.9998) and linear scope is among 5~100μg/ml. Take lidocaine hydrochloride concentration solution of 10, 40, and 100μg/ml and measure them respectively. The recovery rate is (100.05+0.31) %, (100.07+0.25) % and (100.03+0.53) % by calculating, and within-day and daytime RSD is 0.44% and 0.34% (n=3).

Linear scope and the methodology validation results: the recovery rate is (100.10±0.56) %, (100.06±0.43) % and (99.98±0.28) % by calculating and within-day and daytime RSD is 0.42% and 0.54%.

Results of blood samples measurements: after daubing lidocaine hydrochloride ordinary nano-liposomes on mice, lidocaine hydrochloride cannot be detected in the plasma at each time point. And after daubing lidocaine hydrochloride flexible nano-liposome, trace of lidocaine hydrochloride can be detected in plasmain in 30min and 1h. The value is (0.32±0.08) and (0.68±0.13) μg/ml, respectively which is far lower than the concentration of the whole body effect (about 3~6μg/ml (Yao *et al*., 2012). Therefore, it can be ignored.

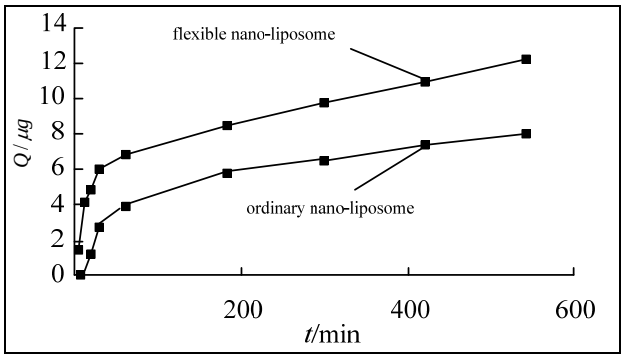

Fig. 1: Accumulation osmotic quantity-time curve (n=3)

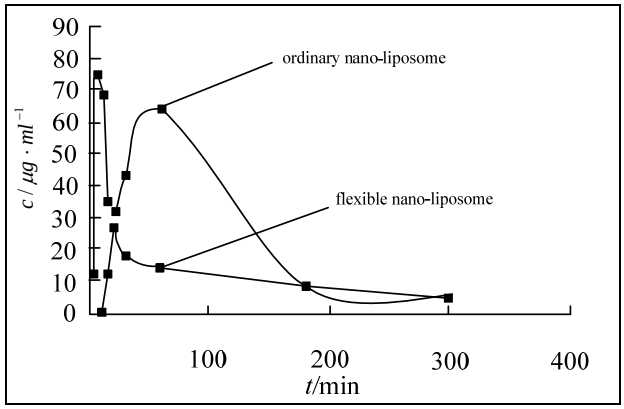

Fig. 2: Medicine in skin after treatment- curve of time (n=3)

Table 1: Experiment results of lidocaine hydrochloride flexible nano-liposome and ordinary nano-liposome

Parameter	Flexible nano-liposome	Ordinary nano-liposome
$J_S / \mu g \cdot cm^{-2} \cdot \min$	0.005	0.004
$P / cm \cdot \min^{-1}$	2.5×10-7	0.2×10-6

DISCUSSION

The vitro penetration test showed that 9h accumulation osmotic quantity of lidocaine hydrochloride flexible nano-liposomeis bigger than ordinary nano-liposome. The reason is that flexible nano-liposome contains surfactant

which has the effect on drug promoting permeability and can be out of shape under the action of skin internal force and produce stronger penetration (Yanhong *et al*., 2010).

Fig. 2 showed that the time of checking out drugs in skin of lidocaine hydrochloride flexible nano-liposome group is earlier than lidocaine hydrochloride ordinary nano-liposome group. The former can be detected in 2min, while the latter is detected in 15min and the tmax of lidocaine hydrochloride flexible nano-liposome group and lidocaine hydrochloride ordinary nano-liposome group is 5 and 60min, the former cmax is 1.2 times of the latter. The results indicated that, lidocaine hydrochloride flexible nano-liposome has better penetration effect, and it can enhance the activity of drug in local. Drug content measurement results in blood showed that lidocaine hydrochloride ordinary nano-liposome cannot deliver drugs to the systemic circulation, lidocaine hydrochloride flexible nano-liposome will not produce the whole body effect because it is far below the effective concentration, though trace of lidocaine hydrochloride can be detected after the treatment.

Flexible nano-liposome can be out of shape under the action of skin internal force and produce penetration effect. *In vitro* experiment, the removal of mice skin changes some physical conditions in the skin, skin contacts with receiving liquid will produce swelling of the skin and damage concentration gradient of skin moisture, therefore the slow fusion and diffusion play an important role. Compared with *in vivo* experiment and *in vitro* experiments, penetration speed of drugs increases significantly. It showed that the skin can keep intactmoisture concentration gradient under normal physiological condition, naturally occurring water together ensure the flexible nano-liposome quickly penetrate the skin given priority to promote penetration deformation mechanism.

CONCLUSION

The local external permeability of lidocaine hydrochloride was studied in mice skin *in vitro* penetration test. In addition, the accumulation osmotic quantity and transdermal rate of lidocaine hydrochloride flexible nano-liposome and ordinary nano-liposome was also compared. We found that, embedding drugs by flexible liposome has faster transdermal rate and better penetrating effect compared with the ordinary one. Moreover, because the drug is absorbed by body, thus systemic side effects are reduced.

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