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Enhanced transdermal delivery of tetracaine by electroporation

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Abstract

The effect of electroporation on the transport of tetracaine through skin in vitro was studied using side-by-side compartment diffusion cells method. After achieving steady state by passive diffusion, fluxes of tetracaine achieved with passive diffusion, electroporative pulse and iontophoresis were compared. Electroporation (square-wave pulse, voltage 130 V, pulse time 0.4 s, pulse frequency 40 pulses min^{-1}) or iontophoresis ($0.2 \cdot mA\ cm^{-2}$, lasting for 4 h) increased the transport of tetracaine through skin. The flux of tetracaine at 0.25 h after electroporation (pulse number 400) was $54.6 \pm 6.0\ \mu g \cdot cm^{-2} \cdot h^{-1}$, that after iontophoresis was $17.4 \pm 5.8\ \mu g \cdot cm^{-2} \cdot h^{-1}$ and that after passive diffusion was $8.2 \pm 0.5\ \mu g \cdot cm^{-2} \cdot h^{-1}$. In addition, the fluxes of tetracaine increased with the increasing of pulse number. From these results, it is clear that electroporation is effective in enhancing transdermal delivery of tetracaine and its function is better than iontophoresis.

Keywords: Electroporation; Transdermal transport; Tetracaine; Iontophoresis

1. Introduction

Electroporation is a phenomenon in which the membranes of cells or lipid bilayers exposed to high intensity electric field pulses are temporarily destabilized and permeabilized. It is routinely used in cell biology and biotechnology to introduce large molecular weight compounds into cells (Weaver, 1993). In the 1990s, high voltage electric field pulses have been shown to rapidly increase transdermal drug delivery (Prausnitz et al., 1993, 1995; Vanbever et al., 1994, 1996, 1998; Riviere et al., 1995; Wang et al., 1998). Prausnitz et al. (1995) compared the transdermal delivery of heparin by electroporation, iontophoresis and passive diffusion. The flux of heparin with passive diffusion could not be detected, but that with electroporation reached $100 \sim 500\ \mu g \cdot cm^{-2} \cdot h^{-1}$. Iontophoresis could also increase the flux of heparin, but its function was weaker than electroporation. According to reports (Prausnitz et al., 1993; Riviere et al., 1995) the skin toxicity of electroporation is less than that of iontophoresis. Electroporation is a prospective method for en-

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hancing transdermal drug delivery. Here, we used tetracaine (TC; $M_w = 300.83$, -1 valence) as the model drug, using type OX4-1 electroporative pulse device, to investigate the influence of electroporation on drug transdermal delivery and compare it with iontophoresis.

2. Materials and methods

2.1. Materials

Tetracaine hydrochloride was purchased from Datong Pharmaceutical Factory of Shanxi Province, Datong, People's Republic of China. Donor solution was prepared by dissolving 0.04 g of tetracaine hydrochloride in 100 ml of 0.9% NaCl solution.

Type OX4-1 electroporative pulse device is a pulse generator made by us, which can produce square-wave pulses. The voltage can be adjusted between 70 and 130 V. The pulse frequency can be selected as 1, 2, 4, 8, 10, 20, or 40 pulses per min. The pulse time is adjustable (Bao et al., 1998).

2.2. Methods

2.2.1. Determination of TC in the samples

TC in the samples taken from the receiver compartment were determined by UV absorbance at 312 nm (which is the maximum absorbance wavelength of TC).

2.3. Diffusion experiments of TC

The in vitro transdermal experiments were performed using rat skin (male, with body weight of 200 g, obtained from the Experimental Animal Center of Zhejiang University, Hangzhou, People's Republic of China). The rats were sacrificed, the fur on the abdomen was shaved off, and full-thickness skin was excised. Subcutaneous fat was removed carefully. The excised skin was rinsed and hydrated in 0.9% NaCl solution for 12 h. After this procedure, the absorbance of skin extract at 312 nm could be ignored.

The skin was mounted between the two compartments of the side-by-side compartment diffusion cells (with effective diffusion area $A_e = 0.5$ cm^2, volume $V = 4.6$ ml) with stratum corneum facing the donor compartment. The donor compartment was filled with the donor solution and the receiver compartment was filled with 0.9% NaCl solution as the receiver solution. The donor and receiver compartments were continuously stirred magnetically and maintained at 32°C. After passive diffusion experiments for 5 h, Ag/AgCl and Ag electrodes were immersed in the solution (unless otherwise noted, the Ag/AgCl electrodes were in the donor solution and the Ag electrodes were in the receiver solution) and connected to the electroporative pulse device (square-wave pulse, voltage 130 V, pulse time 0.4 s, pulse frequency 40 pulses min^{-1}) for skin electroporation or to a constant current source (0.2 mA cm^{-2}, lasting for 4 h) for iontophoresis. Control experiments under similar conditions were carried out without the electric current. Samples (4.6 ml) were taken from the receiver compartment at regular intervals and replaced with an equal volume of drug free receiver solution. The donor solution was replaced with fresh donor solution at the same time. The tetracaine concentration in the samples was determined according to the above-mentioned method. The fluxes of TC were calculated.

3. Results

3.1. Comparison of TC transdermal delivery by electroporation and iontophoresis

The fluxes of TC with passive diffusion, electroporation (square-wave pulse, voltage 130 V, pulse time 0.4 s, pulse frequency 40 pulses min^{-1}, pulse number 400) or iontophoresis (0.2 mA cm^{-2}, lasting for 4 h) were compared. The results are shown in Fig. 1. Compared with passive diffusion, both electroporation and iontophoresis obviously increased the fluxes of TC. There was also a significant difference between electroporation and iontophoresis ($P < 0.01$, t-test, $n = 6$).The flux of TC at 0.25 h after electroporation was $J_e = 54.6 \pm 6.0$ $\mu g \cdot cm^{-2} \cdot h^{-1}$, which was six times

larger than that with passive diffusion ($J_p = 8.2 \pm 0.5$ μg · cm^{-2} · h^{-1}) and twice as large as that with iontophoresis ($J_i = 17.4 \pm 5.8$ μg · cm^{-2} · h^{-1}).

3.2. The influence of pulse number on the fluxes of TC

Fig. 2 shows that when the voltage (130 V), pulse frequency (40 pulses min^{-1}) and pulse time (0.4 s) were kept constant, the fluxes of TC increased with increasing pulse number (25, 50, 100, 200, 400). But the relationship between the fluxes of TC and pulse number was not linear. When pulse number was 50 ~ 400, the fluxes of TC at 0.25 h after electroporation were not obviously different ($P > 0.05$, t-test, $n = 6$), but the fluxes of TC at 0.75 h after electroporation increased with increasing pulse number ($P < 0.05$, t-test, $n = 6$). Additionally, the flux of TC with electroporation tended to that of passive diffusion with increasing time.

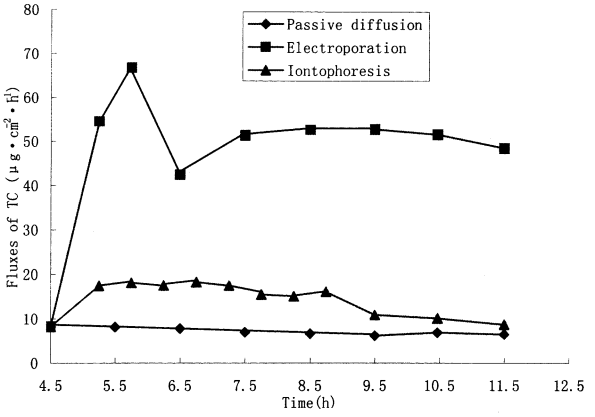

Fig. 1. Influence of electroporation (square wave, 130 V, 40 pulses min^{-1}, 400 pulses) or iontophoresis (0.2 mA · cm^{-2}, 4 h) on the fluxes of tetracaine through rat abdominal skin in vitro, using side-by-side compartment diffusion cells method.

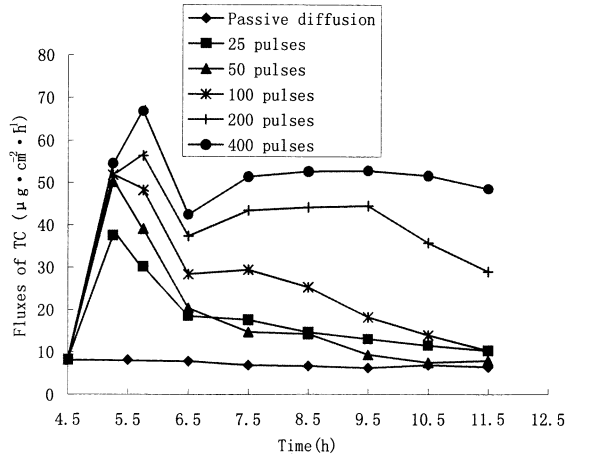

Fig. 2. Influence of pulse number on the fluxes of tetracaine through rat abdominal skin in vitro, using side-by-side compartment diffusion cells method. Donor solution: 0.04% tetracaine in 0.9% NaCl solution. Receiver solution: 0.9% NaCl solution.

4. Discussion

The voltage used here was 130 V. The transdermal voltage was 88 V. It was not high. However, this electroporative pulse (square-wave pulse, voltage 130 V, pulse frequency 40 pulses min^{-1}, pulse time 0.4 s, pulse number 25 ~ 400) could enhance permeation of TC through skin. These results showed that electroporation could make TC, a kind of ionic drug with low molecule weight, transport quickly through the skin. Vanbever et al. (1996) also reported that the enhancing function of exponentially-decaying pulse was better than that of square-wave pulse. On this basis, we made an exponentially-decaying electroporative pulse device, which will be used in our next investigation.

The result that the fluxes of TC with electroporation tended to the level of passive diffusion supported the idea that the skin deformed by the electroporation used here could be repaired. It would not injure the skin. This is accordance with the result reported by Prausnitz et al. (1993).

Both electroporation and iontophoresis could enhance the transdermal delivery of TC. The exposure time of skin to the electric field in iontophoresis (4 h) was longer than that of electroporation (lasting 10 min for 400 pulses, the total pulse time being 2.7 min). So the skin irritation caused by electroporation was far smaller than that caused by iontophoresis. Electroporation is an effective and safe method for enhancing drug transport through skin.

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