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Low-frequency sonophoresis enhances rivastigmine permeation *in vitro* and *in vivo*

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We investigated the enhancement effect of low-frequency sonophoresis on transdermal permeation of rivastigmine *in vitro* and *in vivo*. The *in vitro* permeation study showed that sonophoresis increased steady-state transdermal flux $0.31 \pm 0.03\,\mu g \cdot cm^{-2} \cdot h^{-1}$ and the extent of rivastigmine permeation $6.00 \pm 0.56\,\mu g \cdot cm^{-2}$ though excised skin (both $P < 0.01$). In the *in vivo* experiment, the C_{max} $0.83 \pm 0.16\,\mu g \cdot mL^{-1}$ and $AUC_{0 \to 24\ h}$ $12.35 \pm 1.99\,\mu g \cdot h \cdot mL^{-1}$ of the sonophoresis group was also significantly higher than that of the control group (both $P < 0.01$). These data suggest that low-frequency sonophoresis could be an effective method to enhance rivastigmine permeation.

1. Introduction

Rivastigmine (Riv) is an inhibitor of acetylcholine esterase used for the treatment of mild-to-moderate Alzheimer's disease (AD) (Jann 2000). Riv has been shown to improve or maintain cognitive function, daily living activities and behavior, as well as to decrease overall symptoms of dementia in AD patients (Cumbo and Ligori 2014). Oral administration of Riv is associated with adverse events in the gastrointestinal system: nausea, vomiting and diarrhea (Greenspoon et al. 2011). A transdermal drug-delivery system for Riv may offer tolerability, convenience and therapeutic advantages for AD patients (Boada and Arranz 2013; Seibert et al. 2012). There are Riv transdermal patches available on the market, such as Exelon®. But passive permeation of drugs is always limited by the barrier function of the skin (Wong and Nor 2013). Skin irritation and adhesion loss also occur during the application of Riv patches (Greenspoon et al. 2011; Pregelj 2012). Thus, a novel transdermal system improving permeation and tolerance should be developed. Low-frequency ultrasound has been reported to enhance transdermal absorption of several drugs (Han and Das 2013; Herwadkar et al. 2012; Liao et al. 2014). We investigated the effect of low-frequency sonophoresis on transdermal permeation of Riv.

2. Investigations and results

Penetration profiles of Riv treated by low-frequency sonophoresis or not treated by low-frequency sonophoresis are shown in Fig. 1(A). A. Sonophoresis treatment significantly enhanced Riv permeation through the back skin of suckling pigs. Transdermal parameters are shown in the Table . The steady-state transdermal flux and extent of Riv permeation though excised skin 24 h after sonophoresis treatment was 3.10- and 2.99-times higher than that of the control. These observations suggest that low-frequency sonophoresis enhances Riv permeation significantly *in vitro*. Acoustic cavitation has been identified as primary mechanism of action for sonophoresis mediated enhancement in transdermal delivery (Polat et al. 2011). The effect of ultrasound on skin is heterogeneous thereby creating localized transport pathways (Tezel et al. 2002). Riv permeation could be enhanced through these aqueous porous pathways like other drugs (Polat et al. 2010).

Table: Permeation parameters of rivastigmine through excised pig skin (n=3)

Group	Jss/$\mu g \cdot cm^{-2} \cdot h^{-1}$	ER	Q24h/ $\mu g \cdot cm^{-2}$
Control	0.10±0.02	1.00	2.01±0.35
Sonophoresis	0.31±0.03	3.10	6.00±0.56

Jss, steady state flux; ER, enhancement ratio; Q24h, accumulative amount of permeation by 24 h.

Plasma concentration–time curves after administration of Riv containing the coupling medium (the liquid present between the ultrasound transducer and the skin) are shown in Fig. 1(B). The C_{max} of the sonophoresis group and control group was 0.83 ± 0.16 and $0.28 \pm 0.07\,\mu g \cdot mL^{-1}$, respectively. The $AUC_{0 \to 24\ h}$ of the sonophoresis group and control group was 12.35 ± 1.99 and $4.14 \pm 1.12\,\mu g \cdot h \cdot mL^{-1}$, respectively. The relative bioavailability of the sonophoresis group *versus* the control group was 298%. Sonophoresis was shown to dramatically improve the transdermal absorption of Riv *in vivo*. A good correlation was found between *in vivo* and *in vitro* results like previous results reported by other scientists (Tang et al. 2002), which indicated that the *in vitro* pig skin model can be utilized to predict the *in vivo* transdermal absorption.

3. Experimental

3.1. Materials

Riv containing a coupling medium was obtained by adding 0.216 g rivastigmine tartrate to 10 mL coupling medium containing 2.5% sodium carboxymethyl cellulose and 30% glycerol and stirring uniformly.

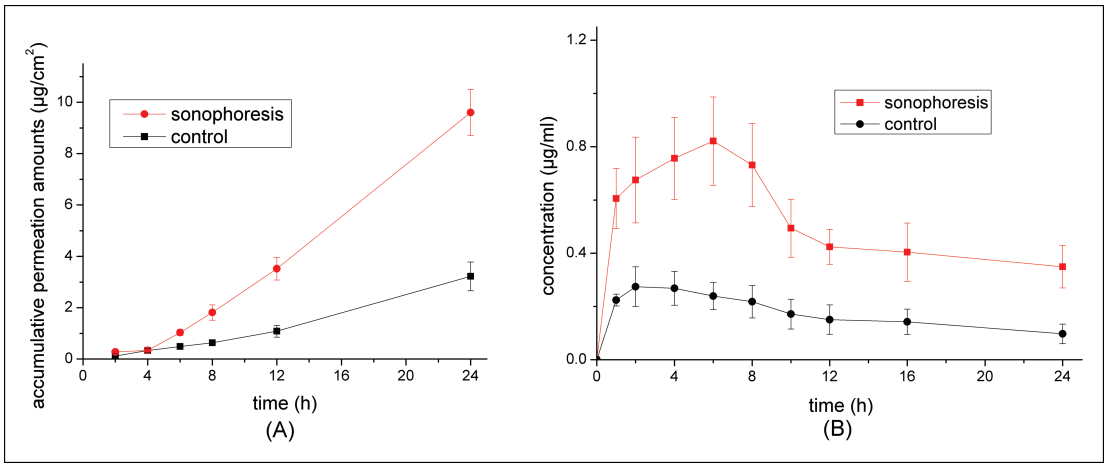

Fig. 1: Transdermal profile (A) of rivastigmine permeated though excised pig skin (n=3) and plasma concentration-time curves (B) following the administration of Rivastigmine containing couple media (n=4).

3.2. *Permeation study*

The *in vitro* permeation study was carried out vertical diffusion cells (diffusion area, 2.83 cm^2; volume of receptor chamber, 6.8 mL). Samples of excised skin from the backs of 9–10-day-old pigs were mounted on diffusion cells such that the epidermal surface faced the donor chamber. Donor compartments were placed on the skin surface. The assembly of diffusion cells was held together with the help of clamps. Saline containing 20% polyethylene glycol was used as the receptor medium. The coupling medium was added to the donor chamber. For the sonophoresis group, an ultrasound probe generating 20-kHz ultrasound working at 4 W treated the skin for 2.5 min. The receptor medium was sampled at predetermined times up to 24 h, with replenishment by equal volumes of fresh medium. The drug concentration in each sample was determined by high-performance liquid chromatography (HPLC).

3.3. *Animal studies*

The *in vivo* study was undertaken with eight male Sprague-Dawley rats (∼200 g). One day before each experiment, skin on the abdomen was shaved without damaging it. A square with a side-length of 3 cm was marked on the skin. The coupling medium was applied uniformly in the square. For the sonophoresis group, the ultrasound probe was allowed to treat the skin for 10 min. Blood samples were collected at predetermined time intervals using heparin as an anticoagulant. Drug concentration in blood samples was determined by a HPLC–UV method reported previously (Yu et al. 2013). Pharmacokinetic parameters were calculated using Thermo Kinetica® v4.4.1 (Thermo Electron, Philadelphia, PA, USA).
All the procedures and care administered to the animals had been approved by Experimental Animal Use Committee of Zhejiang University, and the animal experiments were conducted in full compliance with the Experimental Animal Regulations by the National Science and Technology Commission, China.

3.4. *Statistics*

Data are the mean ± SD. The significance of variation between different groups was determined by the Student's *t*-test using MINITAB v14.1 (Minitab, State College, PA, USA). $P < 0.05$ was considered significant.

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