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*ORIGINAL RESEARCH*

# Microdialysis sampling coupled to HPLC for transdermal delivery study of ondansetron hydrochloride in rats

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ABSTRACT: The transdermal delivery of ondansetron hydrochloride (ON) solution in propylene glycol (PG) with a widely used penetration enhancer, oleic acid (OA), was studied in rats by a microdialysis sampling technique. Dialysate samples collected from the probe were directly injected into the HPLC system without any pre-treatment and no interference occural in the blank sample. A good linearity between the standard concentrations and peak areas within the calibration range was achieved. *In vivo* recovery ($32.52 \pm 1.8\%$) of the probe was assessed with the retrodialysis method, which was used to calculate the ON concentration in the dermis. Oleic acid at the concentrations of 2% and 5% (w/v) increased the steady-state delivery rate from 0.001 to 0.030 and 0.058 μg/h, respectively. OA proved to be an effective enhancer for transdermal delivery of ON in rats. Copyright © 2000 John Wiley & Sons, Ltd.

## INTRODUCTION

Microdialysis, an *in vivo* sampling technique, was established as a method in experimental psychopharmacology and neuropathology at the beginning of 1980s (Ker, 1996). In the past 8 years, the technique has been applied to dermatological studies in humans or animals, in a form called cutaneous microdialysis. It is technically possible to study transdermal or dermal drug delivery *in vivo* with microdialysis (Joseph *et al.*, 1992, 1994; Kenji *et al.*, 1994a, Lutz *et al.*, 1995; Muller *et al.*, 1995; Krogstad *et al.*, 1996; Groth, 1996).

Most studies of transdermal drug delivery are performed *in vitro* by the use of a two-compartment diffusion cell with excised animal or human skin. The data obtained *in vitro*, however, poorly correlates with the actual behavior of the drug *in vivo*. A method for sampling in the dermis *in vivo* would provide more information on the process. Recently, the study of *in vivo* transdermal drug delivery has become a matter of great concern. In this paper, we use microdialysis as a sampling method to study the transdermal delivery of ondansetron hydrochloride (ON) in rats, which is a novel and specific antagonist of serotonin (5-HT) at the 5-$HT_3$ receptor and is used successfully in the prevention and treatment of chemotherapy-induced vomiting in cancer patients (Markham and Sorkin, 1993). HPLC methods have been developed for the analysis of ON in pharmaceuticals and plasma (Brewster *et al.*, 1988; Colthup *et al.*, 1991 Bosso *et al.*, 1992). A modified HPLC method is reported in this paper to determine ON in dialysate samples.

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**Abbreviations used:** 5-HT, serotonin; OA, oleic acid; ON, ordansetron hydrochloride; PG, pizpylene glycol.

## MATERIALS AND METHODS

**Materials.** ON was provided by the Division of Pharmacochemistry of Shenyang Pharmaceutical University (purity >99.0%). Water for normal saline preparation was triple distilled. Acetonitrile was of HPLC grade and was obtained from Shandong Yuwang Co. Ltd (Shandong, China). All other chemical were of analytical grade.

**Microdialysis System.** The microdialysis system consisted of a model KH-1 microsyringe pump with a 1.0 mL glass syringe and a concentric microdialysis probe (10 mm membrane length, 18,000 Da Mw cut-off), which was provided by the Chemistry Institute of Academy Sinica (Beijing, China). The probe was perfused continuously with normal saline or drug solution in normal saline at a rate of 1.0 μL/min in all experiments. After use, the probe was rinsed and perfused with triple distilled water for at least 1 h before storage in 50% aqueous glycerol solution (containing 5% formaldehyde).

Probe characterization methods were described in detail in our other paper. Briefly, the probe implanted in excised rat skin mounted on a Franz cell was used to determine recovery and delivery *in vitro*. Recovery *in vivo* was evaluated by the retrodialysis method based on the probe characterization *in vitro*. Linearity between the perfusate concentration ($C_p$) and the net increase ($C_d - C_p$) of ON concentration in the dialysate ($C_d$) was established, and the slope of the line corresponded to the recovery

($R$). The ON concentration in the dermis were calculated according to the following formula: $C_{dermis} = C_d \cdot R^{-1} \times 100$.

**Animal Experiments.** Male rats, weighing 220–300 g, were anesthetized with urethane (1.3 g/kg, as 20% w/v solution, i.p.) and anesthesia was continued throughout the whole experiment. An Oster clipper (Oster model 5-01, USA) was used to shave the abdominal fur of rats.

The microdialysis probe was inserted into the skin through a stainless steel introducer (i.d. 0.50 mm). Following the probe introducer insertion *in vivo*, a semicircular glass cell was put over the abdomen of rat, glued with 502-glue (cyan-acrylate based glue, Shandong Yuwang Co. Ltd). Then the microdialysis probe was inserted and the introducer was pulled back 1 cm to expose the active dialysis window, which was centered beneath the glass cell. PE tubing was used to connect the implanted probe and the syringe. After the probe implantation, the skin was inspected visually for any puncture.

Before the drug solution was added to the cell, the pump had worked for 1.5 h to wash out, and to provide time for the skin trauma to recover (Groth, 1996). Then 1.5 mL of ON solution (0.15%, w/v), composed of 5% or 3% oleic acid in PG, was applied to the cell. The drug solution in PG without oleic acid was used as the control. The microdialysis samples were collected into mini-tubes at 1 h intervals for 8 h. The surrounding temperature was maintained at 37–39°C.

**Analysis of ON.** The HPLC system used for analysis of ON in dialysate consisted of two Model LC-10A pumps, a C-R6A integrator, a CTO-10A column oven and an SPD-10A spectrophotometric detector (Shimadzu, Japan). The detector was operated at 305 nm. The mobile phase was acetonitrile:buffer solution (0.025 mol/L acetate, pH 3.6, 35:65). The flow rate was 0.6 mL/min. An Alltima $C_{18}$ column (5 μm 200 × 4.6 mm) was used at 35°C. The dialysate (10 μL) was injected into the chromatographic system without any pre-treatment. Samples collected from the probe were stored at −20°C until analyzed.

## RESULTS AND DISCUSSION

The cutaneous microdialysis samples were directly injected into the HPLC chromatograph or diluted with normal saline before being injected, depending on the drug concentration in the dialysate. A typical chromatogram of the cutaneous microdialysis sample in rats obtained by the HPLC method under the chromatographic condition mentioned above is shown in Fig. 1. As can be seen, no interference occurs in the blank sample obtained prior to dosing the rats with ON solution. The microdialysis sampling technique provides several advantages over the conventional technique (Lunte *et al.*, 1991), including a means of continuous sampling with no tissue fluid loss and minimal tissue damage. Samples can often be analyzed by HPLC without 'cleanup', and drugs can be separated from enzymes that might catalyze their degradation. With microdialysis, a single animal can be used for the continuous real-time study of drug flux through the skin, compared with the numerous animals required for each time point, so the number of animals required to perform dermal and transdermal drug delivery research may be reduced significantly. Microdialysis coupled to HPLC is a powerful technique to assay the transdermal delivery rate of ON in rats. The linearity was determined in the concentration range of 0.02–1.00 μg/mL with six standards.

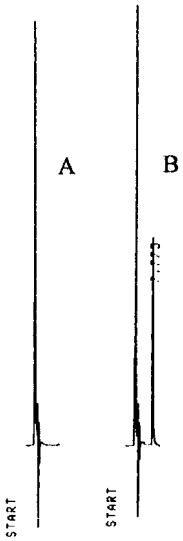


**Figure 1.** Typical HPLC chromatograms of the cutaneous microdialysis samples: (A) blank; (B) sample after drug administration.

A linear relationship was found between the standard concentrations ($C$) and peak areas ($Y$), with a regression equation $Y = 36.099C - 253.090$ ($r = 0.9998$, RSD < 5%).

The in vivo recovery value measured by the retrodialysis method in rats was 32.52% ($n = 3$, SD = 1.8%). The dermal concentration of ON was calculated by the recovery. An important methodological aspect of microdialysis concerns the relationship between 'true' tissue concentration and dialysate (Muller *et al.*, 1995); ie the relationship between the concentration of ON in the dermis and the dialysate.

Based on the probe characterization *in vitro* and *in vivo*, the effect of a widely used penetration enhancer, oleic acid, on the *in vivo* transdermal absorption of ON in rats was examined. Time–concentration profiles of ON in the dialysate and dermis in rats are depicted in Fig. 2 and Fig. 3, respectively. The ON concentration in both dialysate and dermis reached a plateau at about 1.5 h by co-application of 5% OA in the solution of ON in PG, and at about 3.5 h by co-application of 2% OA. The plateau ON concentration in case of 5% OA was about twice that of 2% OA.

In Fig. 4, the accumulative amount of ON in dialysate is plotted against time. The slope of the linear portion of the profile is the transdermal delivery rate and the intercept on the time axis extrapolated from the linear

 

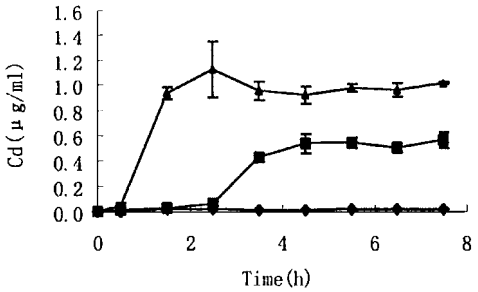


**Figure 2.** Ondansetron hydrochloride concentration–time profiles in the dialysate with or without oleic acid ($n = 3$). (◆) Control; (■) 2%OA; (▲) 5%OA.

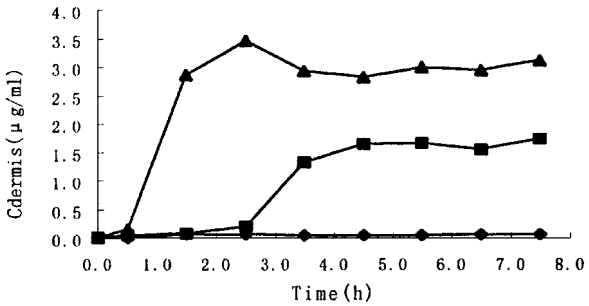


**Figure 3.** Ondansetron hydrochloride concentration–time profiles in the dermis with or without oleic acid ($n = 3$). (◆) Control; (■) 2%OA; (▲) 5%OA.

portion is the lag time. Using 2% and 5% OA resulted in a delivery rate of 0.030 and 0.058 μg/h, respectively, which were significantly different from that of the control (0.001 μg/h), and a lag time of 2.5 and 0.4 h, respectively. The data indicated that OA is an effective transdermal delivery enhancer for ON and its effect is of some concentration dependence.

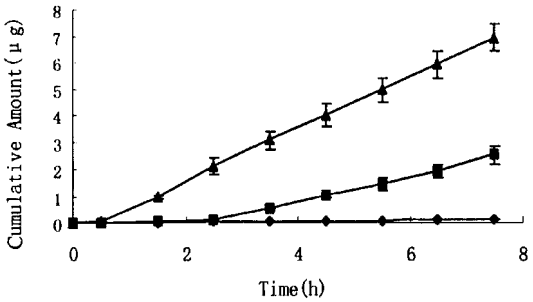


**Figure 4.** Plots of ondansetron hydrochloride cumulative amount in dialysate vs time. The slope of the linear portion is the transdermal delivery rate and the intercept on the time axis is the lag time. (◆) Control; (■) 2%OA; (▲) 5%OA.

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