Evaluation of transdermal permeability of pentoxifylline gel: *in vitro* skin permeation and *in vivo* microdialysis using Wistar rats

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ABSTRACT: The aim of the present work was to evaluate the transdermal permeability of pentoxifylline gel *in vitro* and *in vivo*. Gel was prepared with carbomer 934 as the base, and the Wistar rat was chosen as an animal model. The effects of percutaneous enhancers on the transdermal permeability of pentoxifylline gel were investigated by *in vitro* permeation experiments. Cumulative permeation at different times was determined by HPLC. 3% Azone and 5% propylene glycol were used as collaborative enhancers in an optimal formulation. Topical concentrations at different times were measured by microdialysis *in vivo*. The transdermal process of pentoxifylline fits to a zero-order kinetic equation, and its release profile remains of the zero-order despite the addition of enhancers. In addition, a good *in-vitro-in-vivo* correlation was achieved.

Key Words: Pentoxifylline, gel, transdermal, enhancer, microdialysis, *in vitro*/*in vivo* correlation

1. Introduction

Pentoxifylline (PTX), a derivate of xanthine, is a nonselective phosphodiesterase inhibitor that is commonly used for treatment of symptomatic vascular insufficiency because of its hemorrheological activity (*1*). It can restore erythrocyte deformability, decrease blood viscosity, and prevent thrombocyte adherence and aggregation, which would improve the blood circulation of the brain and the limbs and increase the volume of blood flow in arteries and capillaries. Thus, PTX is used widely in renal transplant recipients (*2*), for the cure of venous leg ulcers (*3*), and for many others diseases related to infection and tumors (*4,5*). However, the manifest first-pass effect results in a low bioavailability of 20% and its half-life is 0.4-0.8 h. Furthermore, oral administration and intravenous injection will cause adverse effects to some extent, including the most common ones of sicchasia, dizziness, headaches, anorexia (*6*). Therefore, PTX is better suited to external use for tropical disease.

The Transdermal Drug Delivery System is a promising method of drug administration that can avoid the variability in rates of absorption and metabolism encountered in oral treatment and that offers several advantages over conventional dosage forms such as tablets and injections, including elimination of first-pass metabolism, minimization of pain and some adverse effects, and possible controlled release of drugs (*7,8*). Additionally, gels are gaining greater popularity with regard to the Transdermal Drug Delivery System due to their bioadhesive properties and biocompatibility (*9*). Based on these considerations, PTX was prepared as a gel for transdermal delivery and the effects of various enhancers, which may increase the diffusion coefficient of the drug into the stratum corneum or improve partitioning between the formulation and the stratum corneum, were studied.

Microdialysis is a semi-invasive, focal sampling method based on the use of probes with a semi-permeable membrane at the probe tip and is a relatively new and effective technology for the assessment of drug distribution and target tissue pharmacokinetics (*10*). Microdialysis has gained more importance due to the fact that tissue concentrations are usually more predictive of clinical outcome than plasma concentrations. In cutaneous microdialysis, a probe is inserted superficially into the dermis, parallel to the skin surface, with or without topical anesthesia at the site of entry. The principle of microdialysis is that a physiological solution pumped through the probe is in equilibrium with the diffusible molecules in the immediate surroundings. This principle can also be used either to remove or to deliver substances to the tissue since the direction of the flux is dependent on the concentration gradient. In this paper, microdialysis was

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utilized *in vivo* experiment.

2. Materials and Methods

2.1 *Materials*

Materials used were carbopol 934 (BF Goodrich, USA), PTX (Shijiazhuang Pharma Group Pharmaceutical Co., China), acetonitrile (Shandong Yuwang Chemical Co., China), propylene glycol (Guangzhou Jietu Chemical Co., China), oleic acid (Dandong Julong Chemical Co., China), glycerin (Anji Haosen Pharmaceutical Co., China), and azone (Shanxi Ruicheng Fine Chemicals, China).

2.2 *Animals*

Male Wistar rats weighing approximately 170-220 g were provided by the Animal Experimental Center of Shenyang Pharmaceutical University. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no. 92-93, revised in 1985) and were approved by the Department of Laboratory Animal Research at Shenyang Pharmaceutical University. Procedures with animals were reviewed and approved by the Animal Ethical Committee at Shenyang Pharmaceutical University.

2.3 *Preparation of PTX gel*

As a base, carbopol 934 was slowly dispersed into distilled water and allowed to swell for 12 h under normal temperature; PTX (200 mg) and enhancer dissolved in 50% glyceryl alcohol solution (8 g) were added to the base while stirring. The carbopol gel was thickened by a few drops of Tris added to adjust the carbopol gel to pH 7. Distilled water was added to the gel for a final weight of 20 g (*11-13*).

2.4 *HPLC determination of PTX*

Drug analysis was performed according to the reverse phase HPLC method previously reported (*14,15*). The optimized mobile phase consisted of a combination of acetonitrile: water (28:72, v/v). The UV detector wavelength was set at 274 nm, the volume of injection was 20 μL, flow rate was 1.0 mL/min, and the temperature of column was 35°C. Under these conditions, the retention time of the PTX peak was found to be 5.1 min.

2.5 *In vitro permeability studies*

2.5.1 *Skin preparation*

Abdominal skin of male Wistar rats, 250 ± 20 g, was used for the permeation studies. The rat was sacrificed with ether and the hair of abdomen was carefully removed using an electric clipper. Full-thickness skin samples were cut, removed, and washed with normal saline. Adhering fat and connective tissues were carefully removed with a blunt-ended forceps. Skin was observed for any damage (*11,16*).

2.5.2 *In vitro skin permeation studies*

Full-thickness skin was mounted on Franz diffusion cells (vertical; available diffusion area, 2.54 cm^2; volume of receiver cell, 13 mL) with a water jacket (32 ± 1°C) to assess skin permeability. The stratum corneum side was facing upward into the donor compartment, and the dermal side was facing downward into the receptor compartment. The receiver cells were filled with distilled water and stirred by a magnetic bar to ensure adequate mixing and maintenance of sink conditions. After the experiment began, all of the solutions were sampled at 1, 2, 4, 6, 8, and 10 h, filtered with micropore film (pore diameter, 0.45 μm), and an equal volume of blank solution was immediately added. Each data point represents the average of five examinations (*17*).

The types of enhancers in gel (1% PTX) were: 1) blank; 2) 1% Azone; 3) 3% Azone; 4) 5% Azone; 5) 5% Oleic acid; 6) 3% Azone + 5% propylene glycol; and 7) 3% Azone + 5% oleic acid.

2.5.3 *Statistical analysis*

The permeation of PTX in gel with different enhancers assayed for 10 h was investigated and plots of the cumulative amount of permeated PTX (μg/cm^2) were plotted versus time. The transdermal flux (J, μg/cm^2/h) was calculated from the steady-state part of the curve and T_{lag} by extrapolation of the linear portion to the x-axis. The effectiveness of penetration enhancers was determined by comparing the flux of PTX in the presence and absence of the enhancer. This was defined as the enhancement factor (EF), which was calculated using the following equation: EF = (drug flux of samples containing an enhancer)/(drug flux of control sample without an enhancer). Data on the cumulative amount of permeation were subjected to a *t*-test at a significance level of $P < 0.01$ (*17,18*).

2.6 *In vivo microdialysis permeability studies*

Microdialysis is based on sampling of analytes from the extracellular space by means of a microdialysis probe that is made of a semipermeable membrane. A microinjection pump is used to deliver normal sodium at a flow rate of 1 μL/min as perfusate to the probe. Once the probe is implanted under the dermis, substances present in the extracellular fluid at concentration (C_{out}) are filtered by diffusion out of

the extracellular fluid into the probe, resulting in a concentration (C_{in}) in the perfusion medium. Samples are collected and determined. For most analytes, equilibrium between extracellular tissue fluid and the perfusion medium is incomplete; therefore: $C_{out} > C_{in}$. The factor by which the concentrations are interrelated is termed recovery (*19*).

2.6.1 *In vivo microdialysis*

2.6.1.1 *Implantation of the probe in vivo*

Male rats were anesthetized with urethane (1.2 g/kg, i.p.). The abdominal fur of rats was shaved. A needle was then inserted into the skin to channel away the probe. After placing the probe under the skin, the needle was withdrawn. The length of the active dialysis window was adjusted to be 1 cm. Environment temperature was kept at 37 ± 1°C using an infrared lamp (*20*).

2.6.1.2 *Permeability of PTX in vivo*

After probe implantation, a hemispherical glass reservoir with the available diffusion area of 2.54 cm^2 was adhered to the abdomen of the rat to be located on the skin above the probe. Two g of gel were added to the reservoir. Primarily, the pump worked for 1 h for washout with normal saline at an optimized flow rate of 1 μL/min. After the gel was added to the reservoir, the dialyzates were collected for 10 h at 1-hour intervals.

2.6.2 *In vitro recovery of PTX*

In order to characterize *in vitro* the influence of drug concentration in the surrounding medium on the transfer rate of the drug across the dialysis membrane, the concentration difference method was used to measure the recovery of the probe (*21*). A custom-made beaker was filled with a 10.0 μg/mL solution of PTX in normal saline, in which a stirrer was rotating. The active dialysis window was immersed in the solution; its length was 1 cm. A series of concentrations (2.0, 5.0, 10.0, 20.0, and 40.0 μg/mL) of PTX in normal saline was used as the perfusate. After each change to a different concentration perfusate, there was a half-hour washout period with the same perfusate, and then the sample was collected for an hour using mini-tubes; accordingly, the volume of the sample was 60 μL.

2.6.3 *In vivo recovery of PTX*

In vivo recovery was assessed according to the retrodialysis method (*22*). The principle of this method relies on the assumption that the diffusion process is quantitatively equal in both directions through the semipermeable membrane, which was verified by the *in vitro* experiment. Therefore, PTX solutions can serve as the perfusate and the elimination coefficient through the probe can serve as the *in vivo* recovery. In the experiment *in vivo*, after the probe was implanted blank normal saline was passed through the probe using an infusion pump as the perfusate to washout the probe for one hour. Afterward, a series of concentrations (2.0, 5.0, 10.0, 20.0, 40.0 μg/mL) of PTX in normal saline was pumped in as the perfusate at a flow rate of 1 μL/min. The dialyzate of each concentration was measured with HPLC. After each change to a different concentration perfusate, there was a half-hour washout period with normal sodium.

3. Results

3.1 *Effect of penetration enhancers*

Data for the *in vitro* permeation of PTX in gel through rat abdominal skin over 10 h are summarized in Figure 1, and J, T_{lag}, and EF are shown in Table 1.

For transdermal delivery, overcoming the barrier of the skin is a crucial step. The use of penetration enhancers offers a simple and convenient method of improving transdermal bioavailability. Several chemical penetration enhancers were examined in order to enhance the permeation of PTX from a carbopol gel base.

The effects of azone and oleic acid were investigated; among the types of enhancers (single enhancers or coalescent enhancers), a coalescent enhancer that consisted of 3% azone and 5% propylene glycol had the most conspicuous effect ($P < 0.01$). Furthermore, it produced a uniform gel.

3.2 *Microdialysis experiments*

Table 1. Effects of penetration enhancers of PTX

	Q (μg/cm^2)	*r*	J (μg/cm^2· h)	T_{lag} (h)	EF
(1)	0.6938t + 0.496	0.9947	0.8010 ± 0.6044	2.143 ± 0.4357	-
(2)	5.682t - 5.236	0.9957	6.755 ± 2.507	2.134 ± 0.5104	8.433
(3)	8.979t - 7.084	0.9995	9.123 ± 3.212	1.852 ± 0.7123	11.39
(4)	6.419t - 2.268	0.9997	6.767 ± 1.514	1.034 ± 0.2586	8.448
(5)	10.64t - 9.377	0.9946	12.79 ± 1.719	2.148 ± 0.3523	15.97
(6)	23.02t - 12.23	0.9995	24.16 ± 11.94	1.409 ± 0.3227	30.16
(7)	11.71t - 7.167	0.9965	15.16 ± 5.228	2.523 ± 0.2867	18.93

Each point and bar represents the mean ± SD of four or five determinations.

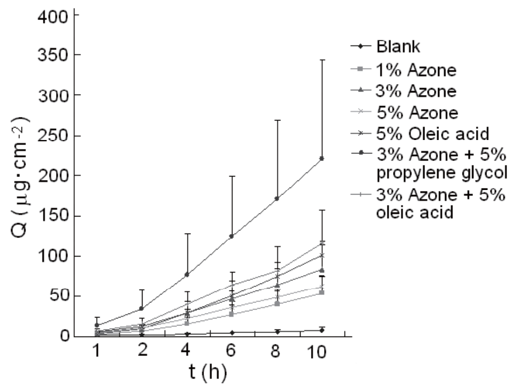

Figure 1. Effects of penetration enhancers of PTX. Each point and bar represents the mean ± SD of four or five.

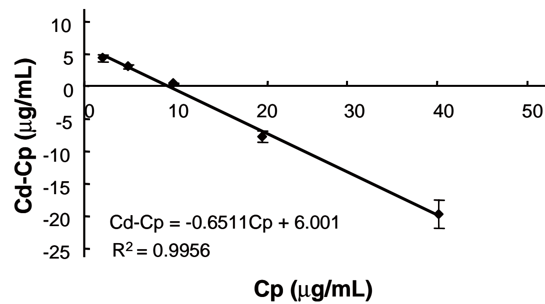

Figure 2. The concentration difference method to estimate *in vitro* recovery of PTX from the microdialysis probe (*n* = 3).

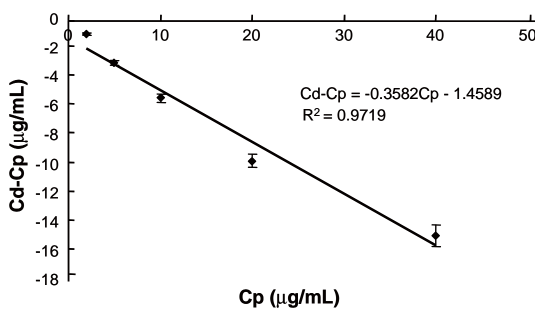

Figure 3. *In vivo* characterization of the probe (*n* = 3). The slope of the line is the recovery.

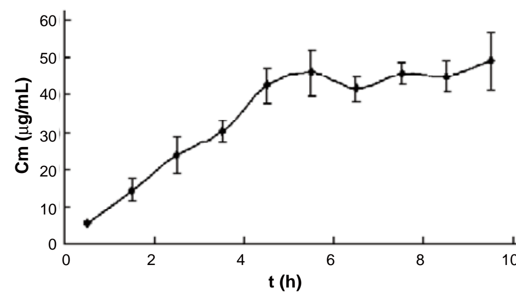

Figure 4. PTX concentration-time profiles under the dermis in rats (*n* = 3).

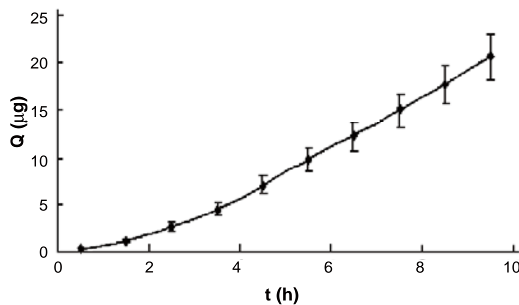

Figure 5. Plots of PTX cumulative amount versus time. The slope of the linear portion is the steady-state flux and the intercept on the time axis is the lag time in rats (*n* = 3).

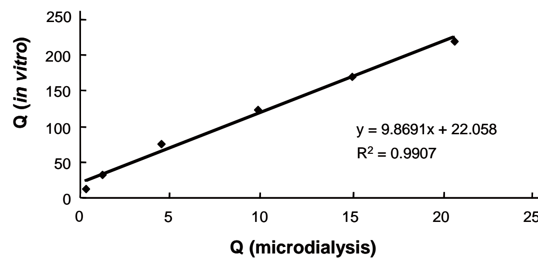

Figure 6. IVIVC model linear regression plots of Q (*in vitro*) *vs*. Q (microdialysis) for PTX.

3.2.1 *In vitro recovery experiments*

Recovery *in vitro* as measured by the concentration difference method is shown in Figure 2. There was a linear correlation for PTX between the difference in concentration in the dialysate and in perfusate (C_d-C_P) and perfusate concentration (C_P) over a wide concentration range *in vitro*. The intersection of the line and horizontal axis showed that the concentration in the surrounding medium was 9.215 μg/mL, which was essentially equal to the assigned concentration (10 μg/mL) (*23*). Accordingly, drug recovery from the tissue to perfusate was verified to be the same as drug loss from perfusate to tissue across the probe membrane in the stated concentration range (*21*). The recovery of PTX from the microdialysis probe was 65.11 ± 0.9%.

3.2.2 *In vivo recovery experiments*

The recovery *in vivo* was measured in rat subcutaneous adipose tissue with the retrodialysis method. Figure 3 shows a linear relationship between (C_d-C_P) and C_P; according to the equation, the recovery *in vivo* was 35.82 ± 1.9% (*n* = 3). The recovery was used to calculate the dermal PTX concentration in the study (*24*).

3.2.3 *PTX experiments*

Concentration profiles of PTX under the dermis versus time are depicted in Figure 4. The concentration under the dermis reached a plateau at about 6 h, which was in the range of 40-50 μg/mL. The cumulative amount of PTX under the dermis is plotted versus time in Figure 5. The slope of the linear portion of the profile is the transdermal delivery rate (2.724 μg/h) and the intercept on the time axis extrapolated from the linear portion is T_{lag} (1.96 h).

3.2.4 *IVIVC*

The relationship between the cumulative amount *in vitro* and *in vivo* was examined. Linear regression analysis was applied to the IVIVC plots. The values of the correlation coefficient (R^2), slope, and intercept were calculated and are given in Figure 6.

4. Discussion

Because of its hydrophilic characteristics, PTX has difficultly permeating the corneum of the skin. During the *in vitro* experiment, a low permeation of PTX resulted without enhancers. To increase permeation, several kinds of enhancers that would affect the corneum of the skin by changing the construction of the lipidic bilayer or by increasing the rheokinesis of lipoids were added to the gel. Accordingly, the transdermal permeability of PTX increased. The results of the experiment indicated that the transdermal permeability of PTX can be improved by various kinds of penetration enhancers; the mixture of azone (3%) and propylene glycol (5%) had the best improvement (P < 0.01) since propylene glycol increased the dissolubility of azone. However, a zero-order kinetic mechanism that was fitted to the transdermal process of PTX was not found to be affected by the addition of penetration enhancers.

In order to investigate the *in vivo* transdermal process of PTX in gel, the sample was microdialyzed. The samples obtained by microdialysis did not contain biomacromolecules, which allowed them to be injected directly into HPLC. The sampling and determination of liber drugs proceeded continuously. Therefore, the variation of the drug concentration was recorded during the whole process in this study.

Results of this study showed that dermal drug concentration can be calculated from the drug concentration in dialysate as a result of recovery, and this is easily assessed by probe characterization *in vitro* and *in vivo*.

Theoretically, the principle of the retrodialysis method is that the probe recovery is equal to the delivery rate for the same drug. However, this must be proven or demonstrated by experiments, for in fact the probe recovery is not always equal to the delivery rate for many drugs (*25*). Thus, use of the retrodialysis method to determine recovery is incorrect. Here, probe recovery was investigated by the *in vitro* experiment. Regarding the linear relationship between (C_d-C_P) and C_P as shown in Figure 2, the portion of the straight line above the horizontal axis is recovery, and the portion of the straight line under the horizontal axis is the delivery rate. The linear relationship indicates that the recovery is equal to the delivery rate for PTX. It also means that there is no interreaction between the drug and the dialyser in normal sodium and that the diffusions from both the obverse and reverse are equal. Furthermore, the concentration of PTX in the surrounding medium has no effect on recovery.

5. Conclusion

PTX gel was prepared by using carbopol 934 as a base and at the same time using 3% azone and 5% propylene glycol as collaborative enhancers. PTX permeates the skin by passive diffusion. Transdermal flux was augmented by the chosen enhancers, but a zero-order kinetic mechanism fitted to the transdermal process of PTX was not affected. Microdialysis was used to investigate the transdermal process of PTX during the *in vivo* experiment. Comparing the evaluations of *in vitro* skin permeation and *in vivo* microdialysis indicates that uniform regularity of the transdermal process was obtained during the evaluation of IVIVC, although the sampling of the two methods differed.

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