약제학회지(2007), 제 37 권 제 5 호
J. Kor. Pharm. Sci., Vol. 37, No. 5, 311-314 (2007)

Buccal Transport of Paclitaxel using Ethanol and Glyceryl Monooleate

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(Received October 9, 2007 · Accepted October 18, 2007)

ABSTRACT – Paclitaxel (PTX) is an antineoplastic agent approved for the treatment of ovarian and breast carcinomas. However, the use of paclitaxel as an anticancer drug is limited by its extremely poor water solubility (below 0.3 μg/mL). Furthermore, it has very low bioavailability when administered orally because paclitaxel is a substrate of P-glycoprotein (P-gp) efflux pump.

In this study, buccal delivery of PTX was investigated as one of the alternatives for PTX delivery. Ethanol and glyceryl monooleate (GMO) were selected as permeation enhancing agents to increase solubility and permeation across buccal mucosa of PTX. At the different concentrations of ethanol solution (30~70 w/w %), PTX permeation was studied, followed effects of GMO in the concentration range of 2.5~25% with ethanol vesicle. The transbuccal ability of PTX was evaluated in vitro using Franz diffusion cells mounted with rabbit buccal mucosa. As a result, incorporation of PTX into ethanol vesicle with GMO significantly enhanced the PTX permeation in rabbit buccal mucosa. Particularly, the mixtures of ethanol : water : GMO at the ratio of 50 : 47.5 : 2.5 showed the most excellent enhancing ability. The results showed a promising possibility for buccal delivery of PTX.

Key words – Paclitaxel, Buccal delivery system, Glyceryl monooleate, Permeation

Paclitaxel (PTX) is a diterpenoid molecule that is derived from the bark of the Pacific yew tree (Taxus brevifolia)[1] and an antineoplastic agent approved for the treatment of ovarian and breast carcinomas.[2] It inhibits cellular growth by both promoting and stabilizing the microtubule assembly by a non-covalent interaction with tubulin, thereby blocking cell replication in the late G2 mitotic phase of the cell cycle.[3,4]

The clinical use of PTX is often limited by its poor water solubility (<0.3 μg/mL)[5] and efflux action of P-glycoprotein (P-gp) for PTX. PTX is currently formulated with a mixture of polyoxyethyleneglyceryl triricinoleate 35 (Cremophor EL) and dehydrated ethanol USP (1 : 1, v/v). But Cremophor EL itself is toxic when administered intravenously and produces vasodilatations, labored breathing, lethargy and hypotension.

There have been numerous reports about the buccal drug delivery as an promising administration route having distinct advantages such as abundant blood supply in buccal area, bypassing the hepatic first pass effect and excellent accessibility.[6,7,8,9] In addition, recent literatures have shown that buccal mucosa is one of regions which do not have functional P-gp at the apical side.[10,11] Thus, it is possible to avoid the action of drug efflux pump and achieve systemic delivery of PTX. Additionally, due to non-keratinized buccal mucosa of human, it is able to be expected a higher permeability of PTX. However, because buccal mucosa shows relative low permeability compared with other mucosa regions such as intestine, it is necessary to improve the permeation using penetration enhancers.

In this study, ethanol and glyceryl monooleate (GMO) were selected as permeation enhancing agents to increase solubility and permeation across buccal mucosa of PTX. Ethanol has not only an ability of increasing solubility of PTX but also has been shown to extract intercellular lipids at high concentration.[12,13] Extraction of buccal intercellular lipids would seem an appropriate mechanism of action of ethanol since ethanol can increase lipid solubility.

Glyceryl monooleate is a mixture of glycerides and oleic acids. It is biodegradable, nontoxic, biocompatible, generally recognized as safe.[14,15] It has attracted great interest in the pharmaceutical area as a penetration enhancer in controlled drug delivery, bioadhesive systems and others.[16] GMO has a similar structure to oleic acid and is a well-known skin penetration enhancer for several drugs.[17,18] It enhances the skin penetration of several compounds such as urea, indomethacin, and steroids.[19,20]

In this study, we thus studied the possibility of the permeation of PTX through rabbit buccal mucosa which is similar to human buccal mucosa, and furthermore, the enhancement of the transport of PTX across the buccal membrane using ethanol and GMO as enhancers.

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Table I–*Permeation Parameters of Paclitaxel with Different Contents of GMO as Penetration Enhancers from 50% (w/w) Ethanol Solution*

Content of GMO (%)	J_{ss} (μg/h/cm^2)	T_L (hr)	K_p ($\times 10^{-3}$ cm/h)	ER*
C (Control)	2.16±0.06	4.6±0.4	6.48±0.17	1.00
2.5	4.05±0.37	4.5±0.1	12.14±1.12	1.87
5	1.78±0.28	4.4±0.3	5.34±0.84	0.82
10	0.811±0.09	4.5±1.1	2.43±0.27	0.38
20	1.065±0.39	4.6±0.6	3.19±1.18	0.49
25	0.72±0.35	3.2±0.9	2.15±1.06	0.33

*ER : enhancement ratio of flux.

Experimental

Materials

PTX (Genexol™) was obtained from Samyang (Taejeon, South Korea), N,N-diethylnicotinamide and phosphate buffer tablet (Sigma-Aldrich Korea Ltd., Kyunggi-do, Korea), GMO (RYLO® MG 19, PHARMA, Danisco, Denmark), ethanol, (Duksan, Kyunggi-do, Korea), Other chemicals and solvents were of analytical grade and used without further purification.

Preparation of Ethanol vehicles

0.4 mg of PTX was respectively dissolved in four mixtures of ethanol : water (30 : 70, 50 : 50, 60 : 40, 70 : 30, w/w %) at room temperature. After confirmation of no precipitation, they were used to permeation studies.

Ethanol vehicles containing GMO

0.4 mg of PTX was respectively dissolved in five mixtures of ethanol : water : GMO (50 : 47.5 : 2.5, 50 : 45 : 5, 50 : 40 : 10, 50 : 30 : 20, 50 : 25 : 25, w/w %) at room temperature. After confirmation of no precipitation, they were used to permeation studies.

Preparation of rabbit buccal tissues

Male albino rabbits (2.0±0.5 kg) were supplied by Han lim Lab (Kyoung-gi do, Korea). After sacrificed by excess ether inhalation, rabbit buccal mucosa was excised and remaining muscle and underlying submucosal tissue were subsequently stripped away using very fine-point forceps and scissors. The tissue was rinsed and stored in cold PBS (pH 7.4) at 5 until used.

Ex vivo rabbit buccal permeation study

Franz diffusion cells were used in the permeation study, which were with a diffusional surface area of 0.63 cm^2 and 5 mL of receptor cell volume. The receptor compartments were filled with PBS containing N,N-diethylnicotinamide (1.5 M), and the receptor phase was stirred at constant speed to mix the contents homogeneously. The basal side of buccal tissue was placed in contact with the receptor compartment fluid and equilibrated for 1 hr to attain a temperature of 37±0.5°C, and then air bubbles were removed. PTX added in various vehicles were applied on the mucosal side of the buccal tissue in donor compartment and covered with parafilm. Aliquots (200 μL) were withdrawn from the receptor compartment at predetermined time intervals for 10 hr and immediately replaced with fresh receptor fluid maintained 37°C for a constant volume. Obtained samples were analyzed simultaneously by HPLC.[18] Experiments were carried out in triplicate for each sample, and results were presented as mean±S.D..

Hydrotropic solubilization of PTX for a sink condition

A Sink condition should be used in the receptor compartment to solubilized PTX permeated across the buccal tissue. To find the condition, the aqueous solubility of PTX was measured in water containing N,N-diethynicotinamide which is one of the hydrotropic agents for PTX.[21] The concentration of PTX was determined by HPLC.[18] If it is possible to achieve sink condition using N,N-diethynicotinamide, we can maintain the sink condition in the receptor compartment without any organic solvents such as ethanol which is not an intrinsic compound.

Assay and HPLC conditions

The cumulative amount of PTX permeated through excised rabbit buccal mucosa into the receptor medium was determined by high-performance liquid chromatography (HPLC) using modification of the method reported elsewhere.[18] The HPLC system consisted of a quaternary pump (Hitachi, L-2130), an autosampler (Hitachi, L-2200), a UV/VIS detector (Hitachi, L-2400) set to 227 nm and an integrator (Hitachi, D-2000, Japan). The column, SunFire C_{18} (3.5 μm, 4.6 mm I.D. × 150 mm, Waters Co., USA) was used at 30°C and the mobile phase was used a mixtures of acetonitrile : 20 mM KH_2PO_4 buffer (5 : 5, v/v %) at a flow rate of 1 mL/min. Samples (50 μL) was injected onto the HPLC.

Data analysis

Steady state flux (J_{ss}, μg cm^{-2} hr^{-1}) of the drug through the rabbit buccal mucosa was calculated from the slope of the Cartesian plot between cumulative amount of drug permeated versus time. Lag time (t_L, hr) was calculated from the intercepts of back extrapolated linear steady state region.

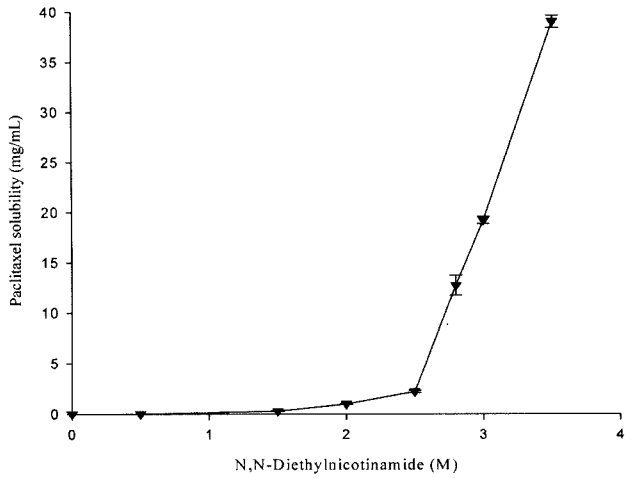

Figure 1–Solubility of paclitaxel in the presence of N,N-diethylnicotinamide. (Mean ± S.D., $n=3$).

$$K_p = J_{ss}/C_d$$

where K_p is the permeability coefficient and C_d represents the drug concentration which remains constant in the vehicle.

Results and Discussion

Sink condition for permeation study

PTX equilibrium solubility in water was reported to be as low as 0.3 μg/mL[5] but it was appreciably soluble in 1.5 M N,N-diethylnicotinamid as shown in Fig. 1. This increased PTX solubility can used to produce sink conditions, which are considered the capacity of a medium to dissolve more than 3 times than 80 μg/mL. Other researchers have demonstrated similar results at over the solubility capacity.[22] Hence, in order to maintain sink conditions during permeation experiments, 1.5M N,N-diethylnicotinamide was included into the receptor phase which was prewarmed at 37 ± 0.5°C.

Effect of ethanol amount within vehicles

To identify the effect of the amount of ethanol on permeation of PTX through rabbit buccal mucosa, the permeation rate of paclitaxle was measured at various concentrations (i.e. 30, 50, 60 and 70 w/w %) of ethanol solution. At lower concentration of ethanol (30 w/w %), PTX was hardly transported to receptor compartments. On the other hand, at high concentration (50, 60 and 70 w/w %), fluxes of PTX were increased at similar levels, which were presented in Fig. 2. The enhancing mechanism of ethanol is mainly caused by traversing the oral epithelium via the polar route.[23] Thus, the enhanced permeability may be expected due to increased solubility of PTX. However, there were not significant differences among high concentrations.

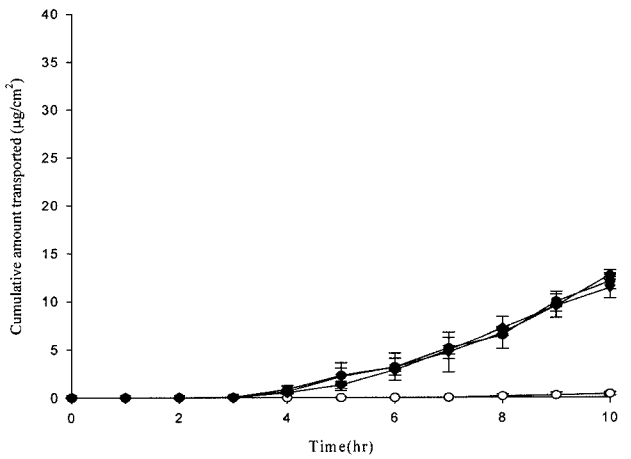

Figure 2–Permeation profiles of paclitaxel through excised rabbit buccal mucosa from ethanol solution with different concentration. Ethanol concentrations were 30% (○), 50% (●), 60% (▽) and 70 % (▼), respectively (Mean ± S.D., $n=3$).

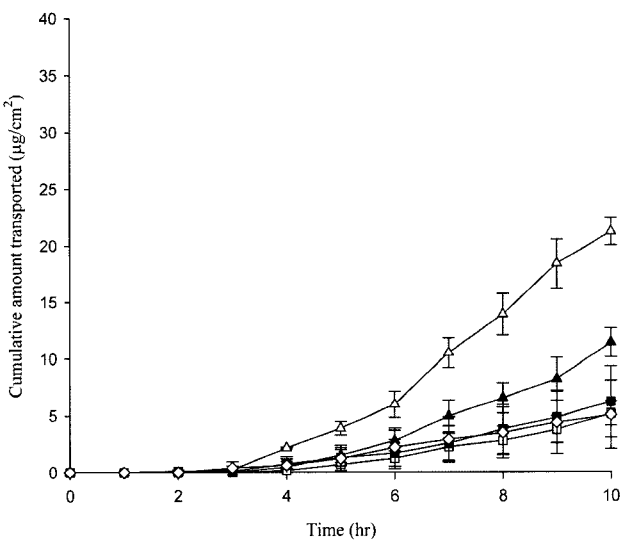

Figure 3–Permeation profiles of paclitaxel through excised rabbit buccal mucosa from ethanol solution containing different amount of GMO. Key: 2.5% (△), 5% (▲), 10% (□), 20% (■) and 25% (◇), respectively (Mean ± S.D., $n=3$).

Effect of GMO for permeation enhancement

In the present study, we investigated the effect of GMO concentration on transport of PTX across buccal membrane at five different levels from 2.5 to 25%. As shown Table I and Fig. 3, PTX transported was elevated by 2-folds at 2.5% and was similar to control at 5%. At higher concentrations, it was decreased rather than increased. These results indicate that relatively high concentrations of GMO do not increase the transbuccal delivery of PTX whereas low concentrations are very effective to do so. For the reason, that lipophilic natures of both GMO and PTX cause a physicochemical interaction between them.[24] Therefore, the phenomenon may result in

drug retention upper layer of buccal membrane where GMO is better partitioned rather than transported to receptor phase.

Conclusions

In this study, transbuccal permeability of PTX was investigated and ethanol and GMO were evaluated for PTX delivery on buccal mucosa. The amount of PTX transported was significantly increased with ethanol and GMO. It is therefore concluded that buccal mucosa is considerable as a promising approach to deliver the high moleculear weight and lipophilic drug, PTX. Ethanol and GMO are effective permeability enhancing agents in buccal delivery.

Acknowledgement

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2005-003-E00330).

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