Original Article

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Assessment of intestinal permeability of paclitaxel in the presence of NSAIDs and P-glycoprotein inhibitors

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Abstract

Paclitaxel is a potent anticancer drug with a unique mechanism of action, which is now administered via IV infusion. Due to the presence of cremophor EL in its formulation it may show anaphylactic reactions. So that oral administration of paclitaxel for removal of these important side effects is the interest of many researches. Intestinal permeation of paclitaxel is restricted because of low solubility, lack of intestinal permeability, and efflux by P-glycoprotein pumps (P-gp) in intestinal wall. In this study the effects of NSAIDs and P-glycoprotein inhibitors on the intestinal permeability of paclitaxel was assessed by everted intestinal sacs technique. The results show that piroxicam, indomethacin, naproxen, mefenamic acid, and ibuprofen increase the intestinal permeability of paclitaxel 15.5, 10.1, 7.5, 5.6 , and 4.5 folds, respectively. Inhibition of the P-gp pumps by verapamil and cyclosporine increase the permeation of paclitaxel 2.9 and 4 folds, respectively. It seems that co-administration of paclitaxel with NSAIDs and P-gp pumps inhibitors could improve the intestinal permeation of paclitaxel.

Keywords: Paclitaxel, NSAIDs, Intestinal permeability, P-glycoprotein, Cyclosporine, Verapamil.

1. Introduction

Paclitaxel (PTX) is a natural diterpenoid originally isolated from the bark of the pacific yew, *Taxus brevifolia*, which belongs to a class of antimicrotubule antineoplastic drugs (1). It has a unique mechanism of action associated with cellular microtubule formation, which increase polymerization of microtubules, stabilizes them and blocks the mitosis in the late G2 or M phase of the cell cycle (2, 3). It has been shown to have clinical activity against many common types of solid malignancies, including ovarian, breast, colon, prostate, non-small-cell lung carcinoma, and AIDS-related Kaposi's Sarcoma (4, 5). Because of low aqueous solubility, it is formulated in polyoxyethylated caster oil (Cremophor EL) containing 50% absolute ethanol and is currently administered by intravenous infusion. This vehicle has shown observed to cause serious, life-threatening anaphylactoid reactions in animals and humans, and is physically incompatible with some intravenous infusion sets, as shown by the leaching of plastisizers (6, 7, 8). Premedication with corticosteroids and H_1 and H_2 antagonists has reduced the intensity and incidence of adverse reactions associated with this vehicle. However, they are not completely eliminated (9). Alternative formulations have been carried out to eliminate the Cremophor vehicle such as microparticles (10, 11), nanoparticles (12, 13), polymeric micelles (14, 15).Currently Abraxane® (albumin microspheres containing paclitaxel) and Genexol® (PEG- PLA polymeric micelles with paclitaxel) are available in the market without cremophor EL (16). Besides these formulations for IV injections, oral delivery has been proposed

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as a simple, convenient, and safer alternative for delivery of PTX. However, oral delivery of PTX is limited by its low water solubility and low intestinal permeability. Since Cremophor EL is not absorbed through gasterointestinal tract (17), oral administration of paclitaxel could prevent the adverse effects caused by the vehicle and may allow the achievement of lasting therapeutic plasma levels. However, reports on the low oral bioavailability of PTX in mice have discowaged the effort toward development of an oral formulation (18). Furthermore, presence of efflux P-glycoprotein (P-gp) pumps in the intestinal wall limit the intestinal permeation of PTX (18, 19, 20). Using solubilizers, permeation enhancers, and inhibitors of P-gp pumps potentially could increase the oral absorption of PTX. Cyclodextrins (CD) are cyclic oligosaccharides with a hydrophobic region. Complexes of hydrophobic drugs with these macromolecules improve the drug aqueous solubility. Solubility of PTX increased by complexation with hydroxyl propyl-β-cyclodextrin (HPβCD) (4). PTX has a bulky and lipophilic molecular structure and it seems that increasing intestinal permeability of it through tight junctions could improve the oral bioavilability of this drug. Experiments with $_{51}$Cr-EDTA and other probes as markers of small intestinal permeability have shown that nonsteroidal anti-inflammatory drugs (NSAIDs) can increase permeation of drugs through the intestinal epithelium by opening of tight junctions (21, 22). Verapamil (23, 24) and cyclosporine (25, 26, 27) are inhibitors of P-gp pumps. Therefore, coadministration of PTX with these P-gp inhibitors could further improve the intestinal permeability and oral absorption of this drug. In present work, we used some of NSAIDs as permeability enhancers (21) and P-gp pumps inhibitors to improve intestinal permeation of paclitaxel by use of everted rat gut sacs technique as an *in vitro* model.

2. Materials and methods

PTX was obtained from Calbiochem-Novabiochem (San Diego, CA, USA). Ibuprofen, mefenamic acid, piroxicam, indomethacin, and naproxen were supplied by Arya, Alhavi and Dr Abidi Pharmaceutical Companies (Tehran, Iran), respectively. Cremophor EL was obtained from Sigma (St Louis, MO, USA) and hydroxyl propyl-β-cyclodextrin (HPβCD) was supplied by Aldrich Chemical Company (Munich, Germany). HPLC grade acetonitrile and methanol were purchased from Merck (Germany). All other solvents and chemicals were of analytical grade.

2.1. Animals

Male Sprague–Dawley rats (weight: 170-250 g) from animal house of Shiraz University of Medical Sciences were used. Animals were kept in controlled condition (standard temperature, humidity, and light-dark cycle) on normal chow pellet diet and free water access. The animal study was approved by the guideline of the ethical committee of Shiraz University of Medical Sciences.

2.2. Preparation of everted intestinal sacs

Animal studies were conducted according to the approved protocols of Shiraz University of Medical Sciences (Shiraz, Iran) for animal handling. All procedures are performed in compliance with ethical standards.

Permeation of PTX across the intestinal wall was studied. Everted intestinal sacs were prepared according to the standard procedure. Male Sprague Dawley rats (250-300 g) were used for the intestinal permeation experiments. Animals were sacrificed and a longitudinal segment as long as 30 cm of the upper section of intestine was quickly cut and removed, rinsed with cold oxygenated physiological Ringer solution and cut into segments of 2 cm length. Each sac tied with cotton thread and filled with 2 mL Ringer solution. For everting the intestine segment a glass rod was used carefully and the serosal side of the intestine was filled with 0.5 mL Ringer solution containing HPβCD 5%(w/v) (acceptor part). Each sac was individually placed in a glass tube containing 40 ml physiologic Ringer solution containing HPβCD 5%(w/v) which continually bubbled with 95% O_2 and 5% CO_2 kept in a water bath at 37 °C (donor chamber).

2.3. Intestinal permeation studies

After equilibration at 37 °C for 30 minutes, the exact volume of PTX solution in cremophor EL: ethanol (1:1 v/v) was added to the

donor chamber (mucosal solution). Final concentration of PTX was 10 µg/ml. 0.5, 1, 1.5, 2 and 3 hours after addition of PTX solution, intestinal sacs were removed (n=3), washed with water and the serosal solution were centrifuged and stored at -20 °C for analysis.

For investigation the NSAIDs effects on the intestinal permeability of PTX, one of the NSAIDs drugs concomitant PTX was added to the donor chamber. Indomethacin, piroxicam, naproxen, mefenamic acid, and ibuprofen were chosen for this purpose. The final concentration of NSAIDs in the chamber was 10 µg/ml. Sampling was done after 3 hours and serosal solutions of rings were collected.

The effect of ibuprofen as selected NSAIDs was evaluated at 2 situations:

One: ibuprofen was administered to alive mice (dose 10 mg/kg) and animals were sacrificed 30 minutes later. Their intestine segments were removed and everted sacs were prepared as mentioned above. PTX solution was added to the donor chamber and the permeated PTX was analyzed.

Two: Everted intestinal sacs were prepared. Ibuprofen and PTX were added to the donor chamber, concomitantly. Sampling was done at determined times and serosal solutions of rings were collected.

For evaluation the effect of P-gp pumps inhibitors verapamil and cyclosporine were added to the donor part of sacs with PTX. The concentration of verapamil, cyclosporine and PTX were 50 µg/ml, 50 µg/ml and 10 µg/ml, respectively.

The concomitant effects of ibuprofen with P-gp pumps inhibitors on permeation of PTX were evaluated. For this purpose, ibuprofen with verapamil or cyclosporine was added to the donor parts of sacs in the presence of PTX. Sampling was done at determined times and serosal solutions of rings were collected.

2.4. Drug analysis

The HPLC system for PTX analysis consisted of a 600 pump and controller (Waters, Milford, MA, USA) with a 486 multi wavelength detector (Waters) and a 746 data module integrator (Waters). The chromatographic separation achieved using a 250-mm C18 column (ShimpackH; Shimadzu, Kyoto, Japan) and a mobile phase consisting of acetonitrile: water 50:50 (water contained 0.05% v/v glacial acetic acid). The mobile phase was pumped to the column at a flow rate of 1.7 ml/min. Detection of PTX was performed at 227 nm. Quantification was performed by measuring the ratios of peak area of the drug to the internal standard.

Calibration curve for determining of PTX in the serosal solution of intestinal rings was plotted as follow: A longitudinal segment of rat intestine (15 cm) was removed and everted on the glass tube. The serosal part of that segment was filled with Ringer solution containing HPβCD 5% (w/v). The segment was placed in a glass tube containing 40 ml physiologic Ringer solution containing HPβCD 5% (w/v) which continually bubbled with 95% O_2 and 5% CO_2 kept in a water bath at 37 °C. After 4 hours the serosal solution was collected for preparation of standard concentration of PTX.

2.5. Statistical analysis

Difference between mean values of parameters was analyzed using Student's t-test and one-way analysis of variance. P-values less than 0.05 were considered significant.

3. Results

3.1. PTX analysis

Validation of the HPLC assay demonstrated that this methodology was linear (r^2=0.9998) in the range of 0.1-10 µg/ ml. CV% was less than 9% and the accuracy was more than 87% within this range of concentrations. Limit of detection and limit of quantification were 0.05 and 0.1 µg/ml, respectively.

Table 1. PTX serosal concentration during 3 hours.

Time(hr)	0.5	1	1.5	2	3
Paclitaxel serosal Conc.(µg/ml)	-	-	-	0.12 ±0.03	0.16± 0.1

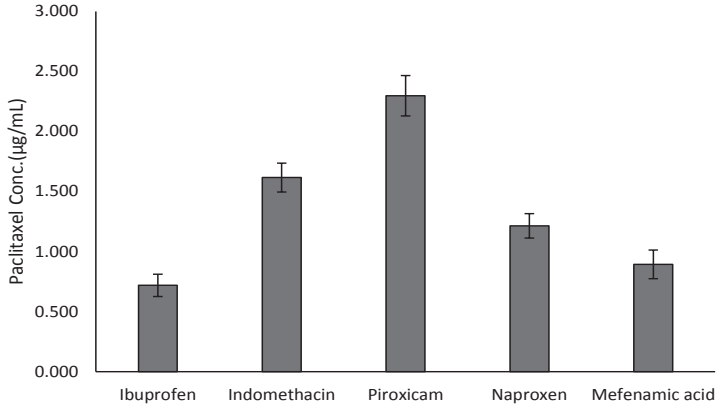

Figure 1. Serosal concentration of paclitaxel 3 hours after addition of NSAIDs to the mucosal part.

3.2. Effect of NSAIDs on intestinal permeation of PTX

Table 1 shows permeation of PTX through intestinal wall is very low even after 3 hours of exposure of drug to the mucosal site of sacs. As shown in Figure 1, NSAIDs improved intestinal permeation of paclitaxel. The amount of PTX that passed through the intestinal wall in the presence of piroxicam, indomethacin, naproxen, mefenamic acid, and ibuprofen was 15.6, 10.1, 7.5, 5.6 and 4.5 folds greater, respectively than the intestinal permeation of PTX alone. Effects of ibuprofen and mefenamic acid on increasing the intestinal permeation of PTX were not statistically different. The effects of other NSAIDs were more than that of ibuprofen on the intestinal permeation of PTX (P<0.05).

Effect of oral administration of ibuprofen to alive rat or addition of it to the *ex-vivo* medium of rat intestine was compared in the Figure 2. In both cases ibuprofen increased the intestinal permeation of PTX at similar rate. The effect of ibuprofen on intestinal permeation of PTX in the *ex-vivo* medium was smaller than the pretreatment of rats with oral administration of ibuprofen.

3.3. Effect of P-gp pumps inhibitors on intestinal permeation of PTX

Serosal concentration of PTX in the presence of verapamil or cyclosporine as P-gp inhibitors was shown in Figure 3. Both agents increased the intestinal permeation of PTX. The effect of cyclosporine was greater than verapamil at all time points (P<0.05).

3.4. Concomitant effect of ibuprofen and P-gp pumps inhibitors on intestinal permeation of PTX

Concomitant effects of ibuprofen and verapamil or cyclosporine were greater than effect of each of agents alone. These effects were pre-

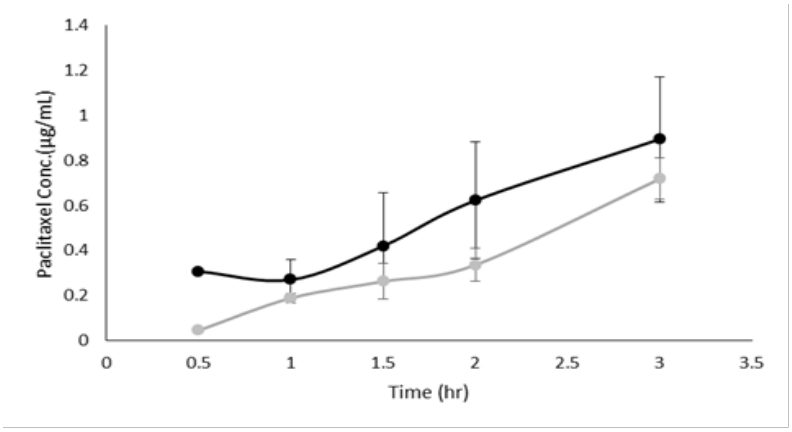

Figure 2. Serosal concentration of PTX after oral administration of ibuprofen to alive rat (●) or addition of it to the ex-vivo medium of rat intestine (●)

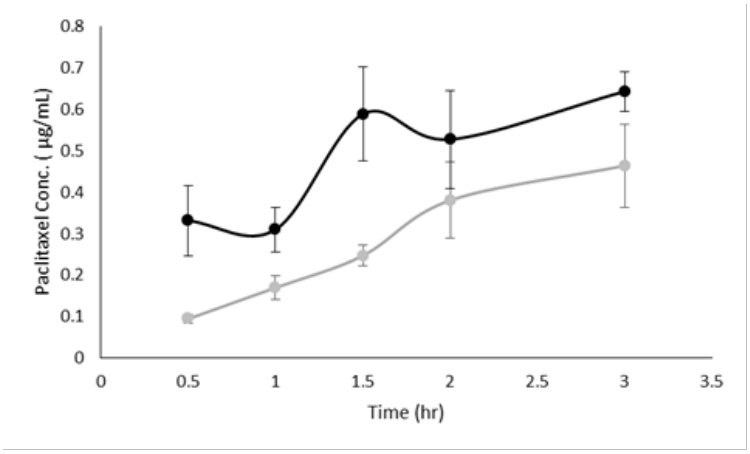

Figure 3. Serosal concentration of PTX in the presence of verapamil (●) or cyclosporine (●) in the mucosal part.

sented in Figure 4.

4. Discussion

Oral administration of chemotherapeutic agents has many benefits in comparison with intravenous rout. Oral treatment avoids the discomfort of an injection and the risk of infection and extravasation that are associated with intravenous access lines. It reduces administration costs and facilitates the use of more chronic treatment regimens. Finally, the patient compliance increases through oral administration. In addition of these cases, oral administration of PTX has a specific benefit which is prevention of side effects of its vehicle, Cremophor EL. Preclinical studies have suggested that PTX is not significantly absorbed after oral administration. Low oral bioavailability could be related to poor absorption or extensive presystemic hepatic metabolism (28). For assessment intestinal permeation of PTX, in this study we applied everted intestinal rings technique. This is a simple and accurate model. Unlike many absorption models which measures transport of drugs through epithelial cell layer, rings are more accurate model for evaluation the uptake or accumulation of drug through intestine. In this model, the access of drugs to the absorption surface reaches maximum by everting the intestinal segment (29). PTX solution in cremophore EL-ethanol with one of the NSAIDs was added to the mucosal site of rings. To prevent of precipitation of PTX following dilution in the incubation medium, HPβCD was used. Cyclodextrins are cyclic oligosacharids which can formation complexes with hydrophobic guests and

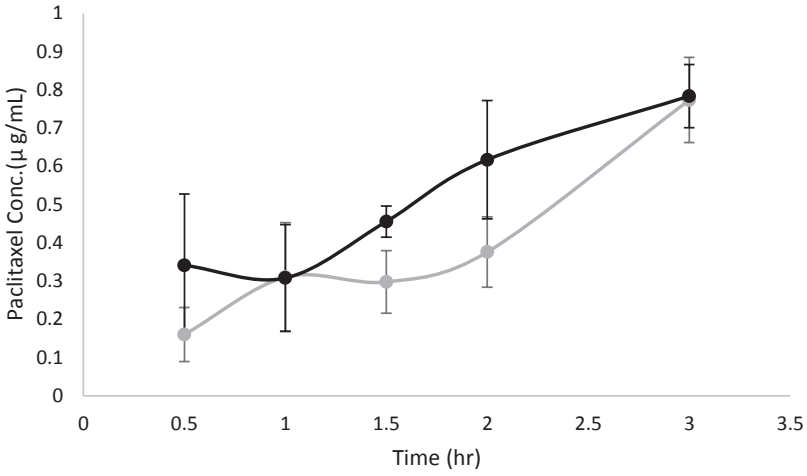

Figure 4. Serosal concentration of PTX in the presence of verapamil and ibuprofen (●) or cyclosporine and ibuprofen (●) in the mucosal part.

improving water solubility of them (30, 31). PTX has a bulky and lipophilic molecular structure and it seems that paracellular pathway is the major path of transportation of it through intestinal wall. NSAIDs have effect on the tight junctions between enterocytes. NSAIDs prevents synthesis of prostaglandins via inhibition of cyclooxygenase enzyme, subsequently they changes cell level cAMP, which will open the tight junctions between cells (21). Our study showed that PTX intestinal permeability 4.5-15.6 times increased when NSAIDs present in the PTX solution medium.

Effect of oral administration of ibuprofen to alive rat or addition of it to the *ex-vivo* medium of rat intestine was compared. In both cases ibuprofen increased the intestinal permeation of PTX at similar rate but the effect of ibuprofen on intestinal permeation of PTX in the ex-vivo medium was smaller than the pretreatment of rats with oral administration of ibuprofen. This phenomenon shows that the results of intestinal permeation studies in the *ex-vivo* model are reliable. Of course better results are expected for in-vivo intestinal permeation experiments.

The intestinal permeability of PTX in the presence of verapamil or cyclosporine improved from 0.1% to 10%, approximately. The effect of cyclosporine was more than verapamil. The potency of cyclosporine in inhibition of P-gp pumps is more than verapamil. It has also been reported that cyclosporine can improve drug transport by affecting the paracellular pathway (32). We observed that when cyclosporine and ibuprofen simultaneously added to the mucosal part of intestinal rings, their effects do not get together. The effect of cyclosporine on paracellular pathway can justify this result. When verapamil and ibuprofen simultaneously added to the mucosal part of intestinal rings, their effects added together, because of different mechanisms of action of these drugs. Verapamil inhibits the P-gp pumps and ibuprofen opens the tight junctions.

5. Conclusion

P-gp pumps are dispersed in many tissues like intestine, liver, kidney and brain. Therefore inhibition of them could change the drug pharmacokinetic. This effect may cause of toxicity. In our results, the effect of NSAIDs on the intestinal permeation of PTX is comparable with effect of cyclosporine or verapamil. Therefore, using NSAIDs especially ibuprofen with lower side effects is suggested for *in vivo* study of oral administration of PTX.

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Conflict of Interest

None declared.

6. References

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