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# Enhanced oral paclitaxel absorption with vitamin E-TPGS: Effect on solubility and permeability in vitro, in situ and in vivo

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**Abstract**

Solubility and permeability being important determinants of oral drug absorption, this study was aimed to investigate the effect of D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) on the solubility and intestinal permeability of paclitaxel in vitro, in situ and in vivo, in order to estimate the absorption enhancement ability of TPGS. Aqueous solubility of paclitaxel is significantly enhanced by TPGS, where a linear increase was demonstrated above a TPGS concentration of 0.1 mg/ml. Paclitaxel demonstrated asymmetric transport across rat ileum with significantly greater (26-fold) basolateral-to-apical (B–A) permeability than that in apical-to-basolateral (A–B) direction. Presence of P-glycoprotein (P-gp) inhibitor, verapamil (200 μM), diminished asymmetric transport of paclitaxel suggesting the role of P-gp-mediated efflux. TPGS showed a concentration-dependent increase in A–B permeability and decreased B–A permeability. The maximum efflux inhibition activity was found at a minimum TPGS concentration of 0.1 mg/ml, however, further increase in TPGS concentration resulted in decreased A–B permeability with no change in B–A permeability. Thus, the maximum paclitaxel permeability attained with 0.1 mg/ml TPGS was attributed to the interplay between TPGS concentration dependent P-gp inhibition activity and miceller formation. In situ permeability studies in rats also demonstrated the role of efflux in limiting permeability of paclitaxel and inhibitory efficiency of TPGS. The plasma concentration of [$^{14}$C]paclitaxel following oral administration (25 mg/kg) was significantly increased by coadministration of TPGS at a dose of 50 mg/kg in rats. Bioavailability is enhanced about 4.2- and 6.3-fold when [$^{14}$C]paclitaxel was administrated with verapamil (25 mg/kg) and TPGS, respectively, as compared to [$^{14}$C]paclitaxel administered alone. The effect of verapamil on oral bioavailability of [$^{14}$C]paclitaxel was limited relative to the TPGS, consistent with the in vitro solubility and permeability enhancement ability of TPGS. In conclusion, the current data suggests that the coadministration of TPGS may improve the bioavailability of BCS class II–IV drugs with low solubility and/or less permeable as a result of significant P-gp-mediated efflux.


*Keywords:* Vitamin E-TPGS; P-glycoprotein; Oral absorption; Pharmacokinetics

## 1. Introduction

Paclitaxel, an antimicrotubule anticancer drug used in wide variety of human cancers, is currently formulated with cremophor EL (polyethoxylated castor oil derivative) and dehydrated alcohol (1:1), is administered through intravenous infusion (Panchagnula, 1998). Ethanol-cremophor EL vehicle required to solubilize paclitaxel in this formulation is toxic and also produces vasodilation, labored breathing, lethargy, and hypotension. In order to develop safer clinical formulations, many studies have been directed to novel oral formulations (Dhanikula and Panchagnula, 1999; Mu and Feng, 2003; Feng et al., 2004; Yang et al., 2004; Win and Feng, 2005). Paclitaxel is very poorly absorbed on *peroral* administration because of its low solubility and low permeability. Apart from its unfavorable physicochemical features for

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passive permeability, it is also believed that P-glycoprotein (P-gp) hinders the transport of paclitaxel from the gut (Varma et al., 2005a). An increasing number of drugs, including HIV protease inhibitors like indinavir, ritonavir, saquinavir and anti-cancer drugs like docetaxel, vinblastine, etc have been reported to be substrates for P-gp (Varma et al., 2003). In vivo studies confirmed that P-gp significantly limits oral bioavailability of several drugs, where intestinal permeability showed dose dependence with increased permeability as lumen concentration increases (Williams and Sinko, 1999; Malingre et al., 2001).

Studies using mdr1a(−/−) mice showed direct evidences that P-gp strictly limits the uptake of orally administered paclitaxel (Sparreboom et al., 1997). Woo et al. (2003) demonstrated that about half of paclitaxel dose administered is extruded to the gut lumen by P-gp and only small amount of drug is lost by gut wall and liver metabolism. Thus, the oral bioavailability of paclitaxel can be significantly enhanced by effectively inhibiting P-gp-mediated efflux.

Solubility and permeability of a drug are the fundamental determinants of its oral bioavailability (Varma et al., 2004). Surfactants are extensively used to increase the absorption of drugs from the intestine; as they show increased solubility of hydrophobic macromolecules, increased membrane fluidity or disruption of tight junctions, interaction with metabolic enzymes and inhibition of efflux transporters (Nerurkar et al., 1997; Rege et al., 2002). Vitamin E-TPGS (TPGS) is non-ionic water soluble derivative of Vitamin E found to enhance the bioavailability of cyclosporin and amprenavir by enhancing solubility and/or permeability, or reducing intestinal metabolism (Sokol et al., 1991; Chang et al., 1996; Yu et al., 1999; Joshi et al., 2003). TPGS form micelles above the critical miceller concentration (CMC) and improve solubility of lipophilic compounds. Previous reports suggested that coadministration of TPGS enhanced oral absorption of cyclosporin A due to improved solubilization by micelle formation (Sokol et al., 1991; Boudreaux et al., 1993). Chang et al. (1996) evaluated the effect of TPGS on the oral pharmacokinetics of cyclosporin A in healthy volunteers, and suggested that enhanced absorption, decreased counter transport by P-gp, or some unknown mechanism is responsible for the observed decrease in apparent oral clearance. Later on, it was demonstrated that TPGS enhanced the cytotoxicity of doxorubicin, vinblastine, paclitaxel and colchicine in the G185 cells, by acting as reversing agent for P-gp-mediated multidrug resistance (Dintaman and Silverman, 1999).

In the light of above discussion, the present work investigated the effect of TPGS on the solubility and permeability of a biopharmaceutic classification system (BCS) class IV drug, paclitaxel in vitro, in situ and in vivo. The functional role of P-gp in limiting the permeability of paclitaxel was determined along with the influence of miceller drug concentration on the effective permeability. Furthermore, we also studied the influence of TPGS on the oral bioavailability of paclitaxel in rats.

## 2. Materials and methods

### 2.1. Chemicals

Paclitaxel was gifted by Dabur India Ltd. (New Delhi, India), and [$^{14}$C]paclitaxel was purchased from Sigma–Aldrich Co. (MO, USA). [$^{3}$H]Imipramine was purchased from NEN Bio (Boston, MA). Hydrochlorthiazide was received from Aristo Pharmaceuticals Ltd. (Daman, India), and propranolol HCl was from Sun Pharmaceutical Industries Ltd. (Mumbai, India). Frusemide was gifted by Dr. Reddys' Lab. (Hyderabad, India). L-Phenylalanine was purchased from Sisco Lab. (Mumbai, India). Other compounds verapamil, imipramine and D-glucose were purchased from Sigma Chemicals Co. (MO, USA). Lecithin and dodecane were purchased from Himedia Lab. Pvt. Ltd. (Mumbai, India). Vitamin E-TPGS was from Eastman, and DMSO was procured from Sigma–Aldrich Co. (MO, USA). Solvents used for quantification were of HPLC grade (JT Baker, Mexico) and all other chemicals and reagents were of analytical grade.

### 2.2. Animals and legal prerequisites

Sprague–Dawley rats (270–350 g) were used for in vitro transport, in situ single-pass perfusion and in vivo pharmacokinetic studies. Anesthesia, surgical and disposal procedures were justified in detail and were approved by the Institutional Animal Ethics Committee (IAEC, NIPER). The studies complied with local and federal requirements for animal studies.

### 2.3. Solubility studies

Equilibrium solubility of paclitaxel in 0–5 mg/ml concentration of TPGS was determined by shake-flask method ($n = 3$). Excess amount of drug was added in TPGS solution in water and equilibrated at $37 \pm 0.2\,^{\circ}C$ with vigorous shaking in shaker water bath (Julabo, Germany) for 48 h. Samples were filtered using 0.22 μm filter (Millipore, USA). Aliquots of each filtrate were diluted appropriately and analyzed by RP-HPLC method.

### 2.4. Measurement of apparent artificial membrane permeability ($P_{PAMPA}$)

Artificial membrane permeability studies were performed in the same manner described previously (Kansy et al., 1998; Bermejo et al., 2004). In brief, a 96-well microplate (acceptor compartment) was completely filled with phosphate buffer (pH 7.4) containing 5% DMSO. Each filter of the donor plate (Millipore Corp., Bedford, MA) was impregnated with 5 μl of 10% (w/v) lecithin in dodecane. A 200 μl of 10 μM [$^{14}$C]paclitaxel (0.4 μCi/ml) in different concentrations of TPGS solution ($n = 4$ for each TPGS concentration studied) was added immediately and incubated