Review Article

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Polysorbate-Phospholipid Complex Micelles as P-glycoprotein Inhibitor Drug Delivery System

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Abstract

Presystemic metabolism and selective transport mechanism are the two important defensive systems with which drug molecules from the time of its release till its elimination through the body interacts. ATP-based transport enzyme P-gp present at the extracellular surface allows selective localization of drugs inside the cytoplasm. This selective transport effluxes the xenobiotics and toxins entering inside thus preventing any harmful effect from occurring but such cohesive effect may result into the shielding of the active drug molecule to produce a desirable pharmacological response. Varieties of inhibitors are available but the use of such molecules to produce a drug delivery system is a confronting task. Polysorbate-Phospholipid complex particles are not considered as the common P-gp inhibiting mechanism but their fruitfulness towards the delivery of drug molecules inside with enhanced residence at the brain, placenta, heart, ovaries and testes tissues proved its efficiency as prominent inhibitors. Coating of Polysorbate 80 is considered as crucial due to its ionic stability and micellization for achieving targeting of the drug at tissues. Use of Phospholipid for the site specific delivery of drugs is very common; they are the building blocks of cells which allow the transport of active drug molecules into the cellular system without any inhibition by any efflux mechanism. Phospholipids also enhance the bioavailability of pharmaceutical products with less membrane penetration potential and less aqueous solubility. In this review, the key points of various literature with more rationalized view are noted to highlight the effectiveness of Polysorbate-80/phospholipid mixed micelles as P-gp inhibitors.

Keywords: Polysorbate-80, Phospholipid, Mixed Micelles, P-gp inhibitors and Bioavailability

1. Introduction

Targeting of the drug to the respective receptor tissues is one of the challenging tasks in the field of formulation development as the presence of barriers in the form of enzymatic proteins has been estimated to cause retardation or resistance to various drugs. The barrier proteins are mainly present for limiting the exposure of toxins, drugs and xenobiotics to the body tissues present [1]. The human P-glycoprotein is one of the multidrug resistance proteins which prevent the accumulation of the drug in brain, heart, placenta, ovaries and testes tissues by extruding the drug molecules into the urine, bile and intestinal lumen [2]. It belongs to a subfamily of ATP-binding cassette transporters and is generally recognized as a surface glycoprotein. In human tissues, P-gp is mainly concentrated in liver, pancreas, jejunum, kidney and colon [3, 4]. This membrane bound protein is an energy-dependent efflux transporter that is mainly provided by the hydrolysis of ATP [5].

Drugs or substrates generally crosses into the cell membrane by simple diffusion, filtration or by the specialized transport mechanism. Drug recognition by P-gp followed by ATP-binding and subsequent hydrolysis and final efflux through the central pore to outside the membrane are the some of the important steps which are responsible for drug transport or efflux. The inhibition of xenobiotics or toxins happens either by the blockage of the ATP hydrolysis process or by the competition of drug to the existing drug binding sites or by both the mechanisms or by an allosteric mechanism [6, 7]. Generally, the concentration of enzymes remains high in the plasma membrane of tumor cells thus causes various cytotoxic drugs to be out of the tumor cells. So, tumors arise from the tissues where over expression of the efflux enzyme is common subsequently their resistance to chemotherapy is also very common. The efflux of doxorubicin, paclitaxel, cisplatin, vinca alkaloids, anthracyclines, epipodophyllotoxins and taxols by P-gp is one of the reasons for the failure of cancer therapy [8, 9]. Restriction in the entry of risperidone and its active metabolite 9-hydroxyrisperidone through the brain cells leads to the failure of therapy in psychosis [10]. The poor bioavailability is one of the effects associated with substrates of P-gp as the efflux transporter is located widely in the various ranges of tissues [11]. Various anticancer drugs such as docetaxel, paclitaxel, and HIV protease

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inhibitors such as saquinavir, ritonavir and indinavir were the reported substrates for P-gp [12]. The unique characteristics of the P-gp have been reported to be responsible for the movement of drugs in or in the opposite direction thus affecting overall drug permeability and bioavailability [13].

Blood brain barrier generally is made up of a tightly connected discontinuous layer of pericytes and brain endothelial cells. The cerebral endothelium allows maintenance of BBB integrity & trans endothelial transport of cell [14]. Brain endothelial cells communicate through specific proteins named tight junctions (TJs) and adherens junctions (AJs) which support the cohesiveness of the barrier [15]. The mechanism of passage happens between endothelial cells is known as paracellular while the one takes place through the endothelial cells is known as transcellular. In most of the cases, transcellular pathway occurs with the passive diffusion of lipophilic molecules which takes place through targeted receptors. In the transcellular process, transcytosis mechanism occurs. In this context, BBB endothelial cells are being explored as an approach for transporting therapeutic drugs into the brain. Conversely, the delivery of several drugs to the brain parenchyma might be reduced by ATP binding cassette transporters. They are active efflux pumps which transport the pharmaceutical drugs into the blood or neurotoxin lipid-solid molecules [16].

The distribution of drugs into the brain is mainly limited by the presence of P-gp present at the junction of blood brain barrier and thus limits the distribution of useful drugs into the CNS which acts as a symptom of a decrease in passive permeability of drug [17]. The P-gp efflux even retards the action of antiepileptic drugs and anti-depressant drugs into the brain [18, 19]. Fetal tachyarrhythmia is one of the life-threatening conditions for unborn and for this digoxin is mainly recommended but an expression of P-gp in placenta decreases fetal exposure to digoxin [20]. So, it can be concluded that if there is a mechanism which can manipulate the transporter enzymes present then pharmacotherapy could be improved and thus combined inhibition of P-gp by doxorubicin, tetrandrine, cyclosporine A and verapamil considered to be an approach for increasing the concentration of drugs in the brain [21]. The different mechanisms used for modulations of P-gp involve direct inhibition by specific competitors, functional modulation and transcriptional modulation [22]. Numerous researches have been published relating to the classification of P-gp inhibitors [23]. Along with P-gp, CYP3A4 is also considered as an asset for the intestinal defense system to protect the body against harmful xenobiotics. Few studies have proved that both enzymes are co-substrates and P-gp can even potentiate CYP3A4-mediated drug metabolism [24, 25]. So, first pass metabolism and efflux transporter enzymes are the two limiting factors affecting formulation development [26, 27]. In this review, the main focus on polysorbate-phospholipid mixed micelles as bioavailability enhancer system by inhibiting P-gp enzymes was made. Several studies are reviewed on the inhibition of P-gp by polysorbate-80 or phospholipid.

2. Inhibition by Polysorbate-80

Nanoparticles as such cannot diffuse freely throughout the blood-brain barrier (BBB) as they require receptor mediated transfer through brain capillary endothelium to distribute their substance into the brain parenchyma [28]. Drug coated with Polysorbate-80 probably may reach into the brain and releases the drug after endocytosis by the brain vessel endothelial cells. It was noted that the nanoparticles with a coating of Polysorbate 80 could be used to target the drugs to the brain [29]. Polysorbate is the most preferred nonionic surfactant. Polysorbates are oleate esters of sorbitol in which each mole of sorbitol is co-polymerized

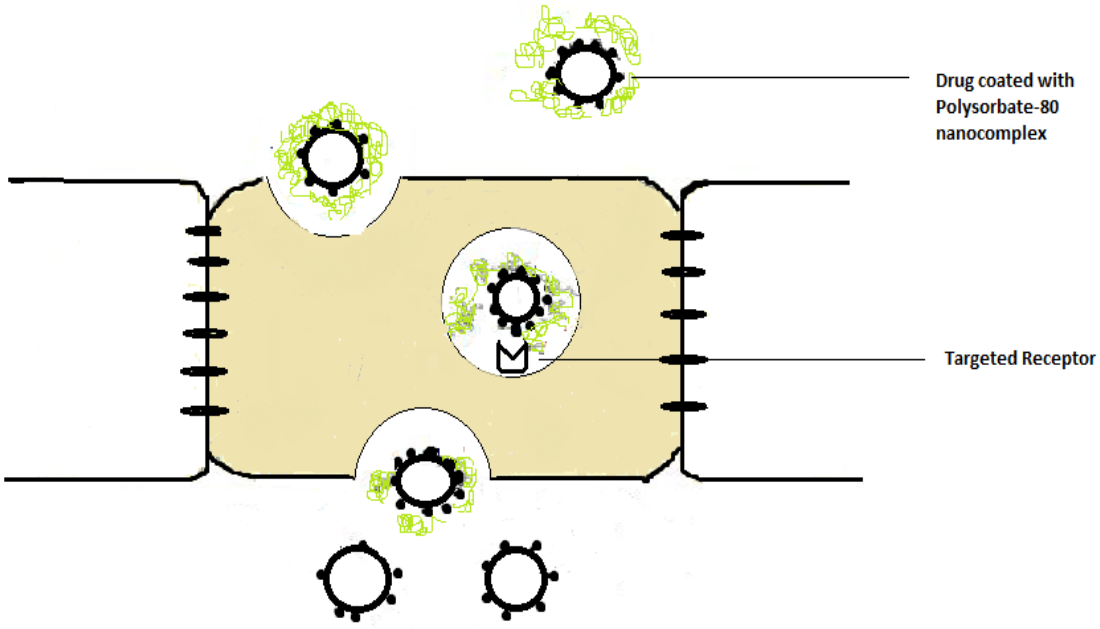

Fig. 1. Delivery of Polysorbate-80 coated drug into the blood brain barrier

with about 20 moles of ethylene oxide [30]. In brain targeting, only a partial exposure of PS-80 is required for targeting. It was reported that for targeting the PS-80, interaction with the brain micro vessel endothelial cells is needed [31]. The delivery of polybutylcyanoacrylate (PBCA) nanoparticles loaded with temozoloamide showed enhanced release into the brain when coated with polysorbate-80 [32].

The thickness of PS-80 coating is also reported to be essential. In a study when dalargin loaded PBCA-NDSs were consecutively double coated as well as single coated with PS-80 and PEG 20000 respectively in varied concentrations and administered orally to mice, it was found that dalargin induced analgesia observed from PBCA-NDSs with double coating of PS-80 and PEG as compared to single coating of either PS-80 or PEG [33].

The possibility of delivery of anticancer drugs into the brain by using colloidal carriers/nanoparticles coated with PS-80 was explored and the results showed significant outcomes in a study conducted on rats by administering the drug through intravenous route in the form of four preparations viz., 1) a solution in saline, 2) a solution in PS-80 1% in saline, 3) a solution bound to poly (butyl cyanoacrylate) nanoparticles and 4) a solution bound to poly(butyl cyanoacrylate) nanoparticles overcoated with 1% PS-80. In this study, animals were sacrificed after 1, 2, 4, 6, and 8 hr and the doxorubicin concentration in lungs, kidneys, spleen, plasma, liver, heart and brain was determined. High brain concentrations of doxorubicin were achieved with the nanoparticles overcoated with polysorbate 80 between 2 and 4 hr. The study demonstrated that the concentration of systemically administered doxorubicin could be enhanced to 60 fold in the brain by biodegradable poly (butyl cyanoacrylate) nanoparticles, overcoated with PS-80 [34].

The drug release mechanism from PS-80 coated particles is mainly based on the endocytosis through the brain blood vessel endothelial cells [35, 36]. There are studies which were conducted on the drug release into the brain inulin spaces in rats and the uptake of fluoresceine isothiocyanate labeled nanoparticles in immortalized rat cerebral endothelial cells (RBE4), were measured. The inulin spaces after i.v. injection of polysorbate 80 coated nanoparticles were significantly increased by 1% compared to controls. In an *in vitro* experiment, endocytic uptake of fluorescent nanoparticles by RBE4 cells was only observed after polysorbate 80 overcoating of particles instead of uncoated particles. These results further support the hypothesis that the mechanism of blood brain barrier transport of drugs by polysorbate 80 coated nanoparticles was of endocytosis followed by possible transcytosis [37].

Nonionic surfactants have huge potential for increasing the bioavailability of poorly aqueous soluble lipophilic drug. The ability to cross blood brain barrier by nonionic surfactants intact is based on their activity as a P-gp inhibitor. PS-80 was reported as the most potent P-gp inhibitor based on the study conducted in Caco-2 cell monolayers [38]. The P-gp mediated transport of peptide drug in Caco-2 cell monolayers showed enhanced permeability by using PS-80 in concentrations up to 30 µM [39].

Furthermore, PS-80 was also showed to increase the digoxin transfer across isolated rat jejuna tissue at a concentration of 0.5% w/v. The increase in concentration of PS-80 to 1% w/v showed 30% increase in AUC [0-10 hr] and for 10% w/v PS-80 the AUC value was increased to 61% [40]. PS-80 was also reported to enhance chylomicron secretion in Caco-2 cell monolayers which in turn enhances the lymphatic transport of lipophilic drugs. Such effect of PS-80 was proved beneficial for P-gp substrates for showing inhibitory action [41].

3. Inhibition by Phospholipids

Along with polysorbate, the use of natural and synthetic polymers especially phospholipids has proved its worth. Phospholipids as a carrier for anticancer drugs are very common but their inhibitory action over P-gp was also reported. Synthetic phospholipids especially 1,2-dioctanoyl-sn-glycero-3-phosphocholine (8:0 PC) and 1,2-didecanoyl-sn-glycero-3-phosphocholine (10:0 PC) were used for determining their influence on the cellular transport of P-gp substrate rhodamine 123 (RH123) on Caco-2 cell layers. The fluorescence anisotropic measurements were then compared with the effects produced by Tween 80 and the P-gp inhibitor verapamil in an *in vivo* study using drug ritonavir. The result showed that the permeability enhancing effect of phospholipid along with P-gp inhibition and also plasma level curve of ritonavir increased tenfold with both phospholipids [42].

The expression of P-gp in the intestinal epithelium resulted in significant impairment in the absorption of active drug into blood plasma. Therefore, some derivatives of phospholipid phosphatidylcholine (PC), either two saturated fatty acid residues of eight (8:0 PC) or ten carbon atoms (10:0 PC), or of two unsaturated docosahexaeonic acid residues (cis-22:6 PC) were reported to be significant P-gp inhibitors in Caco-2 and MDCKII mdr1 and wildtype cell lines [43]. The approach of using lipid nanocapsules loaded with etoposide showed P-gp inhibition activity on glioma cell lines and even the activity was independent of the size of the nanocapsules. The mechanism of drug release from lipid nanocapsules in combination with intracellular P-gp inhibition ensured a higher anticancer drug concentration inside the cancer cells [44].

The efficiency of lipid nanocapsules as a potent P-gp inhibitor was also determined in the case of paclitaxel bioavailability and transport across an intestinal barrier. The study was determined in Caco-2 cells and comparison was made with P-gp inhibitors (verapamil and vinblastine). Experiments were performed with dye-labelled LNCs and

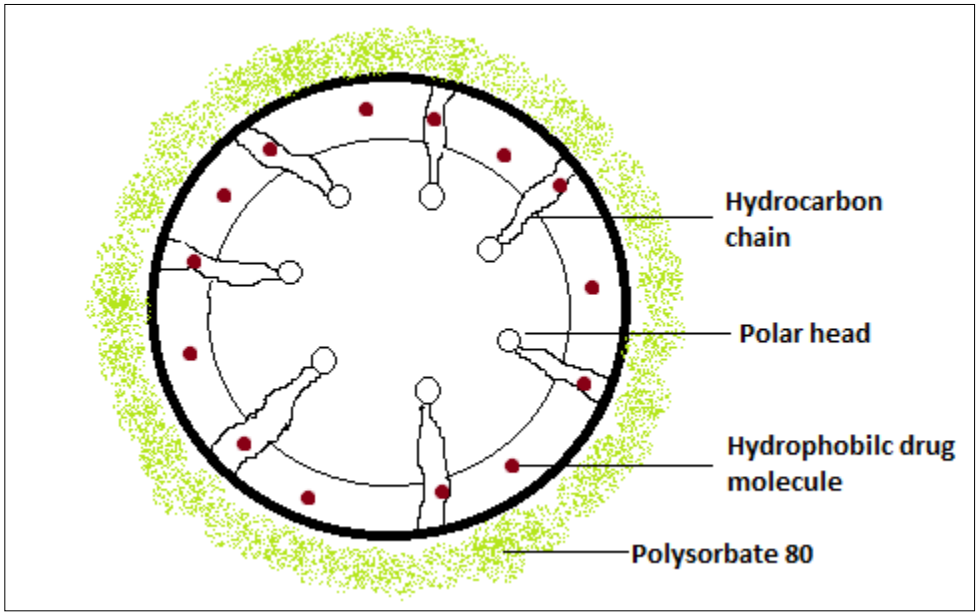

Fig. 2. Schematic diagram of drug entrapped in polysorbate-phospholipid complex

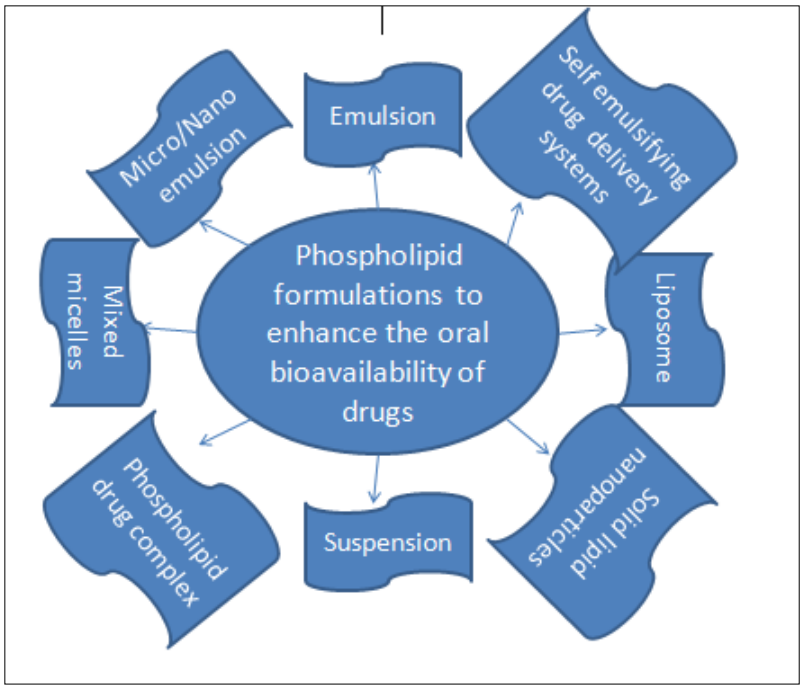

Fig. 3. Formulations of Phospholipid

it was found that the permeability of paclitaxel varied on administration with LNCs on comparison with verapamil and vinblastine [45]. The use of cholesterol as fluidity buffer with phospholipid is very essential for the formulation of liposomes but elevated cellular cholesterol levels reported to increase the P-gp activity in human peripheral blood mononuclear cells (PBMCs) [46].

4. Polysorbate-Phospholipid Mixed Micelles

The drug delivery to brain requires the high permeability of drug to cross blood brain barrier and for such highly lipophilic drugs sufficient hydrophilicity is also required for its transport in the lymphatic system. The use of phospholipids for permeability enhancer and polysorbate for solubilization is very common [47, 48]. The efficiency of both has been proved for their P-gp inhibitory action [49]. The development of polysorbate-phospholipid mixed micelles as drug delivery system is made mainly for improving the stability and therapeutic index of the drug [50]. The basic parameters for such delivery system are micelle size, morphological features, dilution stability and critical micelle concentration (CMC). Miceller delivery systems (less than 100 nm) are bound to show target drug delivery action [51]. Stabilized micelles lead to more sustained release or might circulate for a longer period of time. Stability strategies can be used to stabilize the shell or core of the micelles to make them resistant to dilution [52]. In one study, it was indicated that micelles would be more stable and more easily absorbed with small size and higher zeta potential values [53]. The average zeta potential value

was 28.6 ± 0.2 mv and the mean particle size of the micelles was 90.5 ± 0.8 nm for obtaining stable micelle delivery system [54]. PS-80/Phospholipid mixed micelles were reported to increase the tumor therapeutic effects of docetaxel in kidney, spleen, ovary, uterus, heart and liver and were considered as a potential alternative for clinical intravenous administration of docetaxel [50]. Mixed micelles were also reported safe for intravenous injection as they remain stable at high pH and even micelle size and encapsulation efficiency can be retained upon dilution [55].

5. Conclusion

Polysorbate-80 was proved to be one of the preferred compounds for targeted drug delivery into the brain. The applicability of natural and synthetic phospholipids as a carrier for anticancer drugs was worthwhile. Being biodegradable, biocompatible and non-immunogenic nature prevent its efflux from any kind of selective transport mechanism. The developments of polysorbate-phospholipid mixed micelles proved to deliver active drug molecules to various tissues into the body where the entry of drug as intact is restricted by P-glycoprotein enzymes. Such specific competitive mechanism followed for the modulation of P-gp fundamentally results in enhanced bioavailability, half-life and clearance time of drug molecule through the body. Various research has been published regarding the delivery of drug molecules to target site through polysorbate-phospholipid mixed micelles by inhibiting P-glycoprotein efflux system but still advancement in formulation development is required as optimizing the concentration of polysorbate 80 really requires various factors to be considered. Also, the real mechanism of P-gp inhibition by this system needs to be explored.

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