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Novel formulation approaches for optimising delivery of anticancer drugs based on *P*-glycoprotein modulation

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Considerable research efforts have been directed towards understanding the enigma of *P*-glycoprotein (*P*-gp) in drug development and delivery. *P*-gp is a multi-specific drug efflux transporter that plays a significant role in governing the bioavailability of various anti-cancer drugs. Modulation of this efflux transporter by various traditional 'chemosensitisers' forms a distinctive approach in improving pharmacokinetics and conquering drug resistance. However, such inhibitors show limitations associated with their safety and unwanted pharmacokinetic drug interaction restraining their clinical applicability. To address these concerns, several research groups have used pharmaceutical excipients (functional excipients or additives) to inhibit *P*-gp and enhance drug permeability. This article focuses on such excipients, various co-development strategies for the formulation of cytotoxic drugs with this multi-drug resistance (MDR) reversing additives.

Introduction

Anti-cancer drug treatment is seriously affected by various undesirable properties such as poor solubility, narrow therapeutic index and efflux transporter specificity. Among various efflux transporters, *P*-glycoprotein (*P*-gp) has received enormous attention in both cancer research and pharmaceutical field. *P*-gp transporter impedes the permeability of drugs through physiological barriers producing limited pharmacological response. It influences **absorption**, by expelling drug molecules back into the gastrointestinal (GI) lumen; **distribution**, by preventing entry into tissues like brain; **metabolism**, as it acts synergistically with Cytochrome P450 3A; **excretion**, by affecting both biliary and renal tubular function. Moreover, expression of *P*-gp in the tumor cells also leads to multi-drug resistant phenotype that imposes a big challenge. Such findings have given impetus to the research efforts focused towards circumventing this efflux carrier.

P-gp may be tackled by (i) development of novel agents that are non-*P*-gp substrates, (ii) administration of agents known as *P*-gp inhibitors that inhibit *P*-gp or (iii) designing formulations that allow the drug to bypass efflux pump transport. The (ii) technique is most explored, resulting in the development of several generations of *P*-gp inhibitors over past two decades. Unfortunately, the results of studies aimed at overcoming multi-drug resistance (MDR) and improving oral drug treatment in cancer patients by the concomitant use of first-generation or second-generation Pgp inhibitors and anti-cancer drugs have been disappointing. Their clinical applicability was found to be limited leading to continuous a search for identification of more effective and safe *P*-gp inhibitors. The ideal *P*-gp inhibitor is the one that is non-toxic and does not have any pharmacological activity of its own [1].

Recently it was reported that some excipients, which are commonly added to pharmaceutical formulations, could inhibit the function of *P*-gp in the intestine. These excipients (or additives) offer advantages of being safe, not being absorbed from the gut, pharmaceutically acceptable and have a history of being incorporated in many parenteral and enteral formulations as solubilising or stabilising agents [2].

These findings opened new vistas in oral chemotherapeutics especially to overcome *P*-gp-mediated MDR. This was followed by development of novel drug delivery systems (DDS) such as microspheres, liposomes or nanoparticles that inherently possess

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moderate *P*-gp blocking activity [3]. Recently, combination of both approaches has been explored to further enhance the efficacy of chemotherapy wherein therapeutic agent and chemosensitiser are incorporated into the same carrier for simultaneous delivery into the cell [4,5]. The exact strategy implemented, however, has been varied and till now there has been no consensus regarding which strategy provides the best treatment outcome. In some cases only the chemosensitiser is encapsulated, whereas in other studies, cytotoxic drug is encapsulated and chemosensitiser is free or co-encapsulation of both agents may also occur [6]. STEALTH particles are also reported to overcome *P*-gp-mediated efflux by delivering concentrated levels of drug at the plasma membrane resulting in saturation and reversion of *P*-gp [7]. Polymeric conjugates and mixed micelles constitute another approach to bypass *P*-gp since they are transported into the cell via receptor-mediated endocytosis in contrast to diffusion for free drug [8,9]. The degradation products of carriers may also block *P*-gp by direct interaction and inhibition [10].

This review explores the possibility of using pharmaceutical excipients as *P*-gp inhibitors. Additionally, the mechanisms behind *P*-gp inhibition and the formulation strategies for development of novel drug delivery system are also discussed. This information will further aid in designing oral formulations of anti-cancer drugs.

Pharmaceutical excipients as *P*-gp inhibitors

Various pharmaceutical agents both from synthetic and natural sources, belonging to the categories of cosolvents, surfactants, polymers and lipid excipients have been shown to have *P*-gp inhibitory activity (Table 1). These components increase the absorptive transport of *P*-gp substrates by inhibiting secretion directed transport. The mechanism by which excipients inhibit *P*-gp activity varies with the excipient-type and is currently under investigation. However, several theories have been proposed (Figure 1). Solvents and surfactants interact with the polar head of the lipid bilayers modifying hydrogen bonding and ionic forces and may insert themselves between the lipophilic tails of the bilayers. These membrane perturbations have been shown to modulate *P*-gp activity by causing fluidisation of the lipid membranes [11]. Batrakova and Kabanov showed that pluronics sensitise *P*-gp by inhibiting ATPase activity resulting in ATP depletion [12] while peceol and Gelucire 44/14 downregulate MDR1 gene expression and *P*-gp protein expression in Caco-2 cell culture system [13]. It is also observed that some excipients also affect direct binding to the *P*-gp, inhibit Protein kinase C activity, reduce phosphorylation of *P*-gp, and modulate *P*-gp-mediated efflux [14]. Mixed micelles have reported to bypass *P*-gp drug efflux since the drug accumulation was not influenced by verapamil, a well-known *P*-gp inhibitor [8]. Polymers with thiol groups such as chitosan–thiobutylamidine (chito–TBA) are proposed to inhibit *P*-gp because of interaction with cysteine groups located in the transmembrane region of the *P*-gp. The various categories of excipients may be described under following headings.

Surfactants

The chemosensitising effects of surfactants were first reported using polysorbate 80, towards daunomycin [15]. This was followed by studies from Woodcock *et al.* [16] and since then several non-ionic surfactants such as Tweens®, Spans, Cremophors (EL and RH40), Pluronics and vitamin E TPGS possesses *P*-gp inhibitory activity [17]. Cremophor EL is being used now as a part of commercial formulations of paclitaxel (Taxol), but this formulation is toxic [18]. In general, non-ionic surfactants enjoy the advantage of being more hydrophobic and relatively less toxic to biological membranes, thereby having better capacity to dissolve water-insoluble drugs [19]. Various studies demonstrate the ability of

TABLE 1

Excipients used as *P*-gp inhibitors in anti-cancer formulation development Compiled from [13,14,17,21,28,50–54].

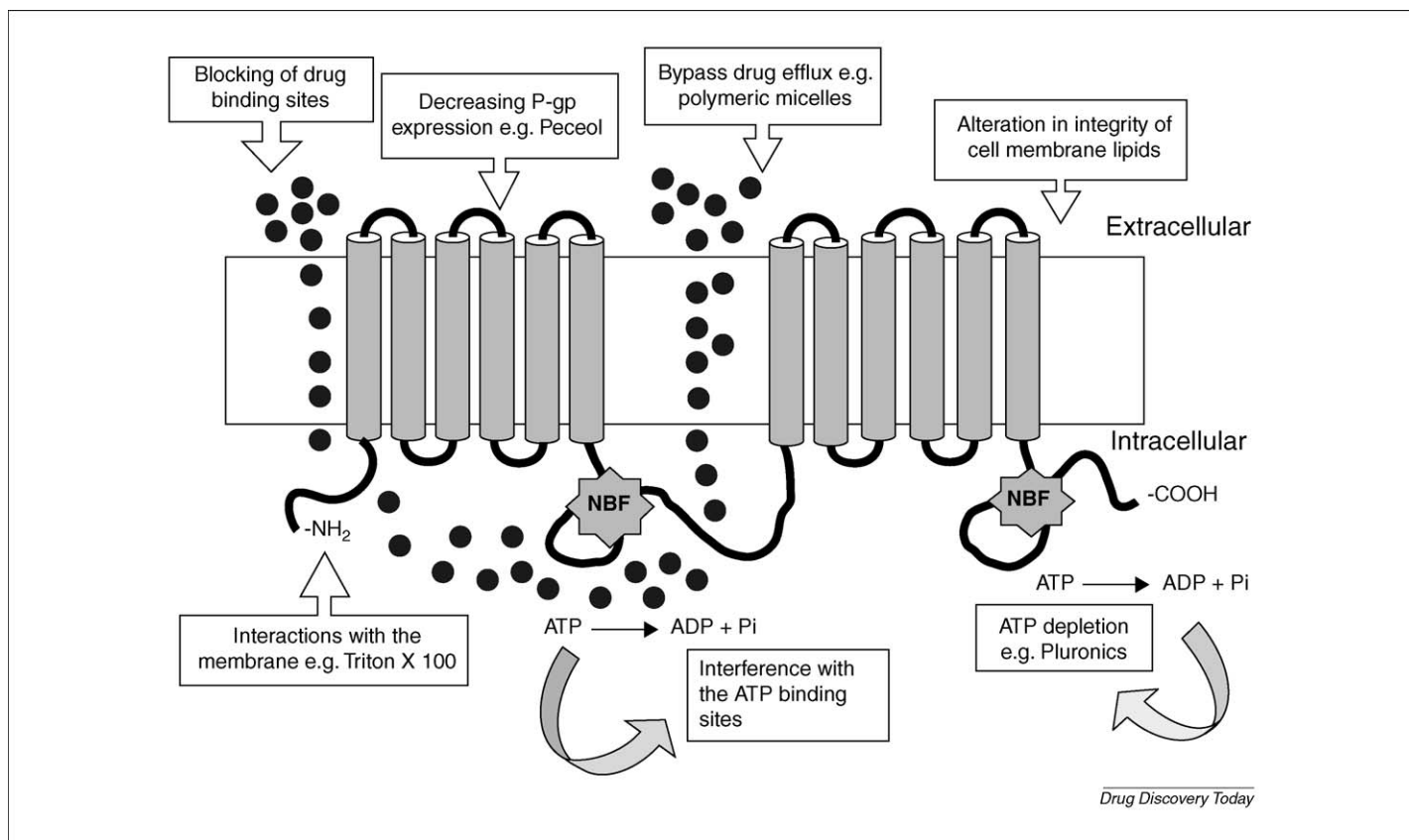| Surfactants	Polymers		Miscellaneous
	Natural	**Synthetic**	
Tween 20/80	Anionic gums	Polyethylene glycols	Detergent
Span 20	Xanthan gum	Polyethylene glycol 300/400/2000/20000/660 hydroxystearate	7-Octyl-β-D-glucoside
Cremophor EL/RH 40	Sodium alginate	Dendrimers	Glycerides
TPGS 200/238/400/600/1000/ 2000/3400/3500/4000/6000	Flavicam	Generation 3 (G3) and lauroyl-G3 polyamidoamine (PAMAM)	Imwitor 742
Gelucire 44/14	Ascophyllum	Thiomers	Solubilising agent
N-octyl glucoside		Chitosan–thiobutylamidine (chito–TBA)	Softigen 767
Acconon E		Poly(acrylic acid) cysteine	Neutral oil
Triton X-100		Amphiphilic diblock copolymers	Miglyol
Solutol HS 15		Methoxypolyethylene glycol–blockpolycaprolactone (MePEG–b-PCL)	Lipid excipients
Labrasol			Peceol
N-lauryl-b-D-maltopyranoside			
Myrj-52			
Brij-30			
Poloxamers			
Pluronic-P85/F68			
CRL-1605			

FIGURE 1

Mechanism by which excipients tackle *P*-glycoprotein-mediated efflux. Modified from [1].

Tweens® to inhibit efflux pumps. Lo demonstrated that Tween 20, Tween 80, Myrj 52 and Brij 30 increased the epirubicin transport and reduced efflux in diffusion chambers with excised rat intestinal mucosa [11].

Two important parameters that govern *P*-gp inhibitory activity include surfactant concentration and hydrophilic–lipophilic balance (HLB). Concentrations that are non-toxic to the intestinal mucosa are most commonly used to inhibit *P*-gp. As these surfactants are primarily used for solubilisation of hydrophobic drug, it would be advantageous if they were more active above the crucial micelle concentration (CMC), since they would provide dual action of solubilising hydrophobic substrates as well as inhibiting efflux [17]. However, the pattern of *P*-gp inhibition varies with the type of excipients. In certain cases, *P*-gp inhibitory effect increases till CMC is reached and after CMC there is loss of the inhibitory effect owing to drug (*P*-gp substrate) entrapment in the micelles. In another scenario, inhibitory effect increases even beyond CMC and this could be attributed to the fact that drug entrapped in micelles bypass *P*-gp-mediated efflux [20].

The optimal HLB value of surfactant systems with suitable hydrocarbon chains and polar groups is an important factor in designing promising drug formulations. The optimal enhancement on the intracellular accumulation of epirubicin was characteristic with intermediate HLB values ranging from 10 to 17 [11]. Collnot *et al.* investigated the influence of the length of the alkyl-chain of various TPGS derivatives on their efflux pump inhibitory activity in order to gain more information regarding its mechanism [21]. Results of 10 different TPGS derivatives ranging from TPGS 200–6000 revealed that the commercially available derivative TPGS 1000 is the so far most potent efflux pump inhibitor. TPGS has also been used to improve bioavailability of paclitaxel [18,22].

Pluronics could enhance Caco-2 cell accumulation of rhodamine at concentrations below the CMC since they show greater permeabilising ability at such concentrations [23]. Pluronics (poloxomers) are very potent, non-toxic, practical and near-market use pharmaceutical excipients. The biological activity of Pluronics is attributed to their ability to incorporate into membranes followed by subsequent translocation into the cells and affecting various cellular functions, such as mitochondrial respiration, ATP synthesis, activity of drug efflux transporters, apoptotic signal transduction, and gene expression. As a result, Pluronics cause drastic sensitisation of MDR tumors to various anti-cancer agents, enhance drug transport across the blood brain and intestinal barriers, and causes transcriptional activation of gene expression both *in vitro* and *in vivo* [12].

Polymers

Polymeric efflux pump inhibitors owing to their high molecular weight, offer advantage of not being absorbed from the intestine and are subsequently free from systemic toxic adverse effects. In addition, they tend to remain in the upper regions of GIT where *P*-gp activity is lower than in the distal parts [24]. Biodegradable polymers [poly(lactide), poly(D,L-lactideco-glycolide) (PLGA) and poly(caprolactone)] provide the safest way of sustained, controlled and targeted drug delivery to improve the therapeutic effects and reduce the side effects of the formulated drugs [25].

Natural polymers include polysaccharides, polypeptides and proteins. Xanthan gum and sodium alginate demonstrated accumulation of P-gp substrates vinblastine and doxorubicin in everted gut sac model [26]. Synthetic polymers belonging to the category of polyethylene glycols (PEGs), dendrimers and thiomers have been extensively reviewed by Werle [27]. PEGs are polyethoxylated excipients added in pharmaceutical formulations to increase aqueous solubility. Polyoxyethylene groups are required to achieve increase in drug transport by P-gp inhibition [14]. PEG 400, 2000 and 20 000 enhanced the absorptive transport of model substrate rhodamine irrespective of its molecular weight. The inhibitory effects by PEGs were concentration-dependent over the range of 0.1–20% [28].

Conjugation of the P-gp substrate propranolol, to generation 3 (G3) and lauroyl-G3 polyamidoamine dendrimers improved absorptive transport and decreased secretory transport through Caco-2 monolayers. The mechanism responsible for this behavior was attributed to bypass the P-gp efflux transport rather than to P-gp inhibition [29]. It has also been demonstrated that thiomer chitosan–thiobutylamidine (chito–TBA) improves apical to basolateral transport and decreases basolateral to apical transport of rhodamine through excised guinea pig ileal mucosa. The effect was even more pronounced when combination of chito–TBA and glutathione (GSH) was used. This is because GSH is capable of inhibiting the enzyme that regulates opening and closing of tight membrane junctions [30].

A family of block graft copolymers of the poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) Pluronic® polyethers and poly(acrylic acid) (PAA) bound by carbon–carbon bonds have been emerged, wherein both polymeric components are generally recognised as safe. At the physiological pH, the Pluronic–PAA copolymers are surface active and self-assemble into intra-molecular and inter-molecular micelles. These micelles efficiently solubilise hydrophobic drugs such as paclitaxel. They inhibit P-gp owing to high surface activity that results in interactions with cell membranes and suppression of efflux pumps [31]. In a more recent investigation, the diblock copolymer enhanced the accumulation of various P-gp substrates, paclitaxel and doxorubicin in P-gp overexpressing MDR cells but did not influence substrate accumulation in non-P-gp expressing cells [32].

Lipid-based excipients

Co-administration of drugs with compounds that inhibit P-gp-mediated efflux or the incorporation into specific lipid excipients can alter the pharmacokinetics of the administered compound. There is a growing body of evidence that certain lipids can inhibit presystemic drug metabolism and can play an active role in P-gp-mediated efflux from the enterocytes. Peceol® and Gelucire 44/14 reduced the secretory efflux at <0.5% concentrations. The inhibitory effect produced was found to be comparable to that of 100 μM verapamil. The excipients have been shown to down-regulate MDR1 gene expression and P-gp protein expression in the Caco-2 cell culture system. However, it remains unclear if and how transporter expression is influenced by formulation components that are assumed to be inert [13].

Formulation approaches based on P-gp modulation

In order to appreciate the potential of P-gp in transport of anti-cancer compounds to tumor cells with more efficiency and specificity, several delivery systems like microspheres, nanosized drug carriers (nanoparticles, nanoemulsions, stealth liposomes, nanogels, polymer–drug conjugates), novel powders, hydrogels, mixed micellar systems intended for systemic and/or localised delivery have been developed (Table 2). The success of these drug delivery systems is attributed to their small size, reduced drug toxicity, controlled drug release, as well as modification of drug pharmacokinetics and/or biodistribution [3]. The other novel formulation approaches such as implants, molecular targeting using immunoliposomes-based antibody-directed binding and internalisation have also been investigated. Formulation development efforts focus towards selecting biodegradable polymers of desired performance, making nanoparticles of smaller size, and coating particles with bioadhesive materials such as carbopol, chitosan, gelatin, pectin, alginate, PEG. In addition, coating of the substrate by P-gp inhibiting material, either by encapsulation or conjugation, may allow nanoparticles to evade P-gp [33]. Following sections discuss the delivery systems developed so far using P-gp inhibitory excipients.

Polymer-based drug delivery systems
Nanoparticulate systems

Co-administration of P-gp inhibitors and the encapsulation of anti-cancer drugs in nanoparticles offer a potential approach for circumventing P-gp-mediated efflux. It allows the drug to evade recognition by P-gp at the plasma membrane, allowing its delivery to the cell cytoplasm or nucleus. Nanoparticles enhance the therapeutic efficacy of an encapsulated drug by increasing and sustaining the delivery of the drug inside the cell. Chavanpatil et al. investigated that paclitaxel, a P-gp substrate; encapsulated PLGA nanoparticles are susceptible to P-gp-mediated drug efflux in MDR tumor cells [34]. Resistance to nanoparticle-encapsulated paclitaxel was reversed by verapamil, a P-gp inhibitor. The study revealed that sustained inhibition of P-gp was necessary for sustained therapeutic efficacy of nanoparticle-encapsulated drug. In another study, Aerosol OT (AOT)-alginate nanoparticles of doxorubicin were formulated, which enhanced the cellular delivery and therapeutic efficacy of P-gp substrates in P-gP-overexpressing cells [35].

Nanogel

A novel drug delivery system (NanoGel^TM) was developed by cross-linking a cationic polymer and a non-ionic polymer for anti-sense phosphorothioate oligonucleotides (SODN), specific to human mdr1 gene. SODN molecules can be easily immobilised in these systems by simple mixing. SODN preserved the ability to inhibit the P-gp expression in a sequence-specific fashion following incorporation in the NanoGel^TM. Modification of SODN-loaded Nano-Gel^TM particles with transferrin targeting moieties additionally increased the mdr1-inhibitory effects. SODN-loaded NanoGel^TM particles were able to cross the Caco-2 intestinal cell monolayers with high efficiency. These NanoGel^TM carriers can be used for effective per oral delivery of anti-sense SODNs [36].

Polymeric micelles

Formulation comprising mixed micelles of a hydrophobic Pluronic L61 and relatively hydrophilic F127 loaded with doxorubicin (SP1049C, Supratek Pharma, Montreal, Canada) via its physical entrapment, has advanced into clinical trials and could be first

TABLE 2

Various strategies implemented for tackling *P*-gp-mediated drug efflux

Strategy	Drug delivery system	Formulation	Excipients	Techniques	Drug/substrate	Cell lines/model	Ref.
Formulation based	Conventional drug delivery	Tablet	Chitosan-4-thiobutylamidine Myrj 52 Pluronic-P85	Compression	Rhodamine-123	Rat intestinal	[24]
	Novel drug delivery system	Micelles	Poly(ethylene glycol) 2000–phosphatidyl ethanolamine conjugate (PEG2000–PE) and D-alfa-tocopheryl polyethylene glycol 1000 succinate (TPGS)	Solubilisation	Paclitaxel	Caco-2 cells	[8]
		Nanoparticles	Poly(D,L-lactide-co-glycolide) (PLGA)	Emulsion-solvent evaporation	Paclitaxel	MCF-7 tumor cells	[34]
		Copolymer conjugate	Doxorubicin-N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer	Chemically modified	Doxorubicin	CEM/VLB, P388-MDR	[39]
		Lipid nanocapsules	Polyethylene glycol–660 hydroxystearate (PEG–HS)	Phase inversion	Etoposide	C6, F98, and 9L glioma cell lines	[42]
		Emulsifying wax nanoparticles	Cetyl alcohol/polysorbate	Microemulsification	Paclitaxel	HCT-15 mouse xenograft model	[43]
		SMEDDS	Vitamin E Cremophor RH 40	Self-emulsification	Paclitaxel	Rat model	[44]
		Microemulsions	Lecithin, butanol, myvacet oil, polysorbate 80	Solubilisation	Paclitaxel	Rats model	[45]
		Polymer–lipid hybrid nanoparticle	GG918	Ultrasonication	Doxorubicin	Breast cancer cell line	[49]
		Nanogels	Hydrophilic poly(ethylene glycol) (PEG)	Emulsification/solvent evaporation	Fludarabine	MCF-7 cells and Caco-2 cell	[55]
		Nanoparticles	Vitamin E TPGS-emulsified poly(D,L-lactic-co-glycolic acid) (PLGA)	Solvent extraction/evaporation	Paclitaxel	Tube shaker	[56]
		Nanoparticles	Tariquidar using poly(D,L-lactide-co-glycolide)	Microemulsification	Paclitaxel	Mouse model	[57]
		Nanoparticles	Poly(lactide)–vitamin E TPGS (PLA–TPGS) copolymers	Dialysis method	Paclitaxel	HT-29; Caco-2 cells	[58]
		Liposome	Verapamil	Encapsulation	Daunorubicin	Breast cancer cell line and resistant sublines	[59]
		Liposomes	PEGylated	Encapsulation	Docetaxel	HT-29 and Igrov1 cell lines	[60]
		Microparticles	Poly(lactic-co-glycolic acid) (PLGA)	Solvent extraction/evaporation	Paclitaxel	Brain tissue	[61]
		Hydrogel	N-(2-hydroxypropyl) methacrylamide (HPMA)	Solubilisation	Doxorubicin	Drug diffusion and Bcl1 leukemia	[62]
Chemical modification	Prodrug	Copolymer conjugate	Doxorubicin-N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer	Chemically modified	Doxorubicin	CEM/VLB, P388-MDR	[39]
	Conjugate	Prodrug	Paclitaxel 2′-ethylcarbonate	Radical copolymerisation	Paclitaxel	Caco-2 and ovarian carcinoma cells	[63]
	Prodrug	Monoclonal antibody	Doxorubicin monoclonal conjugate	Structural Modification	Doxorubicin	Tumor cells	[64]
	Prodrug	Pegylation	Pegylated paclitaxel	Chemically modified	Paclitaxel	Rats model	[65]
Alternative route of administration	Implantable	Films	Chitosan phosphatidyl choline	Homogenisation	Paclitaxel	Human ovarian xenograft model	[66]

FDA-approved chemotherapeutic formulation based on polymeric micelles. It exhibits greater efficacy than non-micellar doxorubicin against a variety of drug-resistant tumors owing to increase in cellular drug influx, inhibition in drug efflux and changes in intracellular drug trafficking [37,38].

Hydrogels

Biodegradable hydrogel could be a potential alternative in the treatment of sensitive as well as resistant cancers, because it allows incorporation of cytostatic drug together with chemosensitiser. Biodegradable hydrogels based on N-(2-hydroxypropyl)methacry-lamide (HPMA) containing the cocktail of doxorubicin with cyclosporine were synthesised. These formulations maintained the level of both drugs to reach the desired pharmacological effect, thereby preventing the toxic effects [39].

Microgels

Novel microgels composed of cross-linked copolymers of poly(acrylic acid) (PAA) and Pluronics in weight ratio of 55:45, were evaluated as possible permeation enhancers for doxorubicin transport using Caco-2 cell monolayers. The developed microgels enhanced the overall cell absorption of doxorubicin by inhibiting P-gp-mediated doxorubicin efflux from the cells and enhancing the passive influx. Pluronic–PAA copolymers exhibited synergism of the doxorubicin transport enhancement with verapamil, a known inhibitor of the P-gp. These copolymers and their combinations decreased the doxorubicin efflux from Caco-2 cells by several fold [40].

Microspheres

Polymeric microspheres with MDR-reversing capability were prepared and characterised using Triton-X-100-immobilised dextran and insulin. Both drug carriers showed a marked increase in drug accumulation by CHRC5 cells, as compared with free Triton solutions at equivalent concentrations [41].

Lipid-based drug delivery systems
Lipid nanocapsules

Recently, etoposide loaded lipid nanocapsules (LNC) as drug delivery device was developed and evaluated for the drug release and their efficiency to reduce cell growth in cell culture for C6, F98 and 9L glioma cell lines. The developed LNC exhibited a very small size (mean diameter 25–100 nm) that facilitates their intracellular uptake. Additionally, the developed LNC was hypothesised to reverse MDR owing to the presence of P-gp inhibiting surfactant PEG–HS (polyethylene glycol–660 hydroxystearate), one of the LNC constituents [42].

Solid lipid nanoparticles (SLN)

Chitosan-solid lipid nanoparticles–microsphere (CSM) made of chitosan and loaded with stearic acid were developed [4]. The composite CSM system is in the form of microparticles can be used for the delivery of phenethyl isothiocyanate (PEITC), for the treatment of lung cancer via pulmonary route. This system provides an initial burst release of the efflux-transporter inhibitors, such as tamoxifen, verapamil or nifedipine (present in a shell) to suppress or modulate the efflux activity of ATPase binding cassette (ABC) transporters followed by the gradual, sustained release of the efflux-transporter substrate, PEITC. Developed composite CSM system provides a one-bullet dosage form for convenient and effective delivery drug into tumors [4].

A solid lipid nanoparticle (SLN) system containing an anionic polymer for the delivery of cationic anti-neoplastic agents and model chemosensitisers (e.g. verapamil) was developed using microemulsion method [5]. Ionic complexation was utilised to enhance the loading of these highly water-soluble drugs. The influence of anionic compounds and polymers on drug partition and loading into SLNs was investigated, and dextran sulfate (DS) was found to be the most suitable among those studied. Dual drug (doxorubicin/verapamil or quinidine/verapamil)-loaded DS-SLNs were also formulated, which released both drugs without noticeable interference to each other.

Paclitaxel entrapped in emulsifying wax nanoparticles (PX NPs) was prepared by Koziara et al. and in vivo efficacy of PX NPs in a HCT-15 mouse xenograft model was studied. Significant inhibition in tumor growth was observed in mice receiving PX NPs treatment [43]. The enhanced efficacy of prepared formulation over conventional Taxol formulation could be attributed to the ability of PX NPs to overcome MDR via enhanced delivery and an anti-angiogenic effect.

Self-microemulsifying drug delivery systems (SMEDDS)

A novel SMEDDS comprised vitamin E as an oil phase, deoxycholic acid sodium salt, TPGS and Cremophor RH 40 as surfactants to increase the solubility of paclitaxel was developed with or without concomitant use of P-gp inhibitors, for enhanced oral absorption of paclitaxel. Compared with Taxol, the oral bioavailability of paclitaxel SMEDDS increased by 28.6–52.7% at various doses. The surfactants might moderately inhibit the P-gp efflux system, leading to a slight improvement of paclitaxel oral absorption. In contrast to this following co-administration with cyclosporine A, paclitaxel SMEDDS showed a higher bioavailability and much longer time above the therapeutic level than Taxol did. The findings indicate that SMEDDS is a promising delivery system for the efficient oral administration and enhancement of oral absorption of paclitaxel, especially when incorporated with an effective P-gp inhibitor and CYP3A4, such as cyclosporin [44].

Microemulsions

Cremophor-free oral microemulsions of paclitaxel using lecithin, butanol and myvacet oil to enhance its permeability and oral absorption were developed [45]. Paclitaxel permeability was significantly increased in the presence of the P-gp/CYP3A4 inhibitor cyclosporine A (CsA). This enhancement may be attributed to the P-gp inhibitory effect of the surfactants, oil and/or the membrane perturbation effect of the surfactants.

Liposomes

Liposomal preparations of PSC 833 provide a useful alternative dosage form for intravenous administration of PSC 833 to be combined with anti-cancer drugs to circumvent drug resistance in cancer chemotherapy [46]. Liposome formulations containing a small fraction of PEG-derivatised phospholipid has been shown to alter dramatically the pharmacokinetic properties of doxorubicin, leading to long elimination half-life and small volume of distribution [47]. Anionic liposomes are internalised by certain cells and provide drug release in intracellular compartments to bypass P-gp.

Neutral phospholipids such as phosphatidylcholine and phosphatidylethanolamine, the primary constituents of many liposomal membranes, have been suggested to be *P*-gp substrates that can compete with drugs for *P*-gp binding [48].

Polymer–lipid hybrid nanoparticle (PLN)

A new lipid-based system was developed from solid lipid nanoparticles by incorporation of an anionic polymer into lipids (PLN), to complex the cationic drug, thus increasing its partition in the lipids [49]. The developed formulation was capable of delivering a cytotoxic drug, doxorubicin (Dox), a chemosensitiser, GG918, or their combination. The results showed that the encapsulation efficiencies of Dox and GG918 in PLN were up to 89% and were not compromised by co-encapsulation of the two agents. Of various combinational treatment approaches, the Dox and GG918 dual agent loaded PLN formulation ((DG)n) demonstrated the highest acute cytotoxic, long-term suppression of cancer cell proliferation, and uptake of Dox by *P*-gp-overexpressing human breast cancer cells while co-administration of two single-agent loaded PLN was least effective.

Conclusions

Great progress has been made in the treatment of tumors by applying the concept of drug delivery systems. Concomitant use of anti-cancer drugs and *P*-gp inhibitors is an effective and safe way to improve oral bioavailability and blood brain barrier delivery. Commonly used pharmaceutical excipients are emerging as a different class of *P*-gp inhibitors owing to advantages of being safe, not being absorbed from the gut and pharmaceutically acceptable. Numerous delivery systems have been developed using polymers, surfactants and lipid-based excipients. There are various mechanisms that can be responsible for efflux pump inhibition, including membrane fluidisation, ATP depletion and interaction with drug-binding or ATP-binding sites. For some excipients, the mechanism still remains unclear. A better understanding of the interaction between efflux pumps and inhibitors would highly contribute to the development of more specific and safe inhibitors. Authors foresee fascinating new developments in this area leading to commercialisation of new and completely bioavailable oral formulations of anti-cancer agents.

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