Chapter 12
Polymer–Drug Conjugates for Targeted Drug Delivery

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Abbreviations

Ara-C	1-b-D-Arabinofuranosylcytosine
CPT	Camptothecin
DDS	Drug delivery system
DOX	Doxorubicin
EPR	Enhanced permeability and retention
HPMA	*N*-(2-Hydroxypropyl) methacrylamide
MA	Methacrylate
MAP	Mucic acid polymer
MDR	Multidrug resistance
PEG	Poly(ethylene glycol)
PG	Poly-L-glutamic acid
RES	Reticuloendothelial system
SMA	Styrene maleic anhydride
TADD	Tissue Activated Drug Delivery
TXL	Paclitaxel

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P.V. Devarajan, S. Jain (eds.), *Targeted Drug Delivery: Concepts and Design*,
Advances in Delivery Science and Technology, DOI 10.1007/978-3-319-11355-5_12

12.1 Drug Delivery Challenges and Pharmacokinetics of Polymer–Drug Conjugates

A drug delivery system (DDS) is defined as a formulation or a device that enables the administration of therapeutic substances in the body and improves its efficacy and safety. Over the past three decades, significant advances have been made in drug delivery technology. Modern technological approach, such as nanotechnology based drug delivery system has profound impact on disease prevention, diagnosis, and treatment in terms of its stability, absorption, therapeutic concentration, and improved pharmacokinetics over native drugs [1–3]. In order to improve the specific delivery of drugs with a low therapeutic index, several nanosized drug carriers have been developed. These nanoformulation vehicles are the polymeric nanoparticles, amphiphilic core/shell (polymeric micelles or liposomes) or hyperbranched macromolecules (dendrimers, drug–polymer conjugates), etc. [4–9].

The concepts of utilizing polymers as therapeutic agents have been widely investigated for a number of decades. In 1975, Helmut Ringsdorf proposed the concept of polymer therapeutics, where a polymer is covalently bound to drug molecules that could improve the aqueous solubility and bioavailability of the drug [10]. These polymer–drug conjugates offers several significant advantages over traditional small molecule therapeutics as they can protect it from degradation, resulting in improved efficacy due to increased drug circulation times, controlled release of drugs in terms of variations in pH, temperature, enzyme concentration, or attachment of ligands for targeting to the desired site of therapeutic need [11, 12]. Further, the polymer–drug conjugates increases the reduction in the uptake by reticuloendothelial system (RES) or macrophages due to stealth effect of the polymer [12]. The increased blood circulation time of the polymer–drug conjugate also enhances the therapeutic index of the drug in tumor tissues, by taking the advantages of leaky vasculature and impaired lymphatic drainage system known as enhanced permeability and retention (EPR) effect (Fig. 12.1) [13–15].

12.2 Important Aspects of Design of Polymer–Drug Conjugates

The basic requirements for the design of polymer–drug conjugates are based on the worldwide screening of the natural and synthetic polymers and its use in vivo screening to find out a biocompatible polymer (i.e., nontoxic, nonimmunogenic, biodegradable, etc.). The identification of the new pharmacological targets arises from the molecular basis of the diseases. A wide range of polymers are available for the delivery of macromolecular drugs [16]. These macromolecular prodrugs comprise a minimum of three components: (1) a natural or synthetic water soluble polymeric carriers; (2) a biodegradable polymer–drug linker; and (3) a bioactive

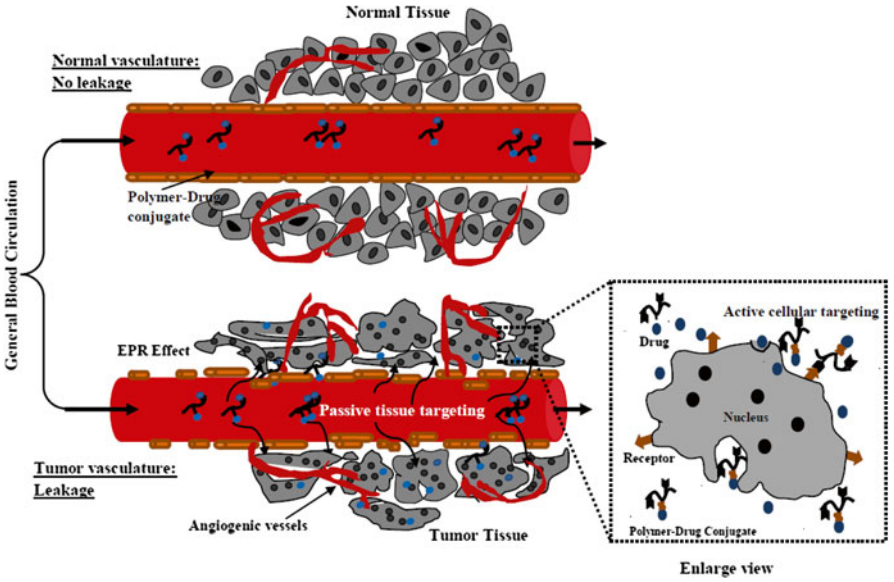

Fig. 12.1 Schematic representative of EPR-mediated tumor targeting

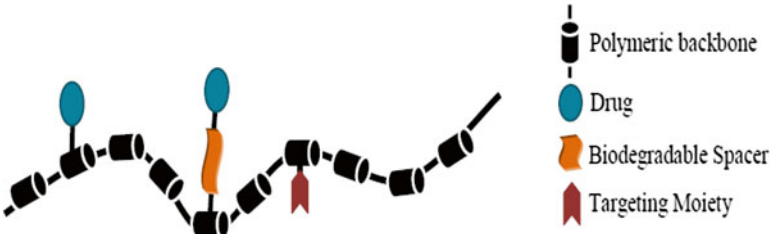

Fig. 12.2 Schematic presentation of polymer–drug conjugates in which a solubility enhancer, a targeting moiety, a spacer, and specific drugs could be attached to the same polymeric chain

antitumor agent (Fig. 12.2). Ligands can also be attached to facilitate active targeting. An ideal targeting system should enable it to overcome the biological barriers that selectively direct it against the diseased cells [17, 18].

12.2.1 The Polymer

The different polymers that are used for the drug conjugation includes *N*-(2-hydroxypropyl) methacrylamide (HPMA), poly-L-glutamic acid (PG), polyethylene glycol (PEG), styrene maleic anhydride (SMA), etc. [19, 20]. These polymers also have been exploited for conjugation to various proteins and drugs. Currently considerable research effort is being focused on the continued development of

improved drug delivery systems. Mostly the polymer–drug conjugate systems are designed for localization of the chemotherapeutic drugs in the targeted mainly cancer tissues to provide a sustained release of drug activity over weeks to months [21]. For this, the choice of polymers should contain the required functional group for the covalent linkage of the drug. Apart from that, the drug carriers for the parenteral administration should have the property of biodegradability, hydrophilicity, and the ability to carry the required payload of the drug for an effective therapeutic application [22].

12.2.2 The Polymer–Drug Linker

In the preparation of the polymer–drug conjugates a biodegradable spacer/linker is inserted to ensure stability in the circulation and subsequently facilitate specific enzymatic or hydrolytic intratumoral drug release. The polymer–drug linker also plays an important role in keeping inactive prodrug until released from the backbone polymer by a disease-specific trigger to help in enhancing the circulatory half-life and biodistribution of the drug [23, 24]. This characteristic feature of disease-specific release from the polymer conjugate has been coined as Tissue Activated Drug Delivery (TADD). Theoretically, the disease specific trigger alters the biodistribution of the active agent, releasing it near the site of pathology. The hydrolytic or enzymatic cleavage of the linker in drug–polymer conjugate is also helpful in the release of the drug, for example presence of the esterases breaks the ester linkage of the conjugation, while amide linkage gives stability in the blood circulation. In some cases the systemic delivery of the drug can be achieved by designing sensitive linkers effective at pH 7.2. Therefore, the choosing desirable option of the linker will be such that it should be stable in the circulation and protect the drug against premature metabolism but amenable to specific enzymatic or hydrolytic cleavage intratumorally.

12.2.3 Ligands

The ligands are used to increase the receptor mediated binding and internalisation of the therapeutic agent into the cells of target tissues. Tumor cells specifically overexpress particular cell surface marker as compared to normal cells, which helps in active targeting and increased cellular uptake. These molecular markers specifically help in active targeting and increased cellular uptake in tumor tissues. Looking at the specificity of the overexpressed markers, the conjugating ligand can be antibody, antibody fragment, peptides, saccharides, or other small molecules. Other targeting moieties such as peptides, sugars, and hormones can generally be readily synthesized at low cost, but they typically have reduced binding affinity and specificity as compared to antibodies and antibody fragments. Ray and coworkers

developed the HPMA copolymers containing cyclic Arg-Gly-Asp (RGD) peptides that target αvβ3 integrins expressed on angiogenic tumor cells and found that the HPMA copolymer–aminohexylgeldanamycin–cyclic RGD had more efficacy via active targeting as compared to conjugates relying solely on "passive" targeting via the EPR effect [25]. Tang et al. used epitope containing HPMA copolymer–doxorubicin conjugates to target the CD21+ Raji B-cells and CD21− HSB-2T-cells. The results showed that, epitope-HPMA copolymer–doxorubicin conjugate have more biorecognition activity towards the Raji cells which demonstrated enhanced cytotoxicity than the free doxorubicin [26].

12.3 Polymer–Drug Conjugates as Drug Delivery Systems

In polymer–drug conjugates the drug is covalently attached to the polymeric backbone. The structure and architecture of the polymer gives scope for designing novel polymer–drug conjugates for drug delivery. Over the past few years, a number of polymer–drug conjugates have entered clinical studies (Table 12.1). In this regard potential therapeutic application of different polymer–drug conjugates are discussed with special emphasis on HPMA, PG and PEG (Fig. 12.3).

12.3.1 N-*(2-Hydroxypropyl)methacrylamide (HPMA) Copolymers*

N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymer belongs to homopolymer group. It has the characteristic features of highly water solubility, non-immunogenic, nontoxic, non-binding activity to blood proteins with long biological half-life. Thus, it can be used as macromolecular carrier for the low molecular weight anticancer chemotherapeutic to amplify the circulation time of the therapeutic agent in blood and limit the side effects. The HPMA copolymer is generally synthesised by free radical polymerization using HPMA and methacrylated (MA)-peptidyl-nitrophenylester (ONp) as co-monomers in a ratio of 95:5 % w/w. The synthesis of narrow molecular weight HPMA copolymer (Mw/Mn = 1.2–1.5 kDa) is possible by careful control of the co-monomers and reaction conditions for polymerization kinetics. The HPMA copolymer backbone has the disadvantage that it is not inherently biodegradable. So there is risk of cellular accumulation due to sequestration in the lysosomal compartment, however the conjugates with molecular weight of <40,000 g/mol could ensure elimination by glomerular filtration after parenteral administration. To overcome these problems the back bone of the HPMA copolymer can be grafted by various functionalized co-monomers, allowing control over the composition of these conjugate systems and reduce the immunogenicity. Kopecek et al. investigated the biocompatibility properties of the HPMA polymer by substituting the α-carbon and the presence of an amide linkage in the side chain to ensure hydrolytic stability [20, 27].

Table 12.1 Polymer–drug conjugates in clinical trials

Conjugates	Indication	Status	Company
N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers–drug conjugate			
HPMA copolymer–doxorubicin (PK1; FCE28068)	Particularly lung and breast cancer	Phase II	Pfizer; CRC UK
HPMA copolymer–doxorubicin–galactosamine (PK2; FCE28069)	Hepatocellular carcinoma	Phase I/II	Pfizer; CRC UK
HPMA copolymer–platinate (AP5280, ProLindac)	Various cancers, particularly ovarian, colorectal cancers	Phase I/II	Access pharmaceutical
HPMA copolymer–DACH–platinate (AP5346, ProLindac)	Various cancers	Phase II	Access pharmaceutical
HPMA copolymer–paclitaxel (PNU166945)	Various solid tumors	Phase I; discontinued	Pfizer; CRC UK
HPMA copolymer–Camptothecin (PCNU166148)	Solid tumors	Phase I; discontinued	Pfizer Inc.
HPMA copolymer–carboplatinate (AP5280)	Ovarian carcinoma, lung and head cancers	Phase I/II	
Poly(α,L-glutamic acid)–drug conjugates			
PG–paclitaxel (CT-2103, Xyotax)	Lung, ovarian, colorectal, breast cancer	Phase III	Cell Therapeutics
PG–camptothecin (CT2106)	Colorectal, lung, and ovarian cancers	Phase I	Cell Therapeutics
Poly(ethylene glycol)–drug conjugates			
PEG–Camptothecin (Pegamotecan)	Solid tumors	Phase I; discontinued	Enzon Pharmaceuticals
PEG–SN38 (EZN-2208)	Solid tumors	Phase I	Enzon Pharmaceuticals
Other polymer–drug conjugates			
Carboxymethyl Dextran–camptothecin (IT-101)	Solid tumors	Phase I	Insert Therapeutics
Carboxymethyldextran–exatecan (DE-310)	Solid tumors	Phase I	Daiichi Pharmaceuticals

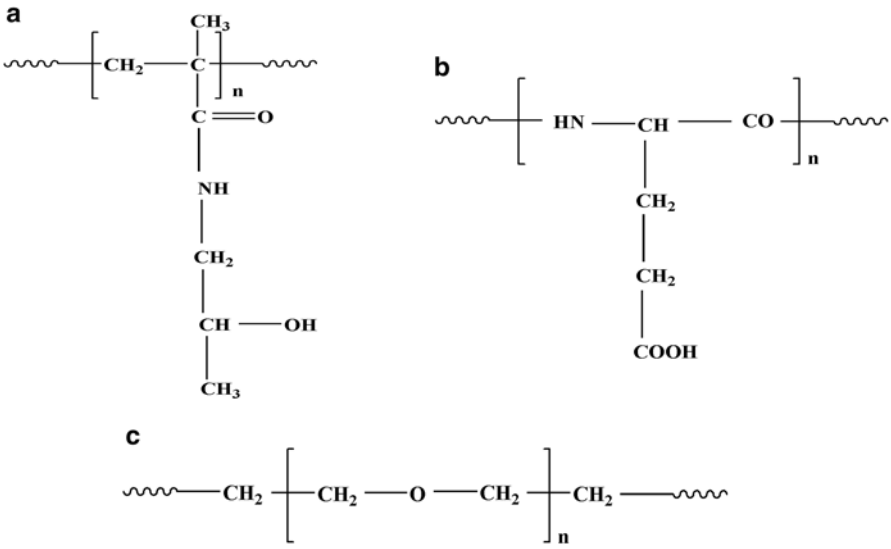

Fig. 12.3 Chemical structures of (**a**) *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, (**b**) poly(α,L-glutamic acid) polymers, (**c**) poly(ethylene glycol) polymers

12.3.1.1 HPMA Copolymer–Doxorubicin

Doxorubicin is an anthracycline antibiotics used in cancer chemotherapy and works by intercalating with DNA along, with the most adverse effect of cardiac toxicity. It is commonly used in the treatment of hematological malignancies, carcinoma, and soft tissue sarcomas. To reduce the toxicity of the doxorubicin, it conjugated with water soluble HPMA copolymer. Malugin and coworkers has developed HPMA-copolymer–doxorubicin conjugate and evaluated the mechanism of apoptosis on the A2780 human ovarian carcinoma cells. The HPMA copolymer–doxorubicin conjugate acts both by the caspase-dependent and caspase-independent pathways of DNA damage and illustrated the enhanced level of apoptosis as compared to free doxorubicin [28]. The promising results of the in vitro study gave scope for the researchers to work further on the HPMA copolymer–doxorubicin conjugate. Seymour et al. reported that, the melanoma tumor bearing mice treated with HPMA copolymer–doxorubicin conjugate (PK1) accumulated ~65-fold of the increased doxorubicin as compared to free doxorubicin [9]. Again the stability of the PK1 was increased by the biodegradable tetrapeptide sequence GFLG (glycylphenylalanylleucylglycine) linkage. The GFLG linkage was designed for cleavage by lysosomal cathepsins, and also helpful in intravenous administration of the drug, which minimizes cardiotoxicity and bone marrow toxicity. Further, phase I clinical trials showed that, the effect of PK1 was marginal with 2 partial and 2 minor responses out of 36 chemotherapy-refractory patients. And in phase II study with patients having breast ($n=17$), non-small-cell lung ($n=29$), and colorectal ($n=16$) cancer. Up to eight courses of PK1

(280 mg/m^2 doxorubicin-equivalent) were given by i.v. administration. Toxicities were tolerable, with grade 3 neutropenia more prominent in patients with breast cancer. Out of 14 anthracycline-naive patients with breast cancer 3 had partial responses. In 26 evaluated patients with non-small-cell lung cancer, 3 chemotherapy-naive patients had partial responses. In contrast, none of the 16 evaluated patients with colorectal cancer responded well [29]. These results supported the concept that polymer–drug conjugates improved anticancer activities of the native drugs.

12.3.1.2 HPMA Copolymer–Doxorubicin–Galactosamine

The galactosamine, a hexosamine derived from galactose is used as promising targeting agent for the hepatocyte asialoglycoprotein receptor. Galactosamine was bound to the HPMA copolymer backbone via a Gly-(D, L)Phe-Leu-Gly linker, which provides hydrophobicity to the HPMA copolymer chain due to the increased content of relative hydrophobic side chains. Seymour and coworkers developed HPMA copolymer–doxorubicin conjugate with the ligand ~27 kDa, doxorubicin content of ~5 wt% and galactosamine content of 1.5–2.5 mol%. For the distribution study they use I^{123} labelled HPMA copolymer–doxorubicin–galactosamine and used gamma camera imaging analogue to follow distribution. The phase I clinical trial conducted in 31 patients, where 23 patients have hepatocellular carcinoma showed a measurable partial response. Again the SPECT gamma camera imaging study indicated that the HPMA copolymer–doxorubicin–galactosamine concentration in liver was of 15–20 % of administered dose at 24 h with lower accumulation in hepatic tumor (3.2 %).With the progress of hepatoma the target asialoglycoprotein receptor is shed out. But still the doxorubicin concentration in hepatoma was 12–50 folds higher than that of free doxorubicin [30].

12.3.1.3 HPMA Copolymer–Paclitaxel

Paclitaxel is one of the most effective anticancer agents known for the treatment of ovarian cancer, breast cancer, and lung cancer. For the effective delivery of the paclitaxel, HPMA copolymers are covalently conjugated which helps in solubilization of the drug. In the conjugation system paclitaxel was linked through tetrapeptide linker to the HPMA backbone copolymer chain. Meerum Terwogt et al. carried out the phase I study, where HPMA copolymer–paclitaxel conjugates illustrated antitumor activity in 12 patients having refractory breast cancer and skin metastases. The plasma pharmacokinetics was measured over 48 h which was linear with dose both for HPMA copolymer–paclitaxel conjugates and the native paclitaxel [31]. In another study, in vitro cytotoxicity study was carried using HPMA copolymer–gemcitabine–paclitaxel conjugate in human ovarian cancer cells (A2780) where the polymer–combined drug conjugate demonstrated synergistic effect, as compared to physical mixture of single polymer–drug conjugate [32].

12.3.1.4 HPMA Copolymer–Camptothecin

Camptothecin is an important class of potent antineoplastic drug having strong cytotoxicity against a wide range of tumors. The topoisomerase I enzyme was the cellular target of camptothecin and that the lactone function is essential for the antitumor activity [33, 34]. The lactone ring preferentially bind to the carboxylate present in serum albumin, camptothecin becomes unstable in the biological systems. This problem was overcome by conjugating camptothecin to HPMA copolymer, which helped in the improvement of the stability of the lactone ring. The C-20 hydroxyl group of camptothecin was attached to HPMA copolymer by ester linkage. The biological half-life of HPMA copolymer–camptothecin was increased up to more than 6 days, depicting its controlled release activity from conjugate system. To seek evidence of tumor targeting, a pilot clinical study was conducted involving ten patients with localized colorectal cancer. They were given HPMA copolymer–camptothecin before surgical removal of the primary tumor. After specified time intervals, the plasma, tumor, and adjacent normal tissue samples were analysed for free and polymer-bound drug. At 24 h after dosing no significant tumor targeting was observed. Further very negligible amount of free camptothecin levels were observed. However, these data's were not consistent with the clinical gamma camera imaging reported. Based on the phase I clinical results and the lack of evidence for tumor targeting, the clinical development of this polymer conjugate were abandoned.

12.3.1.5 Other HPMA Copolymer–Drug Conjugates

HPMA copolymer–aminoglutethimide–doxorubicin was used for metastatic breast cancer. Metastatic breast cancer is incurable but often responsive to treatment. Patients with estrogen-receptor-positive breast cancer may benefit more from endocrine-chemotherapy. Aminoglutethimide is a steroid derived from the cholesterol, which acts as anti steroid drug against metastatic breast cancer. The combination of the steroid with doxorubicin can be used as combinatorial endocrine-chemotherapy. With the use of the HPMA copolymer both the drugs were conjugated and can be simultaneously delivered. Vicent et al. and Greco et al. illustrated in vitro study of the HPMA copolymer–aminoglutethimide–doxorubicin conjugates illustrating enhanced cytotoxicity as compared to HPMA copolymer–doxorubicin in MCF-7 and aromatase-transfected MCF-7Ca cell lines [35–37]. Another polymer conjugate, HPMA copolymer–aminopropyl geldanamycin was used for an antibiotic having anticancer property. Geldanamycin belongs to benzoquinone ansamycin antibiotic having anticancer activity. It binds to heat shock proteins 90 [38] and GRP94 [39], inhibiting their capacity to form complexes with biological oncoproteins, such as p53 protein. The aminopropyl derivative of the geldanamycin, 17-allylamino-17-demethoxygeldanamycin was selected for phase I

clinical trial. Conjugating with HPMA copolymer, it showed modified mechanism of action and decreased nonspecific side effects as compared to the free geldanamycin. Kasuya et al. showed the conjugation of the OV-TL16 monoclonal antibody to HPMA- geldanamycin have more specific and targeted action on the A2780 and OVCAR-3 human ovarian carcinoma cells [40]. Similarly, Greish et al. and Borgman et al. synthesised the HPMA copolymer–aminohexyl geldanamycin conjugates along with antibody conjugation and illustrated more antiproliferative properties towards prostate cancer [41, 42]. Angiogenesis is crucial for tumor growth. Inhibitors of angiogenesis are used as anticancer drugs for the cancer therapy. Folkman and coworkers synthesized and developed polymer conjugate such as HPMA copolymer–O-(chloracetyl-carbamoyl) fumagillol to target and inhibit angiogenesis. This conjugate showed enhanced accumulation due to EPR effect in in vivo tumor and hepatectomy models [43].

12.3.2 Poly(α,L-glutamic acid)–Drug Conjugates

Poly(α,L-glutamic acid) (PG) composed of L-glutamic acid linked together through amide bonds. The pendent free γ-carboxyl group in each repeating unit of L-glutamic acid is negatively charged at neutral pH, which makes the polymer water soluble. PG was found to be more susceptible to lysosomal degradation than poly(L-aspartic acid) and poly(D-glutamic acid). When PG copolymers are sufficiently hydrophilic, they are eliminated primarily through the renal route with limited deposition in the cells of the reticuloendothelial system. Considering these advantageous factors, researchers have given attention towards the development of different PG–drug conjugates. The Poly(α,L-glutamic acid) is non immunogenic and nontoxic, hence Poly(α,L-glutamic acid)–drug conjugates are more deeply investigated in clinical trials.

12.3.2.1 Poly(L-glutamic acid)–Paclitaxel (PG-TXL)

To increase the water solubility and biodistribution, paclitaxel was conjugated with poly(L-glutamic acid), which demonstrated significantly reduced systemic toxicity and remarkable antitumor efficacy, including complete regression of well-established solid tumors in vivo. The antitumor and antimetastatic activities of PG-TXL were studied in four syngeneic murine tumors (breast MCa-4, breast MCa-35, hepatocarcinoma HCa-1, and sarcoma FSa-II) and all tumor models showed significantly better antitumor activities than that of free paclitaxel. The half-life of the PG-TXL was also prolonged ($t_{1/2}$ = 317 min) as compared to free paclitaxel ($t_{1/2}$ = 29 min). Todd et al. carried out the clinical study of PG-TXL, which was detectable in the plasma of all patients having a long plasma half-life of up to 185 h [44, 45]. Based on these results, PG-TXL was subsequently studied in phase I/II

trials. From the clinical findings, it was concluded that the PG-TXL can be delivered through parenteral route in lesser time than that of native paclitaxel (Taxol®) with good patient compliance.

12.3.2.2 Poly(L-glutamic acid)–Camptothecin

poly(L-glutamic acid) (PG) was conjugated at the C20(S) position of camptothecin through ester bonds. PG-camptothecin illustrated delayed growth in H322 human tumor xenograft models which showed fourfold increase in survival rate, as PG protects the lactone structure in camptothecin from the rapid ring-opening process [46]. Another group of researchers demonstrated that, by using the glycine linker between camptothecin and PG, the payload of the drug was increased up to 50 % by weight. There was an improved antitumor effect in B16 murine melanoma tumor model [47].

12.3.2.3 Poly(L-glutamic acid)–Anthracyclines

Anthracyclines (i.e., Doxorubicin, Daunorubicin) compounds are used to treat many cancers. The non-selective reactions with a variety of biomolecules, such as proteins and phospholipids inside the body during its course of action, limits their further clinical applications [48, 49]. Doxorubicin was conjugated to poly(L-glutamic acid) (PG) with an assumption that the conjugates can have greater selectivity and degrade to release Doxorubicin after they are endocytosed by tumor cells. Thus, Doxorubicin was conjugated to PG either directly or through oligopeptide spacers (Gly-Gly-Lec or Gly-Gly-Gly-Leu) via amide bonds. Anticancer activity increased with increasing oligopeptide length and degradation rate of the conjugate. The other potent drug, Daunorubicin inhibits the progression of the enzyme topoisomerase II by breaking the DNA chain replication and thereby stopping the process of cell replication. Daunorubicin have been attached to PG via hydrolytically labile ester bonds and hydrazone bonds. The hydrazone linkage formed by condensing the methylketone in Daunorubicin with hydrazide derivatized PG. It was found that the conjugate was active but less potent then free drug as determined by a [3H]uridine incorporation assay in Yac lymphoma bearing mice model [50].

12.3.2.4 Other Poly(L-glutamic acid) Polymer–Drug Conjugates

Poly (L-glutamic acid) (PG)–1-b-D-arabinofuranosylcytosine polymer conjugate for leukemia, therapy. 1-b-D-Arabinofuranosylcytosine (Ara-C) get conjugated with PG via amide bonds, where N-4 of Ara-C directly combined with the carboxyl

groups of PG and a conjugate in which Ara-C is linked via the aminoalkylphosphoryl side-chain introduced at C-5′ of Ara-C. Studies conducted in murine leukemia L1210 cells where the drug–polymer conjugate illustrated greater antitumor activity than that of the free Ara-C, due to the slow cleavage of free Ara-C from the conjugates and protection of Ara-C from deactivation by cytidine deaminase [51]. Poly(L-glutamic acid) (PG)–Mitomycin C conjugate was developed for leukemia therapy. Mitomycin isolated from *Streptomyces caespitosus* or *Streptomyces lavendulae*, which is also used as a chemotherapeutic agent by acting as a potent DNA cross linker. The Mitomycin C was also conjugated to PG through its aziridine amine using carbodiimide. The in vitro cytotoxicity, and in vivo studies of PG-Mitomycin C conjugates showed higher antitumor activity as compared to native Mitomycin C [52].

12.3.3 Poly(ethylene glycol)–Drug Conjugate

The poly (ethylene glycol) (PEG) was widely used commercial polymer approved by FDA. The specified molecular weight PEG has been developed in large scale. Further, the conjugation was possible by the different structural modifications to PEG backbone. The most important feature of PEG modification is that it greatly extends the half-life ($t_{1/2}$) of most proteins or drugs, and results in increased plasma presence. The PEG–protein conjugates, i.e., PEG–asparaginase (Oncaspar®) [53], PEG–adenosine deaminase (Adagen®) [54], PEG–interferon α-2a (Pegasys®) [55], PEG–interferon α-2b (PEG-Intron®) [56], and PEG growth hormone receptor antagonist (Somavert®) [57] have gained importance due to the reduced uptake by the reticuloendothelial system (RES). Like that the PEG-drug conjugates were exploited to modify the pharmacodynamic and pharmacokinetic properties of the anticancer drugs. The multiarm-PEG is used for the pegylation of the drugs, which ultimately increases the drug payload per PEG molecule as well as the biodegradation of the polymers [58, 59].

12.3.3.1 PEG-camptothecin

As described earlier, camptothecin acts as Topoisomerase I inhibitor in cancer chemotherapy. The PEG was conjugated with camptothecin by alaninate ester linkage at C-20-OH position, which favors the desired lactone ring configuration. Prothecan® (PEG40–Camptothecin conjugate) is now at phase II clinical trials. Enzon Pharmaceuticals, Inc. developed EZN-2208®, which has dendron like structure at the PEGs end chain and used to conjugate SN38 (active metabolite of camptothecin), now has entered to phase I clinical trial [60, 61]. The main limitation of PEG as drug carrier is the presence of only two reactive groups per polymer chain, which led to an intrinsically low drug payload. To overcome this limitation, the

construction of a dendron structure at the PEGs end chain has been proposed. Enzon is currently developing a conjugate of SN38, an active metabolite of camptothecin, with a 40-kDa PEG containing, which provide good results then the Prothecan®.

12.3.3.2 PEG-paclitaxel

PEG-paclitaxel, non-ionic paclitaxel prodrug is highly water soluble (>20 mg equiv. paclitaxel/ml) [62]. PEG-paclitaxel conjugate has difference in antitumor activity related to the changes of PEG molecular size [63]. According to the results of many researchers, the high molecular weight PEG prodrug conjugated with PEG (Mw = 20,000) produce an improved therapeutic effect. Li and coworkers suggested that, in MCA-4 mammary tumor-bearing mice, a single dose of PEG-paclitaxel (40 mg equiv. paclitaxel/kg body weight) significantly delayed tumor growth. In vivo results showed that PEG-paclitaxel inhibited the growth of B16 melanoma cells to an extent similar to that of paclitaxel, where the tumor growth rate was delayed by 0.9 days in case of PEG- paclitaxel conjugated animal as compared to free paclitaxel treated animals [64].

12.3.3.3 PEG–Gemcitabine

Gemcitabine is generally used in lung cancer, pancreatic cancer and breast cancer. However, it shows good results in patients with pancreatic cancer who have successful tumor resections. But the efficacy of the gemcitabine was lowered due to short half-life in blood circulation and rapid metabolism. The conjugation of gemcitabine to PEG demonstrated increased water solubility of the drug which in turn led to higher biodistribution and cytotoxicity. The recent studies on the PEG–gemcitabine conjugate showed a marked improvement in the cytotoxicity and apoptosis-inducing activity in MIA PaCa 2 and PANC 1 pancreatic cancer cell lines [65].

12.3.3.4 Other PEG Polymer–Drug Conjugates

Oridonin has potent antitumor activity, but it possesses rapid plasma clearance and high hydrophobicity. To make it substantially useful for chemotherapy it was conjugated with the PEG polymer. Shen et al. synthesized the PEG–oridonin conjugate by using the succinic acid as spacer moiety. The in vitro results illustrated satisfactory aqueous solubility which further increases with decreased molecular weight of PEG, while more significant sustained-release effect was shown with high molecular weight PEG. In vivo pharmacokinetic studies demonstrated that the elimination half-life was prolonged in comparison with oridonin solution [66]. The other anticancer drug prednisolone, belongs to glucocorticoid class, when inhaled for asthma, bronchitis treatments it is absorbed to blood and decreases its residence

time in lungs. The limitations can be overcome by ester conjugation of prednisolone to PEG. Bayard et al. synthesized PEG–prednisolone conjugate and showed that these conjugates are stable in buffers with a hydrolysis half-lives ranging from 1 to 70 h, depending on the pH and level of substitution. Whereas, the PEG2000 and mPEG2000 conjugates have reduced the maximum prednisolone concentration in the perfusate (C_{max}) by 3.0- and 2.2-fold, respectively. The blood retention time was increased to 40 min as compared to free drug, where it is 20 min. This study demonstrated that hydrolysable PEG drug ester conjugates can be a promising approach for optimising the pharmacokinetic profile of small drugs delivered by inhalation [67].

12.3.4 Other Polymer–Drug Conjugates

There are other polymers which are used in conjugation with various anticancer drugs to enhance its therapeutic activity. Cyclodextrin-based polymer (CDP) has been developed for the improved biodistribution towards tumor tissue. The components of CDP are β-cyclodextrin and polyethylene glycol. Camptothecin is covalently attached to CDP through a glycine linker, which preserves its active form and increases its water solubility. For example, IT-101 is a camptothecin–polymer conjugate. After i.v administration this cyclodextrin based polymer–camptothecin conjugate illustrated prolonged plasma half-life and enhanced distribution in tumor tissues when compared to camptothecin alone. The polymer conjugate also demonstrated 160 fold higher accumulation of active camptothecin released as compared to camptothecin alone [68, 69]. Using mucoadhesive polymers, polymer–drug conjugates are designed for its absorption and prolonged residence time. Soepenberg et al. [70] used DE-310, DX-8951 (exatecan mesylate, a camptothecin analogue) is linked to carboxymethyl dextran (Mw = 340 kDa) via glycyl-glycyl-phenylalanyl-glycyl-peptidyl spacer. The spacer used provides sustained release activity of the active moiety DX-8951 within the tumor as a result of enzymatic cleavage of the peptide by cathepsin B and cathepsin L. Ma et al. developed a multifunctional polymeric carrier for co-delivery of gene and drug. A new cyclodextrin derivative containing poly(L-lysine) dendrons was prepared by the click conjugation of per-6-azido-β-cyclodextrin with propargyl focal point poly(L-lysine) dendron. This conjugate formed a stable nanocomplex with plasmid DNA and exhibit high gene transfection efficiency. To this methotrexate drug was loaded efficiently with sustained release activity. The cyclodextrin derivative may be used directly for the combinatorial delivery of nucleic acid and lipophilic anticancer drugs without a complicated micellization process [71]. Han and Davis, developed a mucic acid polymer (MAP) conjugated camptothecin (MAP-CPT) and herceptin conjugated MAP-CPT nanoparticles and evaluated the efficacy in mice bearing BT-474 human breast tumors. The mice treated with non-targeted MAP-CPT nanoparticles showed significant tumor growth inhibition as compared to camptothecin alone. However,

mice receiving antibody conjugated MAP-CPT illustrated complete tumor regression demonstrating higher efficacy [72].

12.4 Conclusions and Future Prospects

These nanosized multicomponent polymer–drug conjugates has proved potentialities by reaching market and some of them are currently in clinical studies. Their application in cancer treatment is a promising field with growing opportunities to achieve effective medical treatments. However many challenges still exists, which could provide space for further improvement of this technology. Future generation of the polymer–drug conjugates will have to meet number of challenges, such as development of novel polymers, versatile conjugation chemistry which will allow site-specific attachment of targeting molecules and polymerization method as well as improved pharmacokinetic properties to allow accurate control of advance drug therapy. Thus, the polymer–drug conjugate approach is expected to show its greater therapeutic outcome in near future.

Acknowledgements The author A.K.M. thanks the CSIR, Government of India, for providing SRF fellowship. F.D. thanks DST, Government of India for providing women scientist grant and S.K.S. thanks DBT, Government of India for the Tata Innovative fellowship.

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