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AN OVERVIEW OF POLYMER THERAPEUTICS

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ABSTRACT

"Polymer therapeutics" term refers to polymeric drugs, polymer–drug conjugates, polymer–protein conjugates, polymeric micelles, dendrimers and polyplexes. The discovery of the enhanced permeability and retention effect by Maeda, along with the polymer-drug conjugate model that was proposed by Ringsdorf, directed the early steps of polymer therapeutics towards cancer therapy. The high versatility of polymer conjugates and their unique properties allows the design of effective treatments for various diseases. Many PEG-protein complexes have reached the market, while polymer drug conjugates & polymeric micelles have entered different phases of clinical trials. In this article, an overview of polymer therapeutics is discussed with a focus on concepts and examples that characterize the salient features of polymer therapeutics.

KEYWORDS: Anti-cancer medicines, conjugation, dendrimers, EPR effect, polymeric micelles, polyplex.

INTRODUCTION

The term 'polymer therapeutics' describes to nano-sized polymer-based pharmaceuticals, generally in the range of 5 to 100 nm. It includes rationally designed biologically active polymeric drugs, polymer–drug conjugates, polymer–protein conjugates, polymeric micelles to which a drug is covalently bound and polyplexes & dendrimers. The difference between conventional polymer based drug delivery systems & polymer therapeutics is that the bioactive agent is not encapsulated inside polymeric shell or entrapped in a polymer matrix, but covalently linked to a polymeric biocompatible carrier. [1]

In conventional polymer based drug delivery systems the drug is dispersed in a polymer matrix or the drug core is encapsulated inside a polymer coating. Polymer conjugation is an effective technique to facilitate the delivery of drugs and other biological agents. Conjugation protects the body from the drug that is achieved by enhancing its bio-distribution and thus decreasing its toxic effects and it also protects the drug from the body by means of reducing degradation and increasing cellular uptake. In the early 1990's, regulatory authorities in United States authorized the first PEGylated proteins for the purpose of routine clinical application. At about the same time in Japan, a stryene-co-maleic anhydride conjugate of the anticancer protein neocarzinstatin was approved for the treatment of patients with primary liver cancer. Conjugation increased hydrophobicity of the protein. This allowed for the dispersion of protein in a phase-contrast agent that is used for imaging. This formulation is administered locally through the hepatic artery. During related research, a phenomenon called the enhanced permeability and retention (EPR) effect was discovered. It is process by which passive targeting to the tumour tissue is achieved.[24] Polymer therapeutics offer advantages like enhanced solubility in case of the hydrophobic drugs, improved pharmacokinetic profile in terms of increased plasma half-life and volume of distributionas conjugation reduces clearance by the kidneys or liver, owing to increased size of the core drug. The polymer also provides protection to the drug against degradation mainly, proteolytic degradation, Combination therapy in which two or more drugs are conjugated to same polymeric backbone which provide synergistic effect for treatment of various diseases. Passive targeting to tumors by EPR effect (enhanced permeability and retention effect) is the most exploited advantage of polymer conjugates.[1][2]

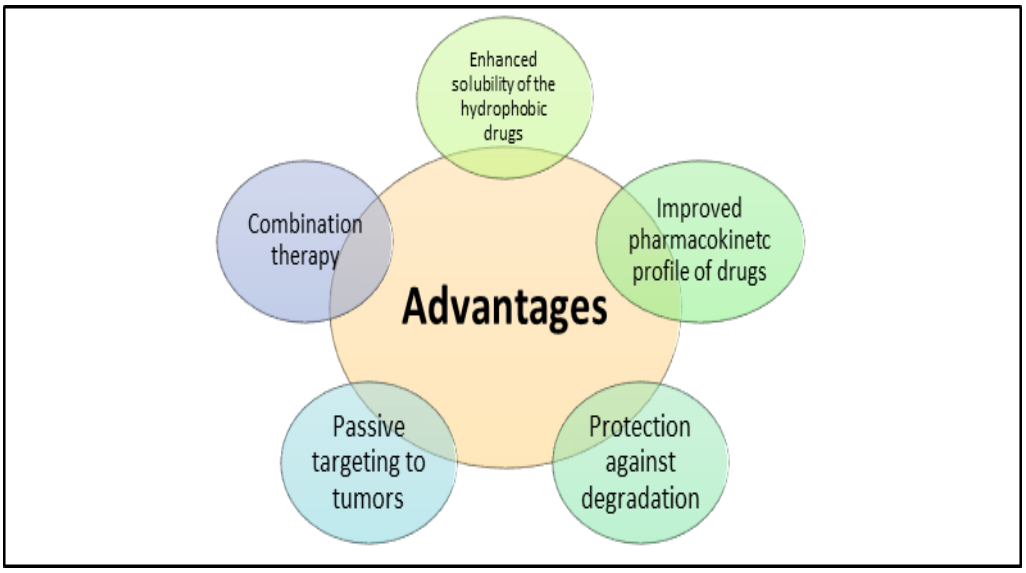


Figure 1: Advantages of polymer therapeutics

Polymer-Drug Conjugates that have Reached Clinical Trials

Table 1: polymer-drug conjugates that have clinical trials

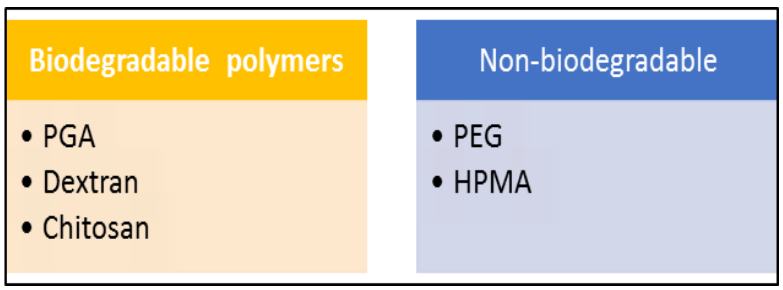
Sr. No.	Polymer-drug conjugate	Company
1	PEG-Naloxone conjugate	Nektar Pharmaceuticals
2	Paclitaxel-PGA conjugate	Cell Therapeutics Inc.
3	HPMA copolymer Doxorubicin conjugate	Pfizer
4	HPMA copolymer Doxorubicin-Galactosamine conjugate	Pfizer

Polymeric Backbone

The drug is conjugated with polymeric structure, which serves as a backbone. This polymeric backbone of the conjugate is either biodegradable, non-biodegradable, or semi-biodegradable. The choice of polymeric backbone for the conjugation largely influences the pharmacokinetic and pharmacodynamic fate of the drug. The polymer properties like molecular weight, polydispersity, architecture, charge and hydrophilicity, influence the solubility and drug loading capacity, its biodistribution, elimination from the body and the interaction with the immune system. Desired properties of such polymers include water- solubility, non-toxicity, non-immunogenicity. Polymers should either be biodegradable or capable of being eliminated from the body. Polymers should exhibit suitable functional groups for attachment of the respective drug or spacer.[4]

Commonly used Polymers for Conjugation of Drugs are Divided into following two Categories.

Table 2: Classification of polymers



1. Biodegradable polymers

As the name suggests, these polymers are degraded in biological system and then exctreted from the body.

PGA (Polyglutamic acid)

It is composed of monomers of glutamic acid. PGA is water-soluble, non-toxic, and biodegradable.PGA undergoes lysozomal degradation. Cysteine proteases, mainly cathepsin

B, plays important role in the lysosomal degradation of PGA. Conjugation takes place at ɤ−carboxyl group of glutamic acid.Example of PGA conjugate is PGA-Paclitaxel conjugate. It is not degraded in lysosomes.

Dextran

It is a natural polysaccharide composed of monomer residues of glucose(α-1, 6& α-1,4linkages). Dextran is water soluble, biocompatible and biodegradable in the blood and gastro-intestinal tract. Dextran has multiple primary and secondary hydroxyl groups that can serve as site for conjugation of drugs or proteins either directly or via spacers. Degradation occurs by dextranase. Dextran- L- asparaginase is one example of dextran conjugate.

Chitosan

Chitosan is a highly basic polysaccharide (poly-D-glucosamine), derived from deacetylation (DA) of chitin. It is biodegradable, biocompatible. Its unique chemical structure (i.e., high content of primary amines) enables chemical modification and formation of large variety of derivatives. [4]

2.Non-biodegradable polymers

These polymers are not metabolized in biological environment and are directly eliminated from the body in unchanged form.

PEG(Polyethylene glycol)

PEG is nontoxic, non- immunogenic, non-antigenic, highly water soluble. Extensive work has been done with respect to conjugation with PEG.PEG is commercially available with either one or two attachment points. It shows functional hydroxyl groups at the chain termini that can be conjugated with the appropriate functional groups of drugs or spacer. It has the ability to mask the antigenic determinants of proteins, thus reducing their immunogenicity.

Table 3: PEG conjugates in the market

Sr. No.	PEG Conjugate	Disease
1.	PEG–asparaginase (Oncaspar®)	Acute lymphoblastic leukemia
2.	PEG–adenosine deaminase (Adagen®)	Severe combined immunodeficiency disease (SCID)
3.	PEG–interferon α2a (Pegasys®)	Hepatitis C
4.	PEG–G-CSF (pegfilgrastim, Neulasta®)	Treating of neutropenia during Chemotherapy
5.	PEG–growth hormone receptor antagonist (Pegvisomant, Somavert®)	Acromegaly
6.	Branched PEG–anti-VEGF aptamer (Pegaptanib, Macugen™)	Macular degeneration (age-related)

HPMA (N-(2-hydroxypropyl)methacrylamide)

HPMA copolymer is water-soluble, neutral, biocompatible, and non-immunogenic. In case of HPMA, conjugation takes place at hydroxyl or amide group. Eg. Doxorubicin- HPMA copolymer conjugate. [4]

Currently, poly(2-oxazoline)s (POx) are being discussed as a potential candidates for conjugation with drugs. They are considered as possible alternative to PEG for the design of next generation polymer therapeutics. The water solubility of poly(2-methyl- or 2-ethyl-2-oxazoline) (PMeOx, PEtOx) is similar to PEG, however, as PMeOx shows no amphiphilicity. PEtOx displays a similar characteristics in terms of elongated blood circulation times of conjugated proteins, reduction of toxicity of conjugated drugs, biodistribution, pharmacokinetics, excretion, endocytosis etc. as both polymers are structurally quite different. For example use of amphiphilic POx copolymers for conjugation of hydrophobic drugs like paclitaxel. [11]

Types of Polymer Therapeutics

The term polymer therapeutics refers to polymeric drugs, polymer–drug conjugates, polymer–protein conjugates, polymeric micelles and polyplexes and dendrimers, each with unique structure and properties. These various types are discussed below. [1]

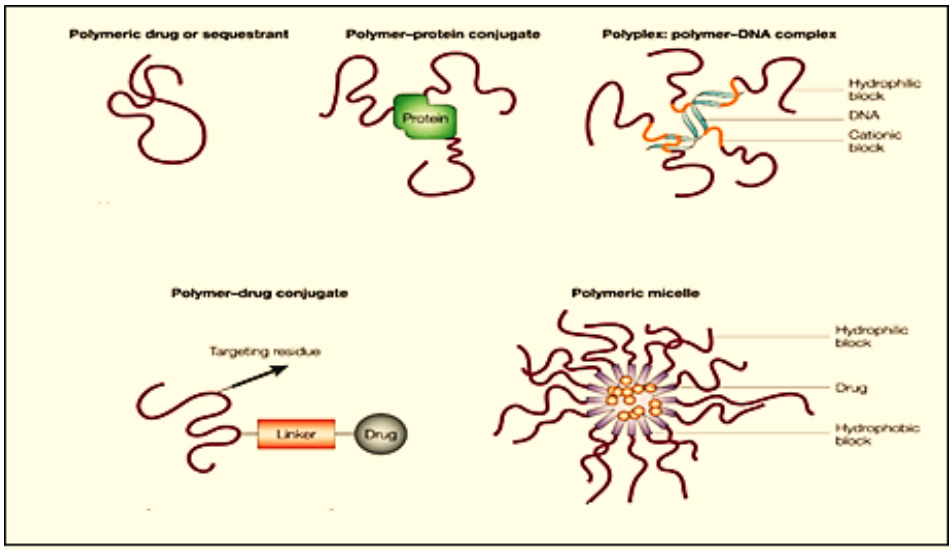


Figure 2: Types of polymer therapeutics

1. Polymeric drugs

These are biologically active polymeric drugs in which polymeric backbone itself displays therapeutic activity. DIVEMA i.e. Divinylethermaleic anhydride is a pyran copolymer. It was

the result of early attempt to prepare a synthetic polyanionic medicine based on the knowledge that the natural polymers extracted from plants, animals and seaweeds, particularly polyanions & poly-sulphates are known for long time antiviral and anti-tumor activity. Although it was known to induce apoptosis, interferon release &activate macrophages to promote the killing of macrophages DIVEMA failed as an anticancer agent in earlystages of clinical trials due to its severe side effects. [1][2]

Glatiramer acetate, which is the active ingredient of COPAXONE consists of the acetate salts of synthetic polypeptides, containing four naturally occurring amino acids: L-glutamic acid, L-alanine, L-tyrosine, and L-lysine.When it is given subcutaneously, it is capable of reducing both the frequency of relapse & progression of disease in multiple sclerosis patients. Its precise mechanism of action is not known, it seems to minimize auto-immune response to myelin that is seen in multiple sclerosis. [16][22]

2.Polymer drug conjugates

Polymer–drug conjugates take all advantages of nano medicine, including solubility enhancement of hydrophobic drugs, prolonged blood circulation, and accumulation at leaky tumor tissues by the EPR effect. Model for the polymer–drug conjugate was proposed by Ringsdorfin 1975 (fig. 4). According to this model conjugate consists of polymeric backbone, drug, spacer, homing device and with proper selection of the spacer, it is possible to control the site and rate of release of drug from conjugate. Pharmacokinetic profile and cell uptake of drug can be improved by attaching homing device. Two types of spacers (or linkers) are used for conjugation of drugs with polymers. pH sensitive linkers which degrade in Acidic environment of endosomes & lysosomes at the pH of 4-5. Examples of such linkers include hydrazone, acetal, cis-aconityl and the second type of linkers are enzymatically cleavable linkers which can be degraded by lysosomal enzymes. Examples include Cathepsin B sensitive Gly-Phe- Leu-Glypeptidyl linker. [2]

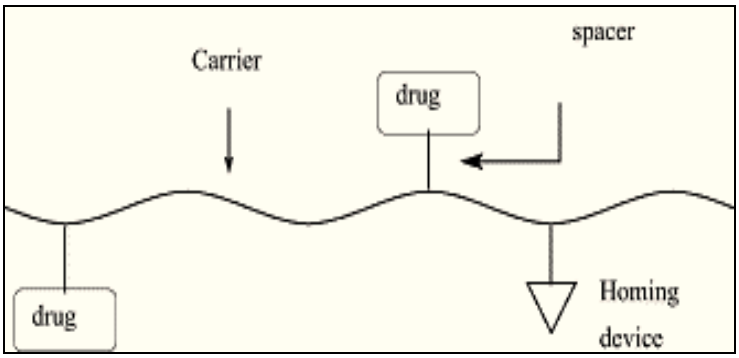


Figure 3: Ringsdorf model for polymer-drug conjugate

Doxorubicin was conjugated to HPMA copolymer by means of the peptidyl linker Gly-Phe-Leu-Gly. The peptidyl linker allowed for the controlled release of the drug in tumors by cleavage by cathepsin B and Glatosamine provided for targeting of the hepatocyte asialoglycoprotein receptor (ASGR).It is used in the treatment primary liver cancer.[17] Combination therapy containing the aromatase inhibitor aminogluthethimide (AGM) and doxorubicin (Dox) was developed for the treatment of cancer. It was found that an HPMA conjugate containing a combination of both Dox and AGM shows significantly improved anti- tumour activity than free drug and HPMA conjugate of individual drug. [8]

3. Polymer-protein conjugates

Protein & peptide drugs show several limitations like short plasma half-life, poor stability and immunogenicity. Linear and branched PEGs of molecular weight 4,000- 5,000 g/mol have been widely used to create polymer- protein conjugates, sometimes multiple PEGs attached per protein. The process is termed as PEGylation.

Main advantages of PEGylated protein are increase in the solubility of hydrophobic protein macromolecules due to PEG hydrophilicity, decreased accessibility for proteolytic enzymes and antibodies, increase in size of core protein due to conjugation which reduces its kidney filtration. Disadvantage of PEGylation of proteins is that it is often accompanied by loss of biological activity. However, this is compensated for by the prolonged body-residence time of conjugates. A typical example is that of PEGylatedα -interferon Pegasys®, which retains only 7% of the antiviral activity of the native protein, but still exhibits improved performance in vivo compared with the unmodified enzyme because of improved pharmacokinetics.[13] First generation protein conjugates used linear monomethoxy PEGs. But it had disadvantages like protein cross-linking (due to contaminating PEG- diol), modification of protein charge due to consumption of $–NH_2$ or –COOH groups during conjugation, unstable protein-PEG linkages, sometimes need for reaction conditions that led to protein denaturation. [1][5]

In 1990, PEG-adenosine deaminase (Adagen®) was the first PEGylated protein to enter market. ADAGEN Injection is indicated for enzyme replacement therapy for adenosine deaminase (ADA) deficiency in patients suffering from severe combined immunodeficiency disease (SCID). Deficiency of ADA is one of the cause for development of SCID. Adagen is bovine ADA, modified with strands of polyethylene glycol. Conjugation slows its clearance, increases its circulation half-life, reduces degradation by protease enzymes, lowers binding

by host antibodies, and reduces immunogenicity, allowing ADA to achieve its full therapeutic effect.

PEG-L-asparginase (Oncaspar®) is used as treatment for acute lymphoblastic leukemia. Compared to native enzyme, this conjugate has advantage of reduced hypersensitivity, longer plasma half-life & slower clearance, less immunogenic.

PEGylated recombinant methionyl GCSF (Neulasta®) has been developed for the prevention of severe cancer chemotherapy-induced neutropaenia. It requires less frequent administration compared to free protein. It is given by a single subcutaneous injection on day2 of each chemotherapy cycle; in comparison with native GCSF, which must be given daily for 2 weeks to achieve the same effect. PEG modification prolongs the plasma half-life and decreases its immunogenicity. [10][17]

4. Polyplexes

The term polyplex is used to describe the complex formed by a polycation and anionic oligo-nucleotide or plasmid. In gene therapy, DNA or RNA is delivered to the cell, which then induces or suppresses a specific genetic function. Polyplexes offer many advantages over viral vectors like they are easy to produce or synthesize, cell or tissue targeting, low-immune response and unrestricted size of DNA to be transported.

Therapeutic polynucleotides i.e. DNA or RNAare mixed with a multifunctional, biodegradable, cationic polymer to synthesize polyplex particles. Polyplex can enter target cells using process named endocytosis. The properties of the polymer must be such that release of polynucleotide from the endosomes and (for DNA) entrance to the cell nucleus is promoted, while protecting the polynucleotide from degradation by lysosomes.

One of the most commonly used polymers for formation of polyplex nanoparticles for gene therapy is positively charged polyethylenimine. Due to the negatively charged cell surface, nanoparticles possessing a positively charged surface generally display better association and internalization rates.

For gene transfer it is required that the vectors can exist in the circulation for longer time periods allowing polyplexes, to reach the target tissues. For this reason, the cationic surface charge of polyplexes needs to be concealed, which usually can be achieved by PEGylation. The PEGylation results in the formation of a hydrophilic corona around

the polyethylenimine- DNA core, and reduces interactions of the polyplex with plasma proteins and erythrocytes. Major problem in the clinical use of these nonviral vectors is their low transfection efficacy. [1][6]

5. Polymeric micelles

Amphiphilic polymers associate in water to form "polymeric micelles" consisting of a hydrophobic core stabilized by a corona of hydrophilic polymeric chains exposed to the aqueous environment. Hydrophilic shell of micelles maintains the micelles in a dispersed state and also decreases undesirable drug interactions with cells and proteins through steric-stabilization effects. Generally the size of polymeric micelles ranges from ~10 to ~100 nm and are composed of synthetic block copolymers or graft copolymers. The polymer concentration at which the association first takes place is known as the critical association concentration (CAC). It is lower by several orders of magnitudes than typical surfactant critical micelle concentration (CMC) values. They can be easily be sterilized by filtration using typical sterilization filters with pore size of 0.45- mm or 0.22-mm, without micron-sized particle's clogging.

They have high structural stability, provided by the entanglement of polymer chains in the inner core. Stability of polymeric micelles have two aspects. First is static stability and is described by a CMC. The second aspect of their stability is dynamic stability and is described by the low dissociation rates of micelles. This aspect is more significant than the static one for in-vivo drug delivery in physiological environments that are in non-equilibrium conditions. Therefore, although they share the root word "micelle," polymeric micelles are very different from low-molecular-weight-surfactant micelles in their physicochemical properties. This difference is critical in the applications for drug carriers. Polymeric micelles allow for incorporation of various chemical species and have high water solubility. Research has been done on polymeric micelles as delivery medium for injectable drug formulations of poorly water-soluble drugs such as paclitaxel, indomethacin, amphotericin B, adriamycin and dihydrotestosterone.

Polymeric micelles increase uptake and enhance bioavailability. The extent of solubilization depends upon the factors like micellization process, the compatibility between the drug and the core forming block, chain length of the hydrophobic block, concentration of polymer, and temperature. It is seen that above CMC, there is a sharp increase in the solubility of drug as it gets more space to occupy in the aggregates of the hydrophobic part of the micelle.

Targeting in this case, occurs by the enhanced permeability and retention effect, stimuli-sensitivity, complexing specific targeting ligand molecules to the micelle surface, Active targeting can be achieved by using immune-micelles by linking monoclonal antibodies to the micelle corona. [7][10][12][14]

6.Dendrimers

A dendrimer is a synthetic polymeric macromolecule of nanometer dimensions, composed of multiple highly branched monomers that emerge radially from the central core. Dendrimers are composed of inner layers which are known as generations with repeated branching units, which are radically attached to the core and exterior surface functional group attached to the outermost interior layers.

Dendrimers are Prepared by two Methods

1. Divergent method: This method starts from a central core and extends towards the surface by a series of reactions, commonly a "Michael reaction".
2. Convergent method: Construction of dendrimer in this method is stepwise process which begins from the end groups and continues inwards. [23]

Dendrimers have easily modifiable surface properties, therefore they can be simultaneously conjugated with many molecules such as imaging contrast agents, targeting ligands, or therapeutic drugs, yielding a dendrimer-based multifunctional drug delivery system. They act as carriers for drugs either by interacting with drugs at their terminal functional groups via covalent bonds or electrostatic forces or by the encapsulation of drugs within the dendritic structure. They are used as coating agents to protect or deliver drugs to specific sites in the body or as time-release systems for biologically active agents. Poly(lysine) dendrimers with attached sulfonated naphthyl groups as antiviral drugs are effective against the herpes simplex virus which can potentially decrease the transmission of HIV and other sexually transmitted diseases. Dendrimers find application in targeted drug delivery. Folic acid is commonly used targeting agent. PAMAM dendrimers modified with carboxymethyl PEG5000 surface chains showed reasonable drug loading, a reduced release rate and reduced haemolytic toxicity compared with the non-PEGylated dendrimer.

They are used as non-viral vector for gene delivery and they are also used as solubility enhancer. Generation 4.5 PAMAM dendrimers of organo-platinum, used in cancer treatment offer significant advantages over unmodified drug like enhanced water solubility, improved

dosage protocols and reduced toxicity. Camptothecin, an anti-cancer agent has poor water solubility. A newly developed dendrimer platform, consisting of poly(etherhydroxylamine) (PEHAM) dendrimers, has been employed to enhance the water solubility of camptothecin. Methotrexate has been encapsulated into generations 3 and 4 PAMAM dendrimers, which had PEG550 and PEG2000 monomethylether chains linked to their surfaces to alter bioavailability & toxicity. [15][16]

Pharmacokinetic Aspects of Polymer Therapeutics

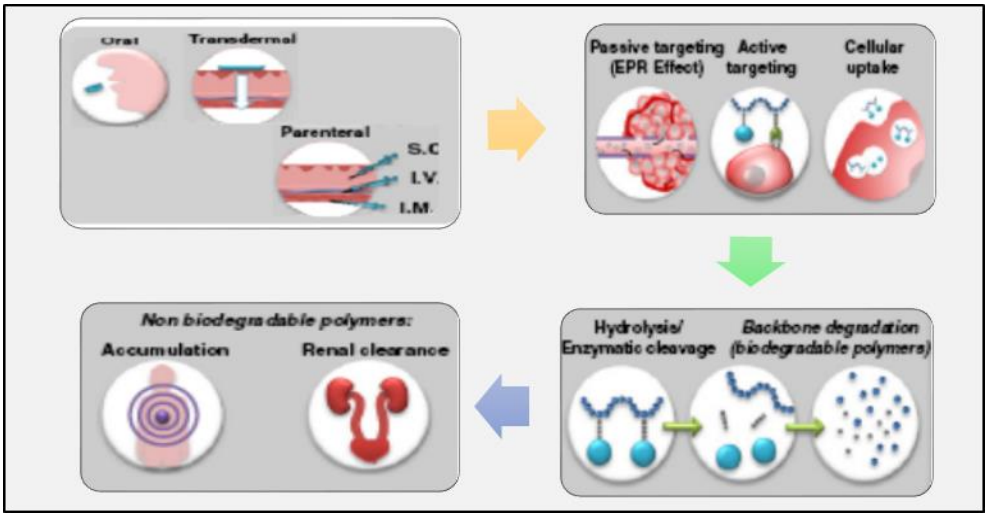


Figure 4: Pharmacokinetic aspects

1. Administration

Most of the products are being developed as injectables. When administered by the IV route, the conjugate directly enters the bloodstream and is distributed throughout the body within few seconds. Therefore IV route of administration is most preferred route of administration. Most of the formulations that have reached market are designed to be administered subcutaneously (SC) and intramuscularly (IM) (Peg-intron®, Pegasys®, Adagen® etc.).These routes of administration allow for slower release of the drug from the injection site to the bloodstream, and therefore less frequent injections are required. Oral administration (eg. chitosan-paclitaxel conjugate) is the best route when it comes to patient compliance. However, there are limitations like, drug administered by oral route has to overcome many barriers before it reaches systemic circulation, such barriers include acidic environment in the stomach, proteases in the gut lumen and brush border membrane, tightly-bound intestinal epithelial cells (enterocytes) and "first-pass metabolism" by liver enzymes. All these factors are responsible for low bio-availability after oral administration as compared to injectable route. [4]

2. Distribution

Once the drug is absorbed from the site of administration, into bloodstream, the next step is to reach the site of action. The drug reaches the target site by either passive targeting (EPR effect) or active targeting.

Passive targeting to tumors

Passive targeting to solid tumors occurs by the EPR effect. According to the EPR concept, macromolecules accumulate at much higher concentrations in tumor tissues than in normal tissues or organs, even higher than those in plasma. Microenvironment of tumor is responsible for EPR effect. Blood vessels in most of the solid state tumors have peculiar structural properties that are different from normal blood vessels. Such properties include extensive angiogenesis and hence high vascular density. High vascular permeability induced by various vascular mediators like bradykinin, nitric oxide (NO), cytokines, prostaglandins and defective vascular architecture.

Additionally the lymphatic drainage system does not operate effectively in tumor tissues. Therefore, these conjugates are selectively retained for a prolonged time in the tumor interstitium. The real dimensions of the gaps in tumor vasculature are not constant but are dynamic and differ greatly between different types of tumors and also between the vessels in the same tumor. Generally the conjugate having molecular weight in the range of 20–200 kDa can pass through these gaps and also avoid rapid renal excretion. For prolonged circulation, accumulation in tumor tissue and enhanced diffusion within tissue, particle size in the range of 20–100 nm was found to be optimum [2][3]

Active targeting

Aim of active targeting is to enhance the delivery of biologicals and drug molecules to the target by using biologically specific interactions, such as antigen-antibody binding and ligand-receptor binding. Active targeting of polymer conjugates utilizes a ternary structure, which is composed of a ligand or antibody as a targeting moiety, a polymer and an active chemotherapeutic drug. While constructing ternary structure of conjugates, some factors must be taken into consideration to create more efficient delivery systems. Ideally, these surface antigens and receptors should have several properties that render them tumor specific targets. These receptors or antigens should be exclusively expressed on tumor cells and not expressed on normal cells. Secondly they should be expressed homogeneously on all target tumor cells. [20]

Cell internalization

Once the conjugate has reached target tissue, the next step is uptake by cells. The usual uptake route of untargeted polymer conjugates is most commonly fluid phase pinocytosis. Following internalization by pinocytosis or by receptor- mediated endocytosis the conjugates are carried to the early endosome, then the macromolecules can be recycled back to the plasma membrane or routed to the late endosome and lysosome for degradation. Here, these ligands are degraded by the lower pH of about 5.0 or by the lysosomal enzymes. The process of recycling from lysosomes occurs relatively slowly and that is why cells are capable of accumulating large amounts of internalized material.

3. Metabolism and degradation of biodegradable polymers

Biodegradable polymers undergo cleavage of bonds in the polymeric backbone, either hydrolytically or enzymatically and then are eliminated from body. PGA, chitosan undergo degradation by lysosomal enzymes. Dextran is degraded by dextranases, α-1-Glucosidases.

4. Elimination of non- biodegradable polymers

Non-biodegradable polymers are directly excreted from the body. PEG or HPMA copolymers are eliminated by glomerular filtration in the kidney, but their molecular weight should be below the glomerular threshold. The properties of polymers like molecular weight, the size and the shape affects its excretion. For example, it is observed that star-shaped HPMA copolymer- bound doxorubicin conjugates were eliminated slower than hyperbranched conjugates. Renal excretion is inversely correlated with the molecular weight of the polymers. The molecular weight thresholds for HPMA copolymer was found to be about 45 kDa and for PEG about 30 kDa. [4]

5. Multidrug resistance (MDR)

MDR by cancer cells is one of the major obstacles in the treatment of cancer. Resistance to such drugs occurs as a result of active transport of these drugs out of the cell. Over expression of P-glycoprotein (Pgp), multidrug resistance-associated proteins (MRP) is primary mechanisms of MDR. Conjugation of cytotoxic agents with polymers reduces its efflux from cells by changing the pathway of drug internalization from diffusion to endocytosis, thus minimizing drug interaction with MDR transporters, leading to increased intracellular concentration of the drug. For example HPMA–copolymer doxorubicin (Adriamycin). [4]

Applications of Polymer Therapeutics

1. Polymer therapeutics as anticancer agents

As deaths due to cancer are persistently increasing, there is need to develop drug-delivery systems capable of guiding drugs selectively to tumour tissue and thus reduce the side effects associated with the chemotherapy, maintain therapeutic concentration at target site over long periods of time. Nanotechnology has large potential to improve healthcare, particularly in cancer. The EPR effect facilitates use of polymer conjugates in the treatment of various types of cancer.

Opaxio® is poly-L-glutamic acid (PGA) – paclitaxel conjugate. It was previously known as Xyotax® from Cell Therapeutics Inc. It is estimated to reach the market in the near future as a potential treatment for ovarian, non-small-cell lung and esophageal cancer. [25]

Maeda and his colleagues prepared SMANCS by covalently linking the anti-tumour protein neocarzinostatin (NCS) to two styrene maleic anhydride (SMA) polymer chains. NCS shows non- specific toxicity which interferes with its clinical development. They synthesized a polymer derivative that was hydrophobic enough to be dispersed in the phase- contrast agent lipiodol. This allowed for local administration of the agent through the hepatic artery to patients with liver cancer. By using X-ray technology, the exact localization of SMANCS to tumour tissue can be monitored. Preclinical research revealed a tumour–blood ratio greater than 2500, much higher than ever reported for other targeting approaches. [1][25]

System based on polymer–drug conjugates and polymeric micelles was developed by Kwon et al. They synthesized an amphiphilic polymer constituted by poly (ethylene glycol)–poly (aspartate hydrazide) (PEG–PAH) block copolymers and Doxorubicin and Wartmannin were attached alone or in combination, at different drug ratios. Studies ensured that the conjugates assembled to form micellar structures. It was observed that the delivery of both agents via the micellar system decreased the amount of drug necessary to produce biological activity.[31]

Apoptosis can be exploited to cure cancer. Apoptotic treatments are divided into proapoptotic, with applications in cancer therapy, and antiapoptotic, which can be exploited in regenerative therapy. Jun activation domain binding 1 (Jab1) controls several pathways involved in cell proliferation, cell cycle progression and apoptosis. Therefore it has been considered as a novel target in cancer therapy, in particular in pancreatic adenocarcinoma. Curcuminis known to be a Jab1 inhibitor, but its use in therapy is limited by its hydrophobic

nature and its poor bioavailability. In order to overcome these drawbacks, Li et al. designed PEGylated curcumin as a new chemical entity. It shows enhanced bioavailability. Free and PEGylated curcumin anti-proliferative activities were tested in four pancreatic tumor cell lines. This conjugate inhibits cell proliferation in a dose-dependent manner, and also shows greater activity than the free drug. The action of PEGylated curcumin is due to partial inhibition of Jab1, as was confirmed by the decreased levels of its target proteins (Smad4, p27 and c-Jun). PEGylated curcumin also makes the pancreatic cancer cells more sensitive towards gemcitabine. US FDA has approved it as chemotherapeutic agent in the treatment of pancreatic adenocarcinoma. [32]

In tumour tissues, anthracyclines like epirubicin (EPI) and the diffusible messenger nitric oxide (NO), can act synergistically. By means of modulating the presence of reactive oxygen species, NO is capable of controlling the pro- and anti-apoptotic properties of chemotherapeutic agents. It has already been demonstrated with PEG–EPI–NO conjugates that in cardiomyocytes, as well as in an *in-vivo* mouse model, NO counterbalances EPI-induced cardiotoxicity. *In-vivo* studies in a model for colon adenocarcinoma confirmed that the PEG–EPI–NO conjugate displayed anticancer activity, but was less cardiotoxic. When these both agents are linked to a same chain, they undergo the same body distribution, thus maximizing the benefits of this combination. Angiogenesis is growth of new capillary blood vessel from pre-existing vasculature and it plays significant role in tumor progression and metastasis. [21]

Anti-angiogenic therapy provides significant novel targeting approaches to treat cancer patients. But most of the antiangiogenic drugs are known to have low-molecular weight, and have a very poor pharmacokinetic profile. These drawbacks can be overcome by conjugating such drugs to polymeric carriers. TNP- 470 is a synthetic analog of fumagillin and an inhibitor of tumor growth. But clinical trials involving TNP- 470 were discontinued due to its high neurotoxicity. Conjugation of TNP-470 with a polymer prevented the drug from crossing the blood–brain barrier (BBB). Caplostatin which is made of HPMA copolymer–TNP-470 was the first developed polymer drug conjugate on the basis of an antiangiogenic therapy and is currently under preclinical evaluation performed by SynDevRx Inc. From *in-vitro* assays of caplostatin, it is evident that it exhibits similar inhibition of vascular hyper-permeability like free TNP-470. But *in-vivo* data indicated that the HPMA copolymer–TNP-470 conjugate was equally effective at inhibiting tumor growth, also shows improved

pharmacokinetic profile and is less toxic when compared with the free drug. *In-vivo* study was performed using different murine transgenic tumor models. Lodamin consists of a diblock copolymer monomethoxy-PEG–polylactic acid (mPEG–PLA) linked to TNP-470, formulated for oral administration. Due to the amphiphilic nature of the diblock copolymer, lodamin self-assembles into micelles with a TNP-470 as a core. This provides protection to the drug from hydrolysis through the gastro-intestinal track, until it reaches the intestine, from where it is mainly absorbed. From the research done in HUVEC cells, it was found that the conjugate significantly inhibited cell proliferation. *In-vivo* study, lodamin inhibited primary tumor growth in mouse models of melanoma and lung cancer and also prevented liver metastasis via oral administration. All lodamin- treated mice survived without apparent side effects. [21]

2. Applications of polymer–drug conjugates in diseases other than cancer

The field of polymer–drug conjugates is now expanding beyond cancer treatment. The use of many drugs effective against different diseases is restricted by a low solubility, a high toxicity, bad pharmacokinetics or a poor bioavailability. Conjugation can alter their properties, thus overcoming these limitations and improving their therapeutic value.

Phloridzin (PRZ) is an oral antidiabetic drug. It is a competitive inhibitor of the sodium–glucose cotransporters (SGLT1). Owing to its toxicity, PRZ is not used for oral administration. Ikumi *et al.* developed a γ-PGA–PRZ conjugate using a non-biodegradable linker, which avoids the absorption of the drug in the small intestine due to its high-molecular weight. Additionally the steric hindrance of the polymer chain provides protection to PRZ against hydrolysis. When the PGA–PRZ conjugates were orally given before D-glucose administration in-vivo the glucose-induced hyperglycemic effects were significantly suppressed. [19][23]

Zidovudine is a nucleoside reverse transcriptase inhibitor used to treat HIV. Gao *et.al.* developed conjugate using sulphated alkyl laminaripentaoside as polymer carrier. It was found that conjugated zidovudine exhibited considerably higher anti-HIV activity than the free drug. This is due to synergism with sulphate functionality in the polymer. Prominent side effect of the free drug is anticoagulant activity. The conjugate exhibited a very low to undetectable anticoagulant activity. [28]

Use of an antifungal agent, amphotericin B (AmB) in therapy is restricted because of its poor water solubility and toxic side effects, especially its high nephrotoxicity. PEG–AmB conjugates with an acid labile linker for the selective release of the drug at the infection were designed. Infection site has an acidic environment. These conjugates exhibited higher lethal dose (LD50) in mice than the free drug. β-glucosidase-sensitive star-PEG–AmB conjugate ensures an efficient delivery of AmB, as most of fungal pathogens possess specific hydrolase β-glucosidases, while these enzymes are not found in healthy human tissues. The conjugate was highly soluble in water, stable in pH 7.4 buffer, also sensitive to hydrolysis in the presence of β- glucosidase. [21]

PEG–naloxol (NKTR-118) is the polymer–drug conjugate used in the treatment of diseases other than cancer that has reached clinical trials. NKTR- 118 is being developed by Nektar Therapeutics. It is currently in Phase II clinical trials as an oral formulation for the treatment of patients with opioid-induced constipation or other opioid- induced bowel dysfunctions. NKTR-118 acts on the peripheral opioid receptors in order to reduce the side effect of opioid therapy, avoiding the penetration of naloxol through the blood brain barrier and the consequent side effects on the CNS. During clinical trials it was observed that NKTR-118 was well tolerated, without any severe side effects. It was found that the drug was rapidly absorbed and had a significantly longer half-life than free naloxone. Also patients with opioid-induced constipation had a significant increase in their spontaneous bowel movements after daily oral administration of NKTR-118 during the first week. The increase in spontaneous bowel movements was maintained during the 28-day treatment. Neither reversal of analgesia nor increase in opiate use was observed. [29]

Glucocorticoid dexamethasone is an anti- inflammatory agent used in the treatment of rheumatoid arthritis. Polymer conjugate takes advantage of two main features. First, the conjugate accumulates in the inflamed joints by a mechanism similar to the EPR effect and second, the acidic environment (intra- and extra-cellular) as a result of inflammation. The conjugate was rationally designed using a pH-sensitive hydrazone linker so that the drug is released at the inflammation site. Maximum release of the drug is observed at pH 5. Following systematic administration it was found that the conjugate evidently exhibited better and longer lasting anti-inflammatory effect, along with a profound bone and cartilage protection when compared with free Dexamethasone. [30]

One rapidly growing area of research is tissue regeneration and repair. In this area, the application of polymer therapeutics can offer promising therapeutic applications. In case of wound healing initial studies with polymer–drug conjugates were performed with the aim to prevent scar tissue formation. Shaunak *et al.* worked on a polyvalent dendrimer conjugate of glucosamine or glucosamine-6-sulphate. Their research revealed immune-modulatory effects for PAMAM gen 3.5-D(+)-glucosamine as this conjugate inhibited the synthesis of key pro-inflammatory chemokines and cytokines. Additionally PAMAM gen 3.5-D(+)- glucosamine 6-sulfate blocked FGF-2-mediated endothelial cell proliferation and neo-angiogenesis. When these dendrimeric systems were combined in a rabbit model they were found to act synergistically, thus preventing scar tissue formation after glaucoma filtration surgery. This combination therapy significantly improved the long-term success of the surgery. [26]

Some estrogens like 17β-estradiol exhibit cardiovascular protective effects. When it is administered systemically, leads to number of side effects, as the estradiol receptors are found in various types of tissues. This problem can be solved by developing a cell-specific targeted delivery system. For this purpose, Rodriguez-Hernandez *et al.* designed a conjugate of a 17β-estradiol linked through an aminocaproic spacer to a modified dextran (2000 kDa). This conjugate offers many advantages such as longer duration of residence in the endothelial lumen and it does not enter into blood cells or organs like the heart, lung or liver. Also the conjugate has slow renal elimination. This conjugate was tested in gonadectomized male Wistar rats and it was observed that it was able to prevent myocardial damage caused by the reperfusion that takes place after coronary ischemia. In comparison with free 17β-estradiol, the conjugate showed the same efficacy and induced the same protection in both intact and gonadectomized male rats. [27]

Bone has a unique micro-structure made of inorganic solids, which can be effectively exploited to target drug molecules to affected area of the bone. Osteoclasts are large multinucleated cells which are responsible for the dissolution and absorption of bone. They sequester portions of bone by sealing off areas called lacunae, then the adjacent membrane to the bone ruffles and consequently cathepsin K and HCl is released. As a result the pH is reduced to 4–4.5 and thus dissolving the bone. Calcium from the bone is then transported to the secretory domain and released into the interstitial space. Endosomes/lysosomes have pH of 5–6 and contain cathepsin B, though this is not limited to osteoclasts. [18]

Dendritic Poly(ethylene glycol) bearing Paclitaxel (PTX) and Alendronate (ALN) is used for targeting bone neoplasms. PTX is potent anticancer drug that has severe side effect and ALN is an aminobiphosphonate used to treat osteoporosis and bone metastases and it also serves as a bone-targeting agent. The PTX-PEG-ALN conjugate was rationally designed, taking into consideration active targeting by the ALN and passive targeting through the EPR effect. It was found that conjugate demonstrates a great binding affinity to the bone mineral hydroxyapatite *in-vitro*. The PTX-PEG-ALN conjugate exhibited an improved pharmacokinetic profile compared with the free drugs in terms of increase in their half-life. [6] HPMA-copolymer–PGE1 conjugates were synthesized containing cathepsin K-sensitive spacers, Cathepsin K enzyme is highly overexpressed by osteoclasts. It is used in the treatment of osteoporosis. PGE1 is an anabolic agent which exerts its actions by enhancing the process of formation of new bone after systemic and local administration. Clinical use of PGE1 is restricted because it also acts on other cells resulting in unwanted side effects. HPMA copolymer–PGE1 conjugates were designed to target the bone resorption lacunas, which are characterized by bone degradation due to the activity of osteoclasts on its surface. In the experiments performed with induced human and murine osteoclasts, osteoblasts, precursor cells and control non-skeletal cells, it was found out that the release of PGE1 mainly occurred in osteoclasts. This phenomenon occurred as a result of selective cleavage of the linker that is the peptidic sequence GGPNle, by cathepsin K enzyme activity. However, a slight PGE1 release from HPMA copolymer–PGE1 was also observed in other cell types owing to ester hydrolysis catalyzed by lipases. A preliminary *in-vivo* efficacy study of osteotropic HPMA copolymer–PGE1 conjugate performed in aged ovarectomized rats. The results were found to be very encouraging when compared with free PGE1. [25]

CONCLUSION

Polymer conjugates are highly versatile nanomedicines that allow for the design of effective treatments forvarious diseases, mainly useful in cancer treatment owing to EPR effect. This 'platform technology' can be developed further for the betterment of human health by controlling the synthetic process, the detailed characterization of the conjugate architecture, the conquest of combination therapy and the discovary of new therapeutic targets. Still there are many challenges to overcome before these novel nano-pharmaceuticals can be used for routine clinical application.

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