Conjugation in Drug Delivery System

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Abstract: Attachment of cellular and sub cellular targeting moiety, delivery-enhancing molecules, or functional entities to drugs or their delivery systems has become an essential and important approach in the field of modern drug delivery. Conjugation involves the linking of two or more molecules to form a new complex having the combined properties of its constituting components. Natural or synthetic compounds with their individual activities can be chemically combined to create novel substances possessing unique characteristics. Drug conjugation can significantly change biodistribution of the therapeutic agent, thus improving its pharmacokinetics (PK) and pharmacodynamics (PD), increasing their therapeutic effects and reducing their side effects, as well as provide a means to circumvent the multidrug resistance (MDR). This article focuses on the potential of conjugation as a platform for developing drug delivery systems in various therapies.

Key words: Conjugation, biodistribution, pharmacokinetics, pharmacodynamics, multidrug resistance, therapies.

1. INTRODUCTION TO CONJUGATION

Drugs are increasingly used in the clinical practice and represent today an important share of the research and development budget of pharmaceutical companies. The efficacy of therapeutic agents is often diminished by various factors such as toxicity, low specificity against targets, low bioavailability, instability, resistance developed by targets such as cancer cells, and insolubility.

One of the main limitations of therapies is their high toxicity which could lead to serious side effects, reducing the administrable and the therapeutic effect. To address this issue, it is essential to transport the therapeutically active molecule mainly to the target where it is needed and at the required time and level. This could be achieved by embedding/conjugating the drugs into nontoxic and biodegradable carriers/polymers from which the drug will be released in a sustained manner.

Conjugation involves the linking of two or more molecules to form a new complex having the combined properties of its constituting components. (Jayant & Tamara, 2006) Natural or synthetic compounds with their individual activities can be chemically combined to create novel substances possessing unique characteristics. For example, a protein able to bind selectively to a target molecule within a complex mixture may be linked to another molecule capable of being detected to form a traceable conjugate as shown in fig. below. The detection component provides visibility for the targeting component, producing a complex that can be localized, followed through various processes, or used for measurement (Na & Si-Shen, 2008).

Figure 1: Protein Drug Conjugate with Targeting Agent

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2. DIFFERENT TYPES OF CONJUGATION USED IN DRUG DELIVERY SYSTEM

(a) Polymer Drug Conjugates

Polymer–drug conjugation is one of the major strategies for drug modifications, which manipulates therapeutic agents at molecular level to increase their solubility, permeability and stability, and thus biological activity. Such a strategy is based on a central assumption that the molecular structure of drugs can be modified to make analogous agents, which are chemically distinct from the original compound, but produce a similar or even better biological effect. Polymer–drug conjugation can significantly change biodistribution of the therapeutic agent, thus improving its pharmacokinetics (PK) and pharmacodynamics (PD), increasing their therapeutic effects and reducing their side effects, as well as provide a means to circumvent the multidrug resistance (MDR).

Polymer drug conjugation has been investigated and some prodrugs have shown promise. The synthetic polymers such as N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers, poly(ethylene glycol) (PEG), and poly(L-glutamic acid) (PGA) have been predominantly utilized as the carriers of anticancer drugs such as doxorubicin, paclitaxel, camptothecin and platinites. Among them, PEG is used most often since it is water soluble, biocompatible and nontoxic, facilitating its application for conjugation with paclitaxel, camptothecin and doxorubicin to improve their water solubility, plasma clearance and biodistribution.

Na Cao & Si-Shen Feng et al have conjugated Doxorubicin to D-α-tocophery polyethylene glycol 1000 succinate (TPGS) and formulate it as nanoparticles to enhance the therapeutic potential and reduce the systemic side effects of the drug.[1] Clinical application of doxorubicin, however, is limited because of its short half-life and severe toxicity, especially gastrointestinal toxicity and heart failure. Also, the intracellular level of DOX can be reduced by the drug resistance. Amphiphilic structure of (TPGS) comprising lipophilic alkyl tail and hydrophilic polar head, is bulky and has large surface areas, enables it to be an effective emulsifier and solubilizer. TPGS is applied in preparation of nanoparticles and as a component of a new biodegradable copolymer TPGS for nanoparticles formulation of anticancer drugs, which achieved high emulsification efficiency, high drug encapsulation

3. ADVANTAGES AND DISADVANTAGES OF CONJUGATION IN DRUG DELIVERY

(a) Advantages

• Conjugation manipulates therapeutic agents at molecular level to increase their solubility, permeability and stability, and thus biological activity.
• Conjugation change bio distribution of the therapeutic agent, thus improving its pharmacokinetics (PK) and pharmacodynamics (PD), increasing their therapeutic effects and reducing their side effects, as well as provide a means to circumvent the (MDR)
• Functional stability
• High affinity
• Defined conjugation chemistry
• Local action/targetability.

(b) Disadvantages

• process of conjugation is very complicated
• take very long time,
• Very expensive.
efficiency, desired drug release kinetics, high in vitro cellular uptake and cytotoxicity, and high in vivo therapeutic effects (Ping-Shan & Pei-Jen, 2007).

Thus TPGS could enhance cytotoxicity of doxorubicin in the G185 cells, which may be attributed to its inhibitory on the p-glycoprotein mediated MDR. TPGS–drug conjugation should thus be an ideal solution for the drugs that have problems in their adsorption, distribution, metabolism and excretion (ADME).

Shinji Sakuma & Takaomi Matsumoto et al have conjugated poorly absorptive drugs with mucoadhesive polymers for the improvement of oral absorption of drugs.[5] To improve the permeability of poorly absorptive drugs include chemical modification with fatty acids to increase lipophilicity, peptidyl derivation to enhance the drug absorption through the H+/peptide co transporter. Drugs with poor permeability are conjugated to mucoadhesive polymers via peptide spacers that can be cleaved brush border peptidases to release the drugs in the vicinity of the intestinal membranes. Fig 4. Schematically shows the behavior of the polymer-drug conjugates in the brush border membranes. Alendronic acid whose bioavailability is about 1% in humans was used as a model drug. It can be readily modified at its primary amino group with peptide spacers. Cationic poly (vinylamine) (PVAm) with strong mucoadhesison due to its electrostatic interaction with anionic intestinal mucosa was selected as the mucoadhesive polymer. A PVAm-alendronic acid conjugate with the brush border peptidase-susceptible spacer was designed and synthesized, and its in vitro / in vivo performance was examined. Conjugation with specific peptides that can penetrate through cell membranes and attachment of mucoadhesive polymer, for improvement of oral absorption (Shinji & Takaomi, 2007).

After oral administration, majority of the intact conjugates reach the small intestine if the spacers are relatively stable against luminal peptidases. The conjugates localize in the vicinity of the intestinal membranes through the mucoadhesion of the polymers, and remain there because intact forms with high molecular weights are not absorbed through the membranes. Spacers of the conjugates are subsequently cleaved by brush border peptidases to release free drugs. The absorption enhancement will result from the increased concentration of free drugs.

(b) Drug/Polymer Receptor Specific Conjugate

Different attempts are being made to explore the potentials of ligand–receptor-mediated delivery systems in many therapies like anticancer therapy. Among others, the receptor has been identified as one of the successful target molecules. In many diseases various receptors have been over expressed e.g. folate receptors are over expressed in cancer tumor.

Transferrin receptors over expressed with a density of 10,000–100,000 molecules per cell
commonly found on tumor cells. Transferrin (Tf) is an 80 kDa serum glycoprotein that traffics ferric ions into cells. Upon binding to its receptor, Tf is internalized into the cell through receptor-mediated endocytosis.

Folate receptor (FR), a 38 kDa glycosyl phosphatidyl inositol-anchored glycoprotein, binds folic acid with nanomolar affinity and transports the captured vitamin into the cell by receptor-mediated endocytosis. Because the FR is often over-expressed in cancers of epithelial origin, it has frequently been exploited for the specific delivery of folate-linked (conjugated) drugs into cancer cells. Indeed, folate-derivatized proteins, chemotherapeutic agents, gene therapy vectors, immunogenic haptens, imaging agents, and liposomes have been efficiently delivered into cancer cells by receptor-mediated internalization (Mani & Jamison, 2009).

It has recently been shown that folate-targeted liposomes containing doxorubicin are more potent than non-targeted liposomes in treating FR-expressing murine leukemias. While these results are very encouraging, it should be noted that the targeted cell type was not identified and, because activated macrophages also express the FR, it is conceivable that the observed therapeutic activity is partially due to the targeted elimination of FR-expressing tumor associated macrophages. Indeed, activated macrophages can be efficiently targeted in vivo by folate conjugates.

Targeted delivery is accomplished by conjugating the surface of drug-carrying carriers, a tumor cell–specific ligand via a lipophilic anchor. Lipophilic derivatives of receptors, a high-affinity ligand for the receptors like FR, can be conjugated / incorporated into carriers like liposomes for targeting tumor cells with amplified receptor expression. Receptor specific-conjugated liposomes preferentially accumulate around the blood vessels within the tumor and effectively eliminate adjacent tumor cells via active receptor-mediated uptake, as well as destroy the endothelial cells by direct cytotoxicity and by stander effect (Tae-il & Jung-un, 2007).

Mani Prabaharan, & Jamison J. Grailer et al have synthesized Folate-conjugated amphiphilic hyper branched block copolymers based on Dox loaded -Boltorn H40, poly (L-lactide) and poly (ethylene glycol) for tumor targeted drug delivery. Remedy is that polymeric micelles can be used as efficient containers for reagents with poor solubility and/or low stability in physiological environments. However, the formation of polymeric micelles is thermodynamically favorable only above the critical micelle concentration (CMC) of the amphiphilic molecules. When the concentration drops below the CMC, the micellar structure becomes unstable and dissociates into free chains. Once the micelles are introduced into the blood stream, they are subjected to severe dilution and become thermodynamically unstable when below the CMC. The disruption of micellar structures leads to the burst release of entrapped drugs, which may cause serious toxicity problems due to the potentially large fluctuations in drug concentrations.

The problem associated with the self-assembled multimolecular polymeric micelles can be potentially overcome by developing an amphiphilic hyper branched block copolymer that has a hydrophobic inner block and a hydrophilic outer block. Boltorn H40 has recently received much attention in designing unimolecular micelles made of amphiphilic hyper branched block copolymers because of its biodegradability, biocompatibility, globular architectures and chain end functionalities.

Folate-conjugated amphiphilic hyper branched polymers were prepared by ring-opening polymerization of 3-caprolactone using H40 as the initiator followed by coupling reactions with PEG-diol and subsequently folate. The resulting products formed nanoparticles in aqueous solutions have great potential for tumor targeted drug delivery (Xue & Jing, 2009). FA is non-immunogenic and has a high affinity for FA-binding proteins that are selectively over-expressed on the surface of many human tumor cells, including ovarian, lung, breast, endometrial, renal, and colon cancer cells.

(c) Peptide Polymer Conjugation

One of the targeting strategies is to use transferring carrier to encapsulate different agents, including
plasmid DNA, proteins, peptides and low molecular weight compounds. Then different targeting moieties are put on the surface of the carriers, which are then physiochemical or biologically directed to the targeted lesions where they can achieve relatively high concentrations. Proteins and peptide are essential body components. So for targeted drug delivery they can be conjugated to various carriers like liposomes, nanoparticles etc.

Guopei Luo, Xianjun Yu et al have synthesized LyP-1-conjugated nanoparticles for targeting drug delivery to lymphatic metastatic tumors. LyP-1 is a 9 amino-acid cyclic peptide identified by in vivo phage display technology on the MDA-MB-435 human carcinoma xenograft tumors. Fluorescein-labeled LyP-1 can accumulate in structures identified as lymphatic vessels and also in tumor cells within hypoxic areas, but not in the blood vessel after an intravenous injection, this peptide also homes to the metastatic lesions of tumors. Therefore, it may serve as a good nanoparticle specific targeting moiety for lymphatic active drug delivery (Guopei & Xianjun, 2010). This peptide also homes to the metastatic lesions of tumors. With these considerations, the purpose of this study is to synthesize LyP-1-NPs (Fig. 6) and examine the possibility of LyP-1-NP serving as a novel targeting drug carrier to lymphatic metastatic tumors.

Preparation of LyP-1-NPs
LyP-1 with sulfhydryl group was conjugated to the maleimide function located at the distal end of PEG surrounding thenanoparticle surface. LyP-1 was mixed with nanoparticles at a peptide: maleimide ratio of 1:3:1 in PBS (pH 7.0). The volume of mixture was 1mL and the conjugation of LyP-1 to maleimidothe nanoparticles was performed overnight on a rotating plate set at a low speed. Nanoparticles were concentrated by centrifuging at 14,000rpm at 4°C for 45 min. In order to examine the conjugation efficiency of LyP-1 to maleimide on the nanoparticles, maleimide-PEG-PLGA and mPEG-PLGA nanoparticles were incubated at RT with LyP-1 presence (+) or absence (−) of FITC to conjugate. Fluorescence intensity of FITC-labeled nanoparticles was quantified by spectrofluorometry (LS 50B luminescence spectrometer, Perkin-Elmer). The schematic representation of preparation of LyP-1-NPs is shown in fig. 6.

Heng Mei & Wei Shi et al. have synthesized EGFP-EGF1 protein-conjugated PEG-PLA nanoparticles for tissue Factor targeted drug delivery (Heng & Wei, 2010).

It is generally accepted that the initial event in coagulation and intravascular thrombus formation is the exposure of cell-surface protein tissue factor (TF). TF interacts with either plasma coagulation factor FVII or FVIIa, and the resulting fibrin serves a trigger for thrombosis formation. Many experimental studies have demonstrated that inhibition of TF/FVIIa can effectively suppress thrombosis. The light chain of coagulation factor FVII begins from the N-terminus with a Gla domain, which is followed by two epidermal growth factor-like domains namely, EGF1 and EGF2.

Here they developed an anti-thrombotic drug delivery system, EGFP-EGF1-NP drug delivery system as shown in figure 7. EGFP-EGF1 domain peptide of plasma coagulation factor FVIIa plays a role in TF binding and the TF/FVIIa complex formation. The intriguing results prompted us to develop an anti-thrombotic drug delivery system that takes advantage of the TF-targeting property of EGF1-EGF2 peptide. So to develop an anti-thrombotic drug delivery system, EGFP-EGF1-NP is effective for targeting. EGFP-EGF1-NPS were prepared by incubating the purified thiolated EGFP-EGF1 with the NPs at room temperature for 9 h, The products were then subjected to a 1.5 20 cm sepharose CL-4B column
and eluted with 0.01 mol/L PBS buffer (pH 7.4) to remove the non-conjugated proteins (Heng & Wei, 2010).

(d) Antibody–drug Conjugates

Targeted therapies such as monoclonal antibodies are an important part of drug development. Humanized antibodies may be used either alone in unlabeled or naked form or conjugated with radioactive isotopes, chemotherapeutics, or toxins to create highly targeted agents. Antibody drug conjugates or monoclonal antibodies linked to a cytotoxic small molecule, provide a niche opportunity for biopharmaceutical companies and contract manufacturers.

Antibody drug conjugates are monoclonal antibodies (mAbs) attached to biologically active drugs by chemical linkers with labile bonds. Antibody–drug conjugates (ADCs) combine the specificity of monoclonal antibodies (mAbs) with the potency of cytotoxic molecules, thereby taking advantage of the best characteristics of both components. By combining the unique targeting of mAbs with the cancer killing ability of cytotoxic drugs, ADCs allow sensitive discrimination between healthy and diseased tissue. Advances in coupling antibodies to cytotoxic drugs permit greater control of drug pharmacokinetics and significantly improve delivery to target tissue. Potent new anticancer drugs can be used to target tissue cancers while minimizing exposure of healthy tissue. Along with the development of the mAbs and cytotoxins, the design of chemical linkers to covalently bind these building blocks is making rapid progress but remains challenging. Recent advances have resulted in linkers having increased stability in the bloodstream while allowing efficient payload release within the tumor cell.

In order to obtain a liposome targeting, organ- or cell-specific homing devices have to be conjugated to the liposome surfaces. Among various opportunities, the coupling of target-specific antibodies to create immunoliposomes (IL) has appeared as most promising way in achieving a liposome targeting. Several methods differing in their chemical basis have been described for the attachment of antibodies to the liposome surfaces.

Clinical demonstration of the antibody–drug conjugate concept is provided by the approval of gemtuzumab ozogamicin (Mylotarg) for the treatment of acute myeloid leukemia. Gemtuzumab ozogamicin is a humanized anti-CD33 IgG4 conjugated to calicheamicin, a highly cytotoxic natural product that induces double-stranded DNA cleavages. Other drugs that have been commonly conjugated to antibodies include auristatins and maytansinoids, which potently inhibit tubulin polymerization (Yelena & Yumie, 2006).

Six engineered monoclonal antibodies [“Rituxan” (rituximab), “Herceptin” (trastuzumab), “Campath” (alemtuzumab), “Avastin” (bevacizumab), “Erbitux” (cetuximab), and “Vestibix” (panitumumab)], two radionuclide-conjugated monoclonal antibodies [“Zevalin” (ibritumomab tiuxetan) and “Bexxar” (tositumomab; iodine I 131 tositumomab)], and one ADC [“Mylotarg” (gemtuzumab ozogamicin)] have been approved to treat cancer.

Yelena V, Yumie Ye et al have conjugated the anti-CanAg humanized monoclonal antibody huC242 with the microtubule-formation inhibitor DM1 (a maytansinoid), or with the DNA alkylator DC1 (a CC1065 analogue), have been evaluated for their ability to eradicate mixed cell populations formed from CanAg-positive and CanAg-negative cells in culture and in xenograft tumors in mice. They found that in culture, conjugates of either drug killed not only the target antigen-positive cells but also the neighboring antigen-negative cells. Furthermore, they showed that, in vivo, these conjugates were effective in eradicating tumors containing both antigen-positive and antigen-negative cells. The presence of antigen-positive cells was required for this killing of bystander cells. This target cell–activated killing of bystander cells was dependent on the nature of the linker between the antibody and the drug. Conjugates linked via a reducible disulfide bond were capable of exerting the bystander effect whereas equally potent conjugates linked via a nonreducible thioether bond were not. Their data offer a rationale for developing optimally constructed antibody-drug conjugates for treating tumors that express the target antigen either in a homogeneous or heterogeneous manner (Yelena & Yumie, 2006).

Anita and Thomas et al have made the antibody–drug conjugates (ADC) trastuzumab–DM1 (T–DM1). It is hoped that by coupling trastuzumab, a humanized monoclonal antibody (mAb) specific for the human epidermal growth factor receptor 2 (HER2; also known as ERBB2) — to the cytotoxic agent DM1 (emtansine; ImmunoGen), T–DM1, and formulating them as Nano delivery could provide more potent anticancer effects than trastuzumab alone, which is a blockbuster therapy for HER2-positive breast cancer (Anita & Julie, 2004).
5. MARKETED FORMULATIONS CONTAINING CONJUGATED SYSTEMS

<table>
<thead>
<tr>
<th>Conjugates</th>
<th>Indication</th>
<th>Year to market</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) High molecular weight drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMANCS (Zinostatin, Stimalamer)</td>
<td>Hepatocellular carcinoma</td>
<td>1993</td>
<td>Yamanouchi Pharmaceutical</td>
</tr>
<tr>
<td>PEG–adenosine deaminase (Adagens)</td>
<td>SCID syndrome</td>
<td>1990</td>
<td>Enzon</td>
</tr>
<tr>
<td>PEG–asparaginase (Oncaaspars)</td>
<td>Acute lymphoblastic leukaemia</td>
<td>1994</td>
<td>Enzon</td>
</tr>
<tr>
<td>Branched PEG–interferon a2a</td>
<td>Hepatitis C</td>
<td>2002</td>
<td>Roche/Nektar</td>
</tr>
<tr>
<td>PEG–growth hormone receptor antagonist (Pegvisomant, Somaverts)</td>
<td>Acromegaly</td>
<td>2002</td>
<td>Pfizer (Pharmacia)</td>
</tr>
<tr>
<td>PEG–G-CSF (Pegfilgrastim, Neulastas)</td>
<td>Prevention of neutropenia associated with cancer chemotherapy</td>
<td>2002</td>
<td>Amgen</td>
</tr>
<tr>
<td>Branched PEG–anti-VEGF aptamer (Pegaptanib, MacugenTM)</td>
<td>Age-related macular degeneration</td>
<td>2004</td>
<td>EyeTech Pharmaceuticals (now OSI Pharmaceuticals) / Pfizer</td>
</tr>
<tr>
<td>PEG–anti-TNF Fab (CDP870; Certolizumab pegol, Cimzia)</td>
<td>Rheumatoid arthritis and Crohn’s disease</td>
<td>2008</td>
<td>UCB (formerly Celltech)</td>
</tr>
<tr>
<td>A PEGylated diFab antibody. TargetsVEGFR-2 (CDP791)</td>
<td>Solid tumors</td>
<td>Phase II</td>
<td>UCB-ImClone System</td>
</tr>
<tr>
<td>(B) Low molecular weight drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPMA copolymer–doxorubicin (PK1; FCE28068)</td>
<td>Cancer, in particular lung, breast cancers</td>
<td>Phase II</td>
<td>Pfizer (CRC/Pharmacia)</td>
</tr>
<tr>
<td>HPMA copolymer–doxorubicin galactosamine (PK2; FCE28069)</td>
<td>Hepatocellular carcinoma</td>
<td>Phase I/II</td>
<td>Pfizer (CRC/Pharmacia)</td>
</tr>
<tr>
<td>HPMA copolymer–camptothecin (MAG–CPT; PNU166148)</td>
<td>Clinical evaluation on several solid cancers</td>
<td>Phase I</td>
<td>Pfizer (Pharmacia)</td>
</tr>
<tr>
<td>HPMA copolymer–paclitaxel (PNU166945)</td>
<td>Clinical evaluation on several solid cancers</td>
<td>Phase I</td>
<td>Pfizer (Pharmacia)</td>
</tr>
</tbody>
</table>

6. CONCLUSION

Conjugation is one of the major strategies for drug modifications, which manipulates therapeutic agents at molecular level to increase their solubility, permeability and stability, and thus biological activity. Such a strategy is based on a central assumption that the molecular structure of drugs can be modified to make analogous agents, which are chemically distinct from the original compound, but produce a similar or even better biological effect.

Drug conjugation can significantly change bio distribution of the therapeutic agent, thus improving its pharmacokinetics(PK) and pharmacodynamics (PD), increasing their therapeutic effects and reducing their side effects, as well as provide a means to circumvent the multidrug resistance (MDR). Various types of conjugation helps in targeting and delivery enhancing of the drug. It also showed great targeting efficiency this delivery system may have great potential for targeting delivery of various therapeutic and cytotoxic agents to body.

References

[5] Jong Eun Oha, Yoon Sung. Conjugation of drug to poly(D,L-lactic-co-glycolic acid) for controlled release


