Journal of Drug Delivery Science and Technology 32 (2016) 132–141

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Research paper

Drug and protein delivery by polymer conjugation

Antonella Grigoletto [a], Katia Maso [a], Anna Mero [a], Antonio Rosato [b, c], Oddone Schiavon [a], Gianfranco Pasut [a, c, *]

[a] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via F. Marzolo 5, 35131 Padova, Italy
[b] Department of Surgery, Oncology and Gastroenterology, University of Padova, Via Gattamelata 64, 35128 Padova, Italy
[c] Veneto Institute of Oncology IOV-IRCCS, Padova, Italy

ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form
10 August 2015
Accepted 15 August 2015
Available online 20 August 2015

Keywords:
Polymer conjugation
Drug delivery
PEGylation
HAylation
Protein conjugation
Enzymatic conjugation

ABSTRACT

Drug and protein delivery techniques and methods, which, in most cases, are concurrently investigated from the very time a new molecular entity is proposed, have become valuable tools for the development of numerous therapeutic molecules. Attracting the attention of academia and industrial researchers, several polymer conjugation methodologies have been shown to have a wide range of applications and a success rate that have been confirmed by the numerous conjugates already in clinical use or under investigation in on-going clinical trials. Having attained a high level of sophistication, this technology is able to generate homogeneous conjugates by exploiting specifically designed coupling strategies (based, for example, on the use of enzymes), which ensure good product characterization, high activity retention, and batch-to-batch reproducibility. Poly(ethylene glycol) (PEG), which is the current gold standard for stealth polymers in the emerging field of polymer-based drug delivery, is in competition with other polymers that have convenient features such as biodegradability. PEG still holds first place as far as PEGylated derivatives under investigation in clinical trials are concerned, and it will presumably remain the polymer of choice even in the future as further improvements such as the development of new copolymers based on PEG or PEG derivatives will overcome for example the hurdle of biodegradability.

1. Introduction

Drug delivery and controlled drug release have been finding increasing applications in several fields and they have remarkably contributed to the success of many chemical and biological therapeutic agents [1–3]. The delivery of a new drug, which is often investigated using parallel, systematic approaches and different methodologies, can lead to unprecedented results in terms of efficacy, safety and patient compliance. In the past, drug delivery was examined for the most part in connection with drugs, proteins, or low molecular weight molecules that present severe therapeutic limitations when they are used in their native form –i.e. low water solubility, fast body clearance, high rate of degradation, etc. Today, the concrete possibility of achieving selective targeting with fewer, less serious side effects is making drug delivery approaches attractive in connection to nearly all classes of therapeutics.

The reader of this volume of the *Journal of Drug Delivery Science and Technology* will find an overview of the different approaches to drug delivery with a special focus on our main area of expertise: polymer conjugation. The technique is very flexible as it can be suited to different therapeutic agents –*i.e.* proteins, small drugs, oligonucleotides– but also to other drug delivery systems (DDSs) such as PEGylation of liposomes or nanoparticles. Polymer conjugation can provide advantages to linked drugs by substantially modifying their *in vitro* and *in vivo* properties. For example, it can: a) improve the solubility of even very insoluble drugs (*i.e.* paclitaxel) [4], b) enhance drug bioavailability due to reduced kidney clearance, a consequence of an increase in hydrodynamic volumes of conjugates [5], c) provide drug protection from degrading enzymes [6], d) prevent or reduce aggregation, immunogenicity, and antigenicity [7], and e) specifically target organs, tissues or cells, using targeted polymers or exploiting the “enhanced permeability and retention (EPR) effect” of solid tumour tissues [8,9].

In general, from a structural point of view, the minimal components of a polymer conjugate are the polymer itself and the drug, either a protein or a small molecule, although a linker, which may have a determinant role in the success or failure of the entire system, can also be present, as disclosed in the known Ringsdorf's

* Corresponding author. Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via F. Marzolo 5, 35131, Padova, Italy
E-mail address: gianfranco.pasut@unipd.it (G. Pasut).

http://dx.doi.org/10.1016/j.jddst.2015.08.006
1773-2247/

model that is better discussed in the next section "PEG conjugation of small drugs". Beyond this composition, there is, in accordance with recent studies, a targeting moiety needed to achieve a ligand-mediated selective targeting at the desired site of action. The polymer has traditionally been regarded as simply a 'platform' that can confer enough size to reduce kidney clearance, shield the protein, and eventually produce passive tumour accumulation. But an increasing number of reports indicate that the polymeric carrier itself plays a key role in biological activity [10]. Initial studies examined libraries of a single polymer to determine the impact of different polymer features on biological activity (see, for example, for HPMA copolymers [11] for PVP [12], for dextran [13] and for PEG [14,15]). Subtle features, such as the impact of polymer architecture [16–18] are also under investigation.

PEG appears to be one of the most important and widely used polymer in the field of drug delivery. PEG's high biocompatibility, low toxicity, and shielding effect are properties that have already been described in several reviews [19–21]. Clinically used PEG conjugates accounts for 15 approved products (Table 1). The numerous studies on PEGylation described in the literature constitute a valuable reference and evidence of its many advantages for researchers working on new projects. While the PEG polymer is now commercially available with different reactive end groups and several molecular weights and architectures (*i.e.* linear, mono- or bi-functional, heterobifunctional, branched, forked, multi-arm, comb-shaped [22], etc.) that are characterized by high purity and low polydispersity. It is non-biodegradable so its *in vivo* use requires the use of molecular weights below the kidney excretion threshold, approximately 40 kDa [23,24].

The presence and role of anti-PEG antibodies, which have been extensively described by some studies involving large PEG conjugates with highly immunogenic enzymes (i.e., asparaginase, uricase, etc.) [25,26], continue to be debated. Some researchers have suggested that the size and structure of conjugates obtained by extensive PEGylation of high molecular weight proteins in which several PEG chains are linked onto the protein surface reassemble the features of small PEGylated nanoparticles for which the so-called accelerated blood clearance (ABC) phenomenon, involving complement activation by IgM, has been documented [27,28]. Findings from another study suggest that the methoxy group of PEG plays a role in the immune response against PEGylated proteins [29]. It is, nevertheless, important to remember that most if not all assays for detecting anti-PEG antibodies are flawed and lack specificity [30]. The biological effects ascribed to anti-PEG antibodies are as yet not entirely defined and require further studies. Based on the good safety profile of the PEGylated proteins currently in clinical use, we can suggest that these concerns regard only a small percentage of the population and are in line the common percentage of adverse immune responses of biologic drugs.

Academic and industrial researchers have presented and investigated new polymers for protein delivery such as hydroxyethyl-starch [31], polyoxazoline [32–34], dextrin [35,36] polysialic acid [37,38], hyaluronic acid [39–42], polypeptides (XTEN technology [43], PASylation [44]), etc. While biodegradable polymers are desirable for drug delivery, biodegradability is not easily controlled. Degradation can, in fact, occur before the drug has been

Table 1
Clinically approved PEGylated therapeutic conjugates and some examples of conjugates being used in phase II and III clinical trials (clinical status from https://clinicaltrials.gov/).

Conjugate	Drug entity	Indication	Approval year/status	Ref.
PEG-Adenosine Deaminase (Adagen)	Protein	Severe Combined Immunodeficiency Disease (SCID)	1990	[97]
PEG-Asparaginase (Oncaspar)	Protein	Leukaemia	1994	[98]
Doxorubicin PEGylated liposome (Doxil)	Small drug	Ovarian cancer, AIDS-related Kaposi's Sarcoma, multiple myeloma	1995	[91]
PEG-Interferon-α2b (PegIntron)	Protein	Hepatitis C	2000	[99]
PEG-Interferon-α2a (Pegasys)	Protein	Hepatitis C	2001	[100]
PEG-Human growth hormone mutein antagonist (Somavert)	Protein	Acromegaly	2002	[101]
PEG-G-CSF (Neulasta)	Protein	Neutropenia	2002	[71]
PEG-anti-VEGF aptamer (Pegaptanib, Macugen)	Oligonucleotide	Wet age related macular degeneration	2004	[102]
PEG-Erytropoietin (Mircera)	Protein	Anaemia associated with chronic kidney disease	2007	[103]
PEG-anti-TNF Fab' (Cimzia)	Protein	Rheumatoid arthritis and Crohn's disease	2008	[104]
PEG-Uricase (Pegloticase; Krystexxa)	Protein	Chronic Gout	2010	[105]
Peginesatide (Omontys)	Peptide	Anaemia associated with chronic kidney disease	2012, withdrawn 2013	[106]
GlycoPEGylated G-CSF (lipegfilgrastim)	Protein	Neutropenia	2013	[107]
Peg-interferon beta 1a (Plegridy)	Protein	Relapsing Multiple Sclerosis	2014	[108]
PEG-Naloxone (Naloxegol)	Small drug	Opioid-Induced Constipation	2014	[109]
Pegylated recombinant coagulation factor VIII (Damoctocog alfa pegol; BAY94-9027)	Protein	Haemophilia, Haemorrhage	Phase III Haemophilia A; Phase II Haemorrhage	[110]
PEGylated recombinant phenylalanine ammonia lyase (Pegvaliase)	Protein	Phenylketonuria	Phase III	[111]
Arginine deiminase replacements (Pegargiminase)	Protein	Hepatocellular carcinoma	Phase III	[112]
GlycoPEGylated recombinant coagulation factor VIII (N8-GP)	Protein	Haemophilia A	Phase III	[113]
Pegylated Recombinant Factor IX (N9-GP)	Protein	Haemophilia B	Phase III	[114]
Pegylated haemoglobin (MP4, Hemospan)	Protein	Blood substitution	Phase III	[115]
PEGylated Recombinant Factor VIII (BAX855)	Protein	Haemophilia A	Phase IIIb	[116]
Pegylated-Proline-interferon alpha-2b (AOP2014)	Protein	Polycythemia Vera	Phase III	[117]
Pegylated-fibroblast growth factor-21 (BMS 986036)	Protein	Type 2 diabetes mellitus	Phase II	[118]
Peginterferon beta-1a	Protein	Multiple sclerosis	Phase IIIb	[119]
PEGylated recombinant human hyaluronidase	Protein	Pancreatic cancer	Phase III	[120]
PEG-SN38 (EZN-2208)	Small drug	Several cancers	Phase II for metastatic breast cancer	[121]
Etirinotecan pegol (NKTR-102)	Small drug	Several cancers	Phase III for metastatic breast cancer	[122]
PEG-polyglutamic-SN38 micelle (NK012)	Small drug	Several cancers	Phase II	[88]