Available online at www.sciencedirect.com
ScienceDirect
Advanced Drug Delivery Reviews 60 (2008) 1–2
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

Preface

Peptide and protein PEGylation III: advances in chemistry and clinical applications☆

The practice of covalent coupling of poly(ethylene glycol) to pharmaceutical proteins, commonly named PEGylation, is now regarded as an extremely useful procedure to overcome certain problems faced in the development and use of protein drugs. The continuing interest in PEGylation is well documented by the number of papers and patents that have appeared since the discovery of this methodology by Davis and Abuchowski in the late 1970s [1]. Indeed, PEGylation has become the dominant protein drug delivery system for the biotech industry, with sales of PEGylated protein drugs reaching over $4 billion [2]. The practical importance of the procedure is clearly demonstrated by the number of protein-PEG conjugates reaching the market or making it to an advanced state of clinical experimentation. Examples of commercial products using PEGylation are Adagen®, Oncaspar®, PEG-Intron®, PEGASYS®, Neulasta® and Somavert®, and a dozen other PEG-proteins which are now in advanced clinical trials [3]. Although this methodology must be considered a mature technique, new developments arising from research and applications justify a new ADDR issue on this subject, following the two previous ones appearing in this Journal in 2002 and 2003 (volumes 54(4) and 55(10)). Originating as a novelty, in recent years there has been a shift from academic to industrial interest in PEGylation technology. This may be understood, considering that this research is close to market interests and, therefore, the technology is now accepted as a standard technique in industrial settings. The present third issue on PEGylation takes into account the results of both fundamental and applied research, with the latter addressing the practical problems of protein drug formulation, as well as the clinical evaluation of new PEGylated products.

The first chapter, by Brocchini and Shaunak (Imperial College of London, U.K.), addresses the important problem of obtaining site-specific protein PEGylation to avoid the loss of biological activity, an inconvenience often observed in cytokine or enzyme conjugation. The proposed method is based on an original and intriguing chemistry that involves only the exposed protein disulphide bonds as sites of modification [4].

The second chapter, by Fontana and Veronese research groups (University of Padua, Italy), illustrates the structural and dynamic features of proteins that enable selective conjugation of an amino derivative of PEG to protein-bound glutamine residues by means of microbial transglutaminase [5]. The authors demonstrate that enzyme-mediated PEGylation requires proper chain mobility or local unfolding at the site of conjugation, as revealed by the clear-cut correlation between sites of modification and sites of enhanced flexibility detected by X-ray analysis. Furthermore, they demonstrate that chain flexibility is required for the action of other enzymes that act on polypeptide substrates, including proteases. These results indicate that it is possible to predict *in silico* the potential sites of enzymatic PEGylation using transglutaminase, if the structural and dynamic features of a protein are known.

In the third chapter, Filpula and Hong Zao (Enzon Inc., Piscataway, NJ, U.S.A.) present an interesting overview of new releasable methods of PEGylation, a recent and promising approach to overcome the often encountered problem of loss of biological activity following permanent linking of the polymer. The authors describe the chemistry used to obtain release of the unaltered original drug under physiological conditions and at the desired rate, and they report examples of favourable biological consequences of this new procedure [6].

Piedmonte and Treuheit (Amgen Inc., Thousand Oaks, CA, U.S.A.) describe in the fourth chapter a seldom-described aspect of the preparation of therapeutic proteins, namely the final formulation of products for market application. They report the experience faced in their company in obtaining a product that could respond to the demands of a labile protein molecule. Neulasta, a PEGylated form of G-CSF, a drug of great therapeutic interest, is the example presented [7].

Sherman et al. (Mountain View Pharmaceuticals Inc., Menlo Park, CA, U.S.A.) report in Chapter 5 the development of Puricase, a PEG-conjugated form of the enzyme uricase that may be of great interest in treatment of gout as well as of some side effect of chemotherapy. Both of these instances are cases in which an overproduction of uric acid takes place. The problem has been solved by performing PEGylation of a suitable non-human enzyme, thus reduced its great immunogenicity. The conjugated enzyme is now in advanced phase three of clinical experimentation [8].

In Chapter 6, Pasut and Sergi (University of Padua, Italy, and Bio-Ker, Italy) reviewed the numerous enzymes that, due to their

☆ This preface is part of the *Advanced Drug Delivery Reviews* theme issue on "Peptide and Protein PEGylation III: Advances in Chemistry and Clinical Applications".

0169-409X/$ - see front matter
doi:10.1016/j.addr.2007.08.003

amino acid depleting activity, may be used in cancer therapy. In different types of tumours there are amino acids that the cancer cell's metabolism is not able to synthesize and therefore, reduction of these amino acids in the blood may represent a new therapeutic approach. Unfortunately most of these enzymes are heterologous and, therefore, their repeated administration may result in immunogenicity; of course, this problem may be prevented by PEG conjugation. This is an approach that, years ago in the case of asparaginase, resulted in a successful product for the treatment of asparagine-dependent tumours [9].

Finally Zappe, et al. (Nektar Therapeutics, Huntsville, AL, U.S.A.) describe a new approach in HIV therapy based on the use of Cyanovirin-N, a potent inhibitor of this as well as of other viruses. Also, in this case, the non-human origin of the peptide makes treatment with the native form risky. However, direct PEGylation of the natural product was not the solution. Rather, it was found that a mutant with an introduced cysteine for site-specific modification was more useful [10].

The topics reviewed in this ADDR issue are only a small selection of the huge production of PEGylation research presently under way. PEGylation, which started from protein modification, now includes delivery of oligonucleotides, genes, microparticles, liposomes and living cells [11,12]. This continuing interest for new applications is well documented in over five hundred patents filed so far in the field, a situation which is not paralleled by any other polymer of pharmaceutical application. The success of PEG modification of proteins is, in a way, imitating nature's post-transcriptional modification of proteins to expand and differentiate their role. It is probable that this strategy is not exclusive for poly(ethylene glycol), as recent studies using polymers of natural origin such as polysaccharides [13,14] and synthetic polymers [15–17] are demonstrating. It is our belief that the future will see interesting discoveries in this field of delivery and therapy, which now are probably limited solely by the availability of alternative polymers, a situation similar to that of several years ago at the beginning of PEG development when only a few laboratories could produce this polymer in a reproducible and pure form. At that time it was interesting to see the collaboration between polymer chemists, protein chemists and motivated industries that made the jump possible. It is likely that we will need a further gathering of interest to go further in the field.

Francesco M. Veronese
(Theme Editor)

Department of Pharmaceutical Sciences, Via Marzolo 5
University of Padua, 35121 Padua, Italy
E-mail address: francesco.veronese@unipd.it (F.M. Veronese)

J. Milton Harris
(Theme Editor)

Serina Therapeutics, Inc.
2215 Mock Road SW Huntsville, AL 35801
U.S.A.
E-mail address: miltonmzee@yahoo.com (J.M. Harris)

References

[1] A. Abuchowski, J.R. McCoy, N.C. Palczuk, T. van Es, F.F. Davis, Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase, J. Biol. Chem. 252 (1977) 3582–3586.
[2] K. Maggon, R&D paradigm shift and billion-dollar biologics, Handbook of Pharmaceutical and Biotechnology, John Wiley, 2007, pp. 161–198.
[3] G. Pasut, F.M. Veronese, Polymer drug conjugation, recent achievements and general strategies, Prog. Polym. Sci. 32 (2007) 933–961.
[4] S. Brocchini, A. Godwin, S. Balan, Ji-won Choi, M. Zloh, S. Shaunak, Disulfide bridge based PEGylation of proteins, Adv. Drug Delivery Rev. 60 (2008) 4–13 (this issue), doi:10.1016/j.addr.2007.06.014.
[5] A. Fontana, B. Spolaore, A. Mero, F.M. Veronese, Modification of PEGylation of pharmaceutical proteins mediated by transglutaminase, Adv. Drug Delivery Rev. 60 (2008) 14–29 (this issue), doi:10.1016/j.addr.2007.06.015.
[6] David Filpula, Hong Zhao, Releasable PEGylation of proteins with customized linkers, Adv. Drug Delivery Rev. 60 (2008) 30–50 (this issue), doi:10.1016/j.addr.2007.02.001.
[7] Deirdre Murphy Piedmonte, J. Michael, Treuheit Formulation of Neulasta® (PEGfilgrastim), Adv. Drug Delivery Rev. 60 (2008) 51–59 (this issue), doi:10.1016/j.addr.2007.04.017.
[8] M.R. Sherman, M.G.P. Saifer, F. Perez-Ruiz, PEG-uricase in the management of treatment-resistant gout and hyperuricemia, Adv. Drug Delivery Rev. 60 (2008) 60–69 (this issue), doi:10.1016/j.addr.2007.06.011.
[9] G. Pasut, M. Sergi, F.M. Veronese, Anti-cancer PEG-enzymes: a 30 years old, but still a current approach, Adv. Drug Delivery Rev. 60 (2008) 70–79 (this issue), doi:10.1016/j.addr.2007.04.018.
[10] H. Zappe, M.E. Snell, M.J. Bossard, PEGylation of cyanovirin_N, an entry inhibitor of HIV, Adv. Drug Delivery Rev. 60 (2008) 80–88 (this issue), doi:10.1016/j.addr.2007.05.016.
[11] F.M. Veronese, G. Pasut, PEGylation, successful approach to drug delivery, Drug Discov. Today 10 (2005) 1451–1457.
[12] K.L. Murad, K.L. Mahany, F.A. Kuypers, C. Brugnara, J.M. Eaton, M.D. Scott, Structural and functional consequences of antigenic modulation of red cells with methoxy poly(ethylene glycol), Blood 93 (1999) 2121–2127.
[13] G. Gregoriadis, S. Jain, I. Papaioannou, P. Laing, Improving the therapeutic efficacy of peptides and proteins: a role for polysialic acids, Int. J. Pharm. 300 (2005) 125–130.
[14] A.I. Fernandes, G. Gregoriadis, The effect of polysialylation on the immunogenicity and antigenicity of asparaginase: implication in its pharmacokinetics, Int. J. Pharm. 217 (2001) 215–224.
[15] F.M. Veronese, P. Caliceti, O. Schiavon, New synthetic polymers for enzymes and lyposomes modification, in: M.J. Harris, S. Zalipski (Eds.), Poly(ethylene glycol) Chemistry and Biological Applications, ACS Symposium Series, vol. 680, 1977, pp. 182–192.
[16] M. Miyamoto, K. Naka, M. Shiozaki, Y. Chujo, T. Saegusa, Preparation and enzymatic activity of poly[(*N*-acylimino) ethylene] modified catalase, Macromolecules 23 (1990) 3201–3205.
[17] F.C. Gaertner, R. Luxenhofer, B. Blechert, R. Jordan, M. Essler, Synthesis, biodistribution and excretion of radiolabeled poly(2-alkyl-2-oxazoline)s, J. Control. Release 119 (2007) 291–300.