Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutical Sciences 24 (2005) 159–168
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
www.elsevier.com/locate/ejps

Synthesis and in vitro evaluation of macromolecular antitumour derivatives based on phenylenediamine mustard

Katleen De Winne[a], Leonard W. Seymour[b], Etienne H. Schacht[a,*]

[a] *Polymer Materials Research Group, Ghent University, Krijgslaan 281 S4-bis, 9000 Ghent, Belgium*
[b] *Department of Clinical Pharmacology, Radcliffe Infirmary, University of Oxford, Woodstock Road, Oxford OX2 6HE, UK*

Received 3 February 2004; received in revised form 3 September 2004; accepted 15 September 2004
Available online 25 December 2004

Abstract

Poly-[*N*-(2-hydroxyethyl)-L-glutamine] (PHEG) and poly(ethylene glycol) (PEG)-grafted PHEG conjugates of *N*,*N*-di(2-chloroethyl)-4-phenylenediamine mustard (PDM) were synthetised. A collagenase-sensitive oligopeptide spacer was selected to link the cytotoxic agent PDM onto the polymeric carrier. First, the oligopeptide-drug conjugate, L-pro-L-leu-gly-L-pro-gly-PDM, was prepared. In a second step, the low molecular weight PDM derivative and PEG-NH_2 were coupled to a *N*,*N*-disuccinimidylcarbonate activated PHEG. Dynamic laser light scattering measurements indicated the formation of aggregates. The presence of human serum albumin had no significant effect on the diameter of the conjugates. The hydrolytic stability of the conjugates was investigated in buffer solutions. The conjugates showed an improved stability compared to the parent nitrogen mustard. The enzymatic degradation studies of the polymeric conjugates were performed in the presence of collagenase type IV (Clostridiopeptidase A; EC 3.4.24.3), cathepsin B (EC 3.4.22.1), cathepsin D (EC 3.4.23.5) and tritosomes. Only the bacterial collagenase type IV was able to cleave the spacer releasing free PDM and its peptidyl derivative, gly-L-pro-gly-PDM. The in vitro cytotoxicity of the conjugates was evaluated against HT1080 fibrosarcoma cells and MDA adenocarcinoma cells. All conjugates showed low toxicity towards these cell lines.

Keywords: Polymeric conjugate; Phenylenediamine mustard; Collagenase; Poly(hydroxyalkylglutamine)

1. Introduction

The main objective of covalent linking a conventional antitumour agent to a macromolecular carrier is to improve the therapeutic index of the parent drug. The concept of water-soluble macromolecular prodrugs was first proposed by Ringsdorf (1975). Such conjugates are designed to circulate in the bloodstream for an extended period of time followed by accumulation within the tumour tissue (Maeda and Matsumura, 1989).

A site-specific drug release can be controlled by selecting a proper drug–polymer linkage. During circulation in the bloodstream the linkage should be stable, but in or near the target cells, e.g. cancer cells, the spacer should be degraded, resulting in the release of the antitumour agent. Consequently, conjugates having oligopeptide spacers, which are substrates for lysosomal and tumour-associated enzymes have been

Abbreviations: PDM, *N*,*N*-di(2-chloroethyl)-4-phenylenediamine mustard; PHEG, poly-[*N*-(2-hydroxyethyl)-L-glutamine]; PEG, poly(ethylene glycol); Z-, benzyloxycarbonyl-; MMC, mitomycin C; EDTA, ethylenediaminetetraacetic acid; NMR, nuclear magnetic resonance; s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; DCC, dicyclohexylcarbodiimide; DCU, diclohexylureum; NHS, *N*-hydroxysuccinimide; TLC, thin layer chromatography; Rf, ratio to front; EtAc, ethylacetate; AcOH, acetic acid; GABA, γ-aminobutyric acid; DSC, *N*,*N*-disuccinimidylcarbonate; ISA, ionic strength adjustor; PBS, phosphate buffered saline; DLS, dynamic light scattering; HPLC, high performance liquid chromatography; DME, Dulbecco's Modified Eagle medium; MTT, 3-(4,5-dimethyl)thiazol-2,5-diphenyl tetrazolium bromide; DMSO, *N*,*N*-dimethylsulfoxide; GPC, gel permeation chromatography; Mw, weight average molecular weight; MMP, matrix metalloprotease

* Corresponding author. Tel.: +32 9 264 44 97; fax: +32 9 264 49 72.
E-mail address: etienne.schacht@ugent.be (E.H. Schacht).

0928-0987/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.ejps.2004.09.006

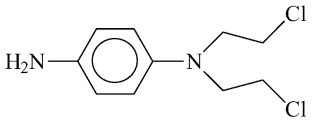


Fig. 1. Structure of *N*,*N*-di(2-chloroethyl)-4-phenylenediamine mustard (PDM).

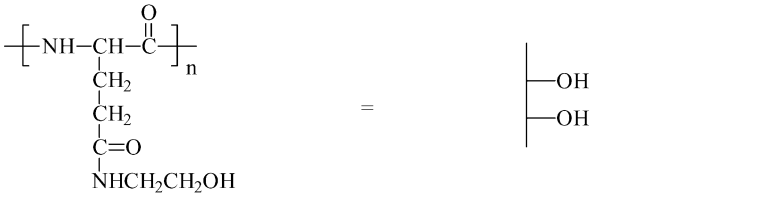
investigated by several groups (Duncan, 1992, 2003; Kopecek et al., 2000; Subr et al., 1992; Rejmanova et al., 1983; Soyez et al., 1996a,b; Matthews et al., 1996).

N,*N*-Di(2-chloroethyl)-4-phenylenediamine mustard (PDM; Fig. 1) is an alkylating antitumour agent which was selected in this work because of its high cytotoxicity. Due to the formation of an aziridium ion, it is highly reactive against nucleophiles (Connors and Knox, 1995). The biological activity of PDM is a result of the crosslinking of DNA in the nucleus of the cell (Panthananickal et al., 1978; Connors and Knox, 1995; Palmer et al., 1990). For aromatic nitrogen mustards the substituent in para position has a major influence on the chemical stability and reactivity of the mustard. In case of PDM, where the para substituent is an amine, the reactive aziridinium ion is stabilised, resulting in a fast hydrolysis or a fast rate of alkylation. By acylation of the aniline function of the nitrogen mustard the highly toxic agent can be converted into a less toxic compound (Palmer et al., 1992; Ross, 1955; Johnson and Owen, 1961).

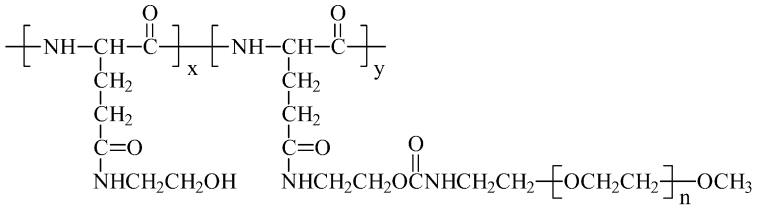
Marquisee and coworkers developed nitrogen mustard peptide derivatives such as Z-L-pro-L-leu-gly-L-pro-gly-PDM which were designed to serve as a substrate for tumour-associated collagenase (Marquisee and Kauer, 1978). The in vitro results showed to be promising. However, the in vivo tests using Sarcoma 180 cells were disappointing. This in vivo lack of specific antitumour activity may be due to competing cleavage by collagenases in certain normal tissues. Another possible explanation is a premature rapid elimination of the low molecular weight derivative from the body through glomerular filtration, which results in low therapeutic concentration of the drug in the tumour tissue. Therefore, attaching this oligopeptide derivative of PDM to a polymer carrier is a plausible approach to improve the efficiency.

In this work, poly-[*N*-(2-hydroxyethyl)-L-glutamine] (PHEG) and poly(ethylene glycol) (PEG)-grafted PHEG (Fig. 2) were selected as drug carriers for the preparation of the macromolecular prodrugs. PHEG is a synthetic poly-α-aminoacid, introduced by the group of Neri as a plasma expander (Gerola et al., 1970). The lack of toxicity, its low immunogenicity, water solubility, biocompatibility, biodegradability and the presence of hydroxyl groups makes PHEG a suitable carrier (Pytela et al., 1989, 1994, 1998; Rypacek et al., 1985). In our research group, PDM and mitomycin C (MMC) conjugates of PHEG were already synthetised and evaluated (De Marre et al., 1994, 1995; Soyez and Schacht, 1997; Soyez et al., 1996a,b, 1997, 1999; Hoste et al., 2000, 2002). Due to the poor water solubility of hydrophobic drugs like MMC and PDM, soluble conjugates are only obtained for low degree of substitution. In order to maintain the water solubility of the conjugates and to increase the degree of substitution of the drug, PEG was used as solubilising entity.

It was shown in the work of Hoste et al. that PEG-grafted dextran and PEG-grafted PHEG are promising candidates as macromolecular drug carriers (Hoste et al., 1994, 1996, 2000; Schacht and Hoste, 1997). The substitution of PEG onto the PHEG resulted in a better solubility of the conjugates. Moreover, the in vivo evaluation of MMC-PHEG-PEG conjugates showed a strong tumour regression within colorectal SW380 bearing mice (Hoste et al., 2002). Therefore, it was decided to synthesise PDM conjugates with PEG-substituted PHEG as carrier.



PHEG



PEG-PHEG

Fig. 2. Structure of poly-[*N*-(2-hydroxyethyl)-L-glutamine] (PHEG) and poly(ethylene glycol)-grafted PHEG (PEG-PHEG).