Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 277 (2004) 119–131
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Polymeric prodrugs

K. Hoste, K. De Winne, E. Schacht*

Polymer Materials Research Group, Department of Organic Chemistry, Ghent University, Krijgslaan 281 (S4bis), 9000 Gent, Belgium

Received 30 January 2003; received in revised form 15 April 2003; accepted 17 July 2003

Abstract

In 1975 Prof. H. Ringsdorf proposed a model for rational design of polymeric prodrugs [J. Polym. Sci. Symp. 51 (1975) 135]. The model has been the most important basis for research in the field, since it was the first model that took into account both the chemical and biological aspects needed for the design of polymeric prodrugs. This paper deals with the most important properties that were discovered by designing polymeric prodrugs: prolongation of action of the drug, controlled release of the drug, passive tumor accumulation by the EPR-effect and alteration of body distribution and cell uptake. Over the years, other objectives have been formulated and other properties of polymer–drug conjugates were discovered. One recent example, the immunoprotective ability of polymeric prodrugs, is described in more detail in this paper.

Keywords: Prodrug; Controlled drug release; Conjugate; Tumor-associated enzymes; Targeting

1. Introduction

In the last decades polymer chemists have been actively involved in designing polymer materials for biomedical applications.

One field of application that has attracted polymer chemist's attention from the late 1960s onwards is the need for advanced drug delivery systems to improve drug efficacy. Polymer materials were designed and proposed as matrices or depot systems for injectable or implantable systems or devices. One particular approach towards an improved use of drugs for therapeutic applications is the design of polymeric prodrugs or polymer–drug conjugates.

It was already early in the 1950s and 1960s that polymer chemists started to link drugs onto polymers to improve their efficiency (Jatzkewitz, 1955; Panarin and Ushakov, 1968). At that time however, they were mainly concentrating on the chemistry itself and almost any class of polymers was covalently combined with any class of drugs. The biological aspects for the design of polymeric prodrugs were hardly taken into account.

It was for the first time in 1975 that a rational model for pharmacologically active polymers was proposed (Ringsdorf, 1975). Prof. H. Ringsdorf was the first to recognise the immense potential of polymeric prodrugs, if only polymer chemists and biologists would work together in the field. The proposed model consists mainly of five components: the polymeric backbone, the drug, the spacer, the targeting group and the solubilising agent.

The polymeric carrier can be either an inert or a biodegradable polymer. The drug can be fixed directly or via a spacer group onto the polymer backbone. The proper selection of this spacer opens the possibility of

* Corresponding author. Tel.: +32-9-264-4497; fax: +32-9-264-4990.
E-mail address: etienne.schacht@rug.ac.be (E. Schacht).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2003.07.016

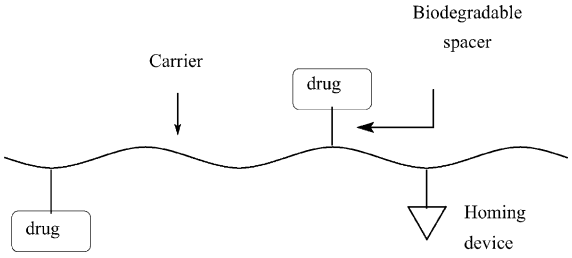

Fig. 1. The Ringsdorf model.

controlling the site and the rate of release of the active drug from the conjugate by hydrolytic or enzymatic cleavage. The most challenging aspect of this model is the possibility of altering the body distribution and cell uptake by attaching cell-specific or nonspecific uptake enhancers (homing devices).

This model, although still oversimplified, has been an important mark in the history of polymeric prodrug design. It made clear that a more rational design was needed based on information arising from biological work. This remarkable paper has also catalysed the interest of biologists and pharmacists in synthetic polymers. As more information becomes available from cell biology and molecular biology, polymer chemists are trying to design tailor-made polymeric carriers that better fulfil the specified requirements.

In time it has been shown that there is a clear relationship between the structural elements of the Ringsdorf model and the properties of the synthetised polymeric prodrugs based on it. In fact, the properties that polymer chemists want to reach with the design of their polymer–drug conjugates, are translated in the components of the model. Some of these properties and their relationship with the components in the model will now be discussed in more detail in this paper (Fig. 1).

2. Properties of polymeric prodrugs

2.1. Prolongation of action of the drug

The profile of plasma concentration of drugs is an important determinant of their quantitative access to peripheral targets. The plasma profile is usually measured as the area under the curve (AUC). In general, slow renal elimination and metabolic inactivation promote better access of drugs to remote targets, although this can also cause elevated toxicity. Many drugs in routine use are membrane permeable because their sites of action are intracellular and such drugs typically exhibit high volumes of distribution and rapid plasma clearance.

By linking a drug onto a polymer, a conjugate is obtained with a higher hydrodynamic volume. This results in a slower renal excretion, longer blood circulation and an endocytotic cell uptake (Maeda et al., 1992; Seymour et al., 1990).

To select polymers as candidate drug carriers a number of requirements should be fulfilled:

- availability of suitable functional groups for covalent coupling with drugs;
- biocompatibility: preferably nontoxic, nonimmunogenic;
- biodegradability or a molecular weight below the renal excretion limit;
- availability.

A number of reviews cover what has been done over the past 20 years in the field of soluble polymers as potential drug carriers (Vert, 1986; Okano et al., 1994; Barry, 1983; Donaruma, 1974; Bat, 1977). The polymers selected for preparing macromolecular prodrugs can be categorised according to: (a) the chemical nature (vinylic or acrylic polymers, polysaccharides, poly(α-amino acids)); (b) the back bone stability (biodegradable polymers, stable polymers); (c) the origin (natural polymers, synthetic polymers); and (d) the molecular weight (oligomers, polymers).

Vinyl polymers can be easily prepared by radical polymerisation of the corresponding vinyl monomer. They are interesting drug carrier candidates. Since copolymerisation of selected monomers results in polymers with a variable composition, different polymer properties can be achieved. In a way, the candidate carriers can be tailor-made to fulfil the requirements for the design of the polymer–drug conjugate. However, vinyl polymers are not biodegradable. Hence, in order to avoid undesirable storage, the molecular weight should at least be below the renal filtration limit (40–50 kDa).

At present, the most intensively studied vinyl polymers are copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) (Duncan et al., 1983a,b; Lloyd et al., 1983; Seymour et al., 1991; Flanagan et al.,

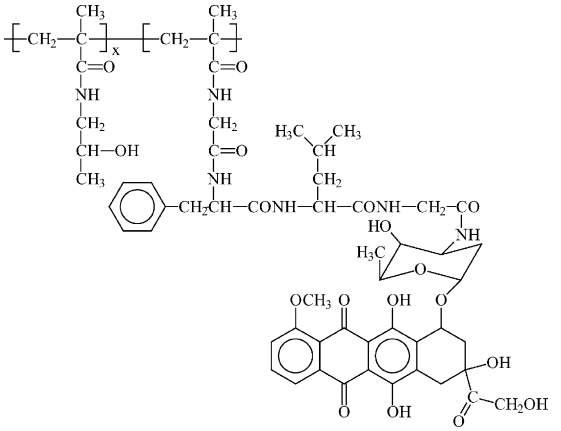

Fig. 2. Structure of PHMPA copolymer containing adriamycin (PK1).

1989; Seymour et al., 1987). HPMA homopolymer was originally developed in Czechoslovakia as a plasma expander (Kopecek and Bazilova, 1973). It is hydrophilic and non-toxic in rats. HPMA copolymer with adriamycin as antitumor agent linked onto it with the peptidyl linker Gly–Phe–Leu–Gly (PK1) (Fig. 2), was developed by Duncan and Kopecek (Duncan, 2001).

It was demonstrated that HPMA–adriamycin conjugates are remarkably less toxic than the free drug and accumulate within solid tumor models. PK1 reached phase II clinical trial for the treatment of breast, colon and non-small-cell lung cancer (Duncan, 2001). The only polymeric prodrug with targeting moiety, that entered early clinical trials for the treatment of primary and secundary liver cancer is PK2 (Seymour et al., 2002). This conjugate is a HPMA copolymer

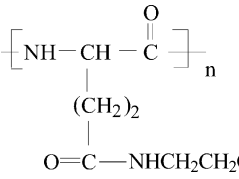

Fig. 4. Structure of poly(*N*-(2-hydroxyethyl-L-glutamine)).

with adriamycin as antitumor agent and *N*-acylated galactosamine as targeting group. It is developed for targeting to the liver by facilitating the interaction with hepatocyte asiaglycoprotein receptors.

Poly(styrene-*co*-maleic acid/anhydride) (SMA) is a vinyl polymer introduced by Maeda and co-workers. It was used to synthetise the prodrug, SMANCS (Fig. 3), a conjugate of a low-molecular-weight styrene maleic anhydride copolymer (SMA, 1.6 kDa) and the antitumor protein neocarcinostatin (NCS). It is already marketed in Japan for the treatment of hepatocellular carcinoma (Maeda, 1991a,b).

Synthetic poly(α-amino acids) like poly(L-lysine), poly(L-glutamic acid), poly((*N*-hydroxyalkyl)glutamines) can be made by ring-opening polymerisation of the *N*-carboxyanhydride monomers (Kricheldorf, 1987; Blout and Karlson, 1956). These polymers have functionalities in their side groups (amine, hydroxyl, carboxyl) that allow covalent coupling with drug molecules (Fig. 4). Generally, poly(L-amino acids) are biodegradable, whereas their D-enantiomers are not.

In our research group poly(*N*-(2-hydroxyethyl-L-glutamine)) (PHEG) is already used for many years in the development of polymeric prodrugs. PHEG was originally designed by Neri as a plasma expander (Neri et al., 1973; Gerola et al., 1970). It is non-toxic,

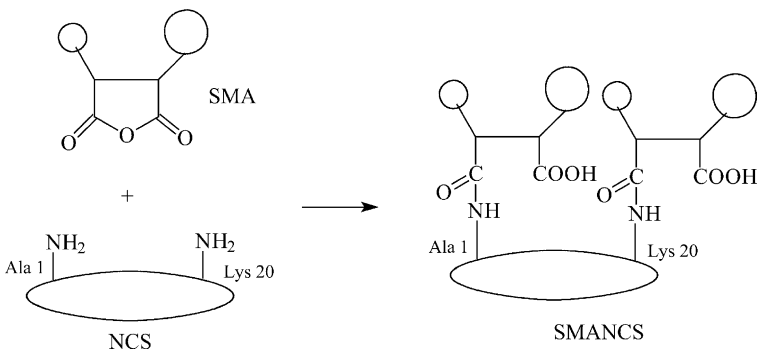

Fig. 3. Diagrammatic representation of the reaction between SMA and NCS to produce the conjugate SMANCS.

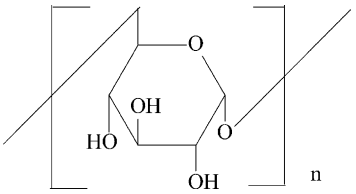

Fig. 5. Structure of dextran.

biocompatible and degradable by lysosomal enzymes. The synthesis and evaluation of PHEG-based drug conjugates are described in a large number of articles (Roseeuw et al., 1999; De Winne et al., 2001; De Marre et al., 1994b; Hoste et al., 2000).

Polysaccharides are another interesting class of drug carriers. Much attention has been directed to the use of dextran. Sezaki and co-workers prepared dextran–mitomycin conjugates with either amino-caproic acid or 6-bromohexanoic acid as spacer (Sezaki and Hashida, 1984; Matsumoto et al., 1986) (Fig. 5). The pharmacokinetics of these conjugates proved to be dependent on the molecular weight and the electrical charge of the polymer derivative.

Dextrans are a family of polysaccharides mainly composed of 1,6-linked α-D-glucose units. Dextran with a molecular weigth below 100,000 is not immunogenic and is clinically used as blood substitute. Dextran was claimed to be biodegradable. However, it was demonstrated by Vercauteren that the in vitro degradation of dextrans in presence of lysosomal glucosidases or endodextranases is rather slow. Moreover, it was shown that chemical modification of the dextran further reduces its biodegradability (Vercauteren et al., 1992).

Proteins such as serum albumin have also frequently been used for preparing polymeric prodrugs. An interesting example is the work of Meyer and co-workers who used mannosylated serum albumin as carrier for antiviral drugs (Franssen et al., 1994; Seymour, 1994). A disadvantage of proteins is their complexity in chemical composition, which complicates the identification of the final conjugates.

Poly(ethylene glycol) (PEG) has been used to modify a number of therapeutically interesting proteins. PEG is a polymer with many useful properties, it is soluble in water and in organic solvents, it is not toxic and not immunogenic. It is approved by the FDA to be used in nose sprays, food and cosmetics. It has

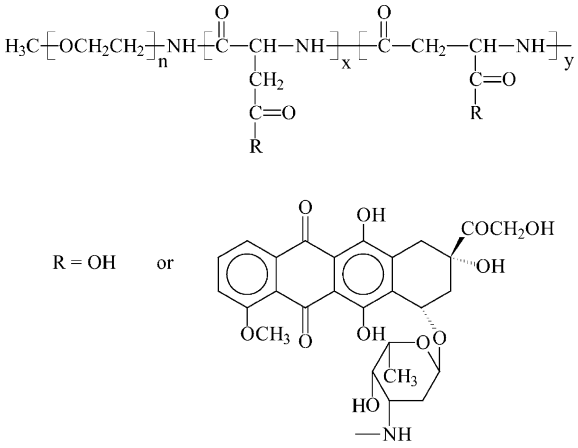

Fig. 6. Adriamycin-conjugated poly(ethylene glycol)–poly(aspartic acid) block copolymer.

been clearly demonstrated by Abuchowski et al. (1977, 1984) that grafting of PEG onto proteins reduces their immunogenicity, improves their resistance to proteolytic degradation and improves their thermostability.

Micelle-forming block copolymers have been introduced by Kataoka and co-workers (Yokoyama et al., 1990, 1991; Yokoyama, 1992). Conjugates of adriamycin with poly(ethylene glycol)–poly(aspartamide) block copolymers tend to form micelles (Fig. 6). The hydrophilic PEG chains form the outer shell and the hydrophobic poly(aspartic acid)–doxorubicin components form the inner core. It was demonstrated that these systems have a very high in vivo antitumor activity and show a reduced non-specific accumulation in heart, lung and liver.

2.2. Controlled drug release

After administration, it is necessary that the macromolecular prodrug is stable during circulation in the bloodstream but the cytotoxic drug should be released from the macromolecular drug conjugate intracellulary in the lysosomes (lysosomotropic drug delivery) and/or intratumorally (tumoritropic drug delivery). This controlled release from polymeric drug conjugates by enzymatic or hydrolytic cleavage can only be achieved by proper selection of linkage between drug and polymeric carrier.

In the development of spacers, the most interest has been focussed on pH-sensitive spacers

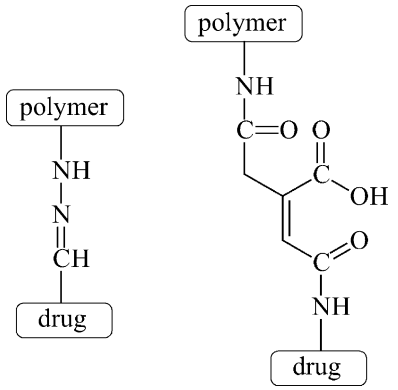

Fig. 7. The hydrazon and *N-cis*-aconityl spacer.

(pH-controlled drug release) and oligopeptide spacers (enzyme-assisted drug release).

2.2.1. pH Controlled drug release

When the macromolecular drug conjugate is taken up by the cell through endocytosis, the conjugate is predestined to be exposed to the acidic pH of the lysosome. Also in or near the tumor tissue the pH is slightly acidic in comparison with healthy tissue (Thistlethwaite et al., 1985). This relatively low pH can be exploited to design pH-sensitive spacers. Two types of acid-sensitive spacer have been frequently studied and reviewed (Kratz et al., 1999): the hydrazon linkage and the *N-cis*-aconityl spacer (Fig. 7).

The acid labile spacers were first explored by Shen and Ryser (1981). They reported on the synthesis of daunorubicin-linked aminoethyl polyacrylamide beads and poly(D-lysine) via a *N-cis*-aconityl spacer. The pH-controlled hydrolysis of the *cis*-aconityl was demonstrated by measuring the half lives at pH 5 ranging from 1 to 4 h, depending on the conjugate and the used buffer. The *cis*-aconityl spacer was readily hydrolysed at pH 4 but release was not noticeable at pH 6.

Another extensively studied acid labile spacer is the carboxylic hydrazon linkage. Kaneko and co-workers synthetised a series of conjugates with hydrazon spacers in order to study the relationship between the acid-sensitivity and cytotoxicity of adriamycin-immunoconjugates (Kaneko et al., 1991). The immunoconjugate with propanoyl-hydrazonspacer showed the highest in vitro and in vivo antitumor activity. The research group of Kratz developed and evaluated a series of transferrin and albumin conjugates with anthracyclines (Kratz et al., 1998). Coessens and co-workers linked the antibiotic streptomycin to dextran and to poly-(*N*-(2-hydroxyethyl)-L-glutamine) via a carboxylic hydrazon linkage (Coessens et al., 1996). Release of streptomycin was demonstrated at lysosomal pH.

2.2.2. Drug release by lysosomal and/or tumor-associated enzymes

After the cell uptake of the polymeric prodrug through endocytosis and after fusion of the endosome with the lysosome, the drug conjugate is not only exposed to the acid environment but also to the degrading nature of the lysosomal enzymes. When the lysosomal hydrolases degrade the spacer—most likely an oligopeptide spacer—the drug is released inside the cell. The lysozymes are not only present in normal cells but are often overexpressed in tumor tissues. Several lysosomal proteases such as cathepsins B, D and metalloproteinases, play a very important part in tumor growth and formation of metastases (Vassalli and Pepper, 1994; Rochefort et al., 1990; Osmak et al., 1997; Keppler et al., 1988; Stetler-Stevenson, 1990; Ginestra et al., 1997).

If the substrate is a specific oligopeptide for lysosomal proteases, the cytostatic drug can be released by these enzymes in or near the tumor tissue. Subsequently, the tumor cells can be selectively destroyed. For the design of a specific polymer drug conjugate, the site and the rate of the cleavage will then depend on the amino acid composition of the oligopeptide.

Jatzkewitz was the first to use a dipeptide Gly–Leu to couple mescaline to poly(vinylpyrrolidone-*co*-acrylic acid) (Jatzkewitz, 1955). The attachment of the drug onto the polymer resulted in a drastic increase in the biological half-life of mescaline. Trouet and co-workers synthetised albumin–daunorubicin conjugates with oligopeptide spacers (Trouet et al., 1982).

A major contribution to the understanding of lysosomal release was made by Duncan and Kopecek (Duncan, 1987; Kopecek et al., 2000; Putnam and Kopecek, 1995). The release of model drug *p*-nitroaniline from HMPA copolymers, catalysed by chymotrypsin, was studied by Kopecek and Chytry (1981). The oligopeptide sequence was later optimised for lysosomal thiol proteases, such as cathepsin B (Rejmanova et al., 1983; Duncan et al., 1983a).

Kopecek and Duncan also evaluated a series of HPMA–doxorubicin conjugates with different oligopeptide spacers (Duncan et al., 1983a; Kopecek et al., 1985; Subr et al., 1992). In vitro release studies in media containing lysosomal enzymes clearly demonstrated that drug release can be tailored by the length and composition of the peptidyl spacer. As a result of a study with cathepsin B, the oligopeptide sequence Gly–Phe–Leu–Gly was incorporated in the HPMA–doxorubicin copolymers with and without *N*-acetylated galactosamine as targeting moiety (PK2 and PK1, respectively), which are currently being used in clinical trials (Duncan, 2001).

The influence of the oligopeptide composition of the spacer on the drug release was also demonstrated by De Marre and co-workers for poly((2-hydroxyethyl)-L-glutamine) PHEG–peptide–mitomycin C conjugates (De Marre et al., 1994a,b). Conjugates with a spacer having glycine as C-terminal amino acid are less hydrolytical stable in aqueous buffer or serum than those having a more hydrophobic terminal amino acid such as leucine or phenylalanine. Tetrapeptide spacers were more susceptible to cleavage by lysosomal enzymes than tripeptides (Table 1).

2.3. The "enhanced permeability and retention effect"

It is known by several studies that macromolecules (natural and synthetic) and macromolecular prodrugs are taken up by solid tumors. Rapid pinocytosis by tumors cells was originally suggested as an explaination for this passive tumor uptake (Mego and Mc Queen, 1965). In 1986 Matsumura and Maeda proposed that this passive targeting can be ascribed to the combination of the poor tissue drainage and an increased tumor vascular permeability (Matsumura and Maeda, 1986; Seymour et al., 1995; Maeda et al., 2001; Maeda, 1991a,b). It is termed the 'enhanced permeability and retention effect' (EPR effect). Due to permeability enhancing factors, such as vascular endothelial growth factor (VEGF) and bradykinin, the endothelium of the tumor vasculature becomes discontinous (Senger et al., 1983; Maeda et al., 1988). This leads to the extravasation of the macromolecules from the bloodstream towards the tumor tissue. Additionally, the lack of effective lymphatic drainage prevents the macromolecules or macromolecular prodrugs from being removed and subsequently this results in an extravascular retention of the macromolecules or the macromolecular drug conjugates. This so-called 'enhanced permeability and retention effect' (EPR effect) was also observed in inflamed tissue (Yamaoka et al., 1994). The EPR effect is now generally accepted and considered as a major rational for using polymeric prodrugs (Fig. 8).

Table 1
Release of mitomycin C (MMC) by hydrolysis of PHEG–tripeptide or tetrapeptide–MMC conjugates by tritosomes at pH 5.5 after 3 h

Tripeptide spacers	MMC release (%)	Tetrapeptide spacer	MMC release (%)
Gly–Phe–Leu	2.4	Gly–Gly–Phe–Leu	3.1
Gly–Gly–Leu	2.5	Gly–Phe–Leu–Gly	57.7
Gly–Phe–Phe	2.7	Gly–Phe–Ala–Leu	74.6
Gly–Phe–Gly	7.1	Ala–Leu–Ala–Leu	81.0

2.4. Alteration of the body distribution and the cell uptake by active targeting

2.4.1. Antibody conjugates

The use of monoclonal antibodies to direct drug conjugates is based on the fact that surfaces of tumors contain a wide variety of proteins, some of which are specific to the tumor type. The monoclonal antibodies used as targeting group selectively seek out the tumor cells by binding to such tumor-specific antigens. As a result, the drug conjugate should bind very specifically these tumor cells (Ram and Tyle, 1987). Frequently, however, the quantity of drug that can be selectively targeted is limited by the number of antigens available. Hence, in cancer therapy the targeted-drug approach has been most successful for extremely potent agents such as the plant toxins, which in conjugation with antibodies have been termed the 'immunotoxins' (Wawrzynczak and Derbyshire, 1992). Further problems associated with the use of monoclonal antibodies as targeting moiety are lack of tumor selectivity, tumor access and immunogenecity (Blakey, 1992).

One antibody-based targeting strategy is antibody-directed enzyme prodrug therapy (ADEPT). An enzyme, capable of converting a non-toxic prodrug into a potent cytotoxic drug, is covalently attached to a tumor selective monoclonal antibody (Bagshawe et al., 1988; Springer et al., 1991). Following localisation of the antibody enzyme conjugate at the tumor site and

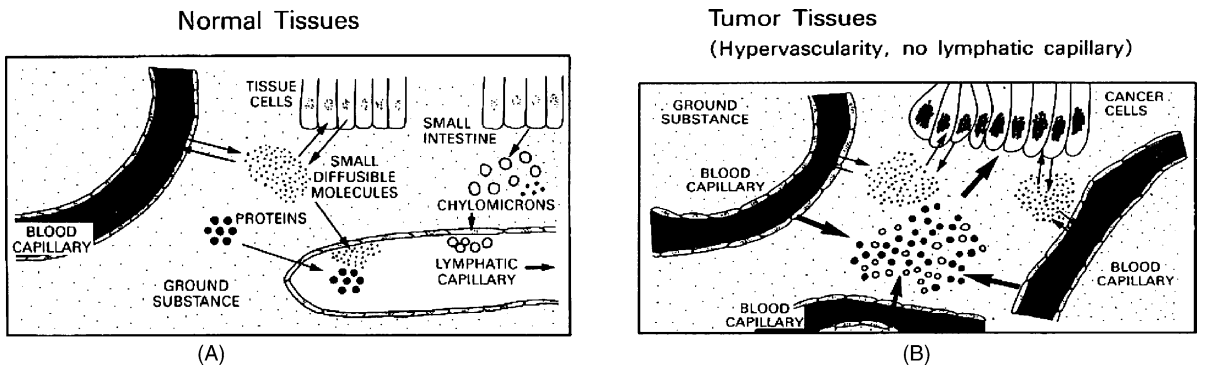

Fig. 8. The EPR effect (Sezaki and Hashida, 1984).

clearance of residual conjugate from the bloodstream, the prodrug is administered. This prodrug can be converted by the enzyme into a potent cytotoxic drug at the tumor site, so minimising non-specific toxicity (Fig. 9).

One major advantage over conventional antibody-targeting is the inherent amplification stage, meaning that for every successful enzyme-targeting event a very large number of prodrug molecules can be activated (Senter et al., 1991; Sharma et al., 1991). Initial results have been promising, though dogged with such problems as poor water-solubility of prodrugs, and the approach is currently being refined for further development.

2.4.2. *Macromolecular glycoconjugates as carrier systems*

Sugar-specific receptors are plasma membrane components (either glycoproteins and glycolipids, called lectins; Goldstein et al., 1980) of many mammalian cells. The first membrane lectin was characterised on hepatocytes by Ashwell and Harford (1982). Endogenous lectins, generally multivalent in their binding and recognition capacities, are found on numerous normal and malignant cells (Table 2).

The possibility of using hepatic lectins, such as the asialoglycoprotein receptor (ASGP-R), recognising galactose as targets for drug delivery is particularly attractive and has been studied intensively as a possible target for the treatment of various liver diseases such as hepatitis, parasitic infections and liver metastasis.

The ASGP-R (Schwartz, 1984) is easily accessible to the vascular circulation, being situated predominantly on the blood-facing surfaces of hepatocytes. Moreover, it is present in relatively large numbers, between 100,000 and 500,000 per cell.

The feasibility of liver targeting is well documented. As an example, Duncan and Kopecek prepared a series of co-polymers using methacryloylated galactose units as co-monomer (Chytry et al., 1987), resulting in materials containing pendent galactose units. It was found that these conjugates are rapidly

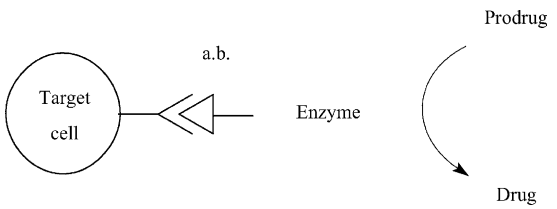

Fig. 9. Schematic representation of the ADEPT concept.

Table 2
Membrane lectins from various sources (Sezaki and Hashida, 1984; Ogino et al., 1988)

Origin	Sugar specificity
Murine liver macrophages	D-Mannose, L-fructose, D-galactose and *N*-acetyl-D-glucosamine
Rat and human hepatocytes	D-Galactose
Mouse spleen	D-Galactose/ *N*-acetyl-D-glucosamine
Human fibroblasts	D-Mannose-6-phoshate
Mouse L1210 leukaemia cells	L-Fructose

cleared from the blood and accumulate into the liver Duncan et al. (1983c, 1986), more specifically in the hepatocytes. Galactose was also introduced on the HPMA–doxorubicin derivative (PK2). O'Hare demonstrated that these derivatives associate in vitro with human hepatoma cell lines (O'Hare et al., 1989). After intraveneous administration of PK2 70% was taken up by the liver, making this conjugate possibly useful in the treatment of liver cancer. PK2 is now in clinical trial phase I (Pimm et al., 1996).

Vansteenkiste et al. (1991) prepared dextran and poly-HEA conjugates with pendent mono- or triantennary glycosides. Subcellular distribution experiments (Anderson et al., 1994) indicated that mono-galactosylated dextran is accumulated within the lysosomal compartment of liver hepatocytes. In contrast, the tris-galactose-substituted polymer shows a greater affinity for the galactose-specific receptor in vivo and also shows a high level of association with the cell surface of hepatocytes. This can be explained by the so-called "*clustering effect*". Binding to the ASGP-R depends strongly on the structure of the oligosaccharide ligand: mono-, bi- and triantennary sugar units bind with increasing affinity.

Drug delivery to macrophages (e.g. Kupffer cells) offers a second potentially attractive goal in the development of targeted treatment of various malfunctions, notably parasitic disorders such as Leishmaniasis or enzyme deficiencies such as Gaucher's syndrome. Moreover, since macrophages are part of the immune system, they can be activated and rendered tumoricidal by immunostimulating agents (e.g. *N*-acetylmuramyldipeptide, MDP).

Mannosylated carriers can also fulfil an important role not only in active drug targeting but also in receptor blocking. It was demonstrated that mannosylated dextrans were useful as transient receptor blockers in vivo for a 791T/36-ricin toxin A immunotoxin (Vansteenkiste et al., 1992). The circulation half-life of the immunotoxin was prolonged by a factor 3–4 up to 40 min following co-injection of an excess of mannosylated dextran. The liver disposition of the immunotoxin was markedly reduced from 43 to 18% of the recovered dose. The influence of the molecular size as well as the sugar loading of the competing polysaccharide was demonstrated to be small.

2.4.3. Targeting to angiogenic vessels

Angiogenesis is a fundamental process by which new blood vessels are formed. It is essential in reproduction, development and wound repair. However, many diseases are also driven by persistent unregulated angiogenesis, like artritis and several eye diseases. Angiogenesis is also an important process for tumor growth and metastasis of solid tumors (Weidner et al., 1991). Endothelial cells in angiogenic vessels of solid tumors show an increased expression of several cell surface proteins that stimulate cell invasion and proliferation (Yancopoulos et al., 1998; Eliceiri and Cheresh, 2001). These proteins include receptors for different angiogenic growth factors (Martiny-Baron and Marme, 1995) such as the vascular endothelial growth factor (VEGF) and they also include the $\alpha_v\beta_3$ integrin receptor (Brooks et al., 1994). The $\alpha_v\beta_3$ integrin is highly expressed in most growing tumor vasculature but has very low expression in normal vasculature and most other normal or benign human tissue.

One type of integrin receptor binding peptides is the RGD (arginine–glycine–aspartic acid) containing peptides. These peptides bind to the integrin receptors with high affinity and can therefore be used as targeting moieties for drug delivery. Moreover, it is known that peptides containing the RGD sequence inhibit experimental metastasis (Humphries et al., 1986). Arap and co-workers showed that the coupling of cyclic RGD and NGR peptides (CDCRGDCFC and CNGRCVSGCAGRC) to the anticancer drug adriamycin resulted in an increased efficacy of the drug against human breast cancer xenografts in mice (Arap et al., 1998). The research group of Mayumi prepared conjugates of RGD-peptides (RGD and RGDS) and poly(ethylene glycol) (PEG) (Maeda et al., 1997). The inhibitory effect of these conjugates, examined on experimental metastasis in mice, was demonstrated to be superior to the free RGD peptides.

2.5. Immunoprotective therapy

Recently it was found that the use of polymeric antitumor drug derivatives may play an important role in the protection of the cancer patient's immune system. One of the mechanisms that induces the programmed cell death or apoptosis of cancer cells, is the Fas–Fas ligand interaction (Maher et al., 2002).

Fas and Fas ligand (FasL) are both transmembrane proteins (Nagata, 1997). Both receptor and ligand are expressed either constitutively or after activation on most of the cells of the immune system (Daniel et al., 1998; Restifo, 2000). Fas is also expressed on cancer cells. Interaction between Fas and FasL triggers a cascade of signals, that eventually results in apoptosis (Krammer et al., 1994; O'Connell et al., 1999). It has been reported however that several tumor cell lines can express FasL (O'Connell et al., 1996; Hahne et al., 1996; Strand et al., 1996; Niehans et al., 1997; Von Bernstorff et al., 1999; Bennett et al., 1998; Mitsiades et al., 1998; Friesen et al., 1996). Hence, they are able to kill cells of the immune system expressing Fas. This mechanism is called the Fas counterattack. The counterattack of the tumor cells not only prevents the eradication of the cancer cells, but participates also in the destruction of the immune system.

The counterattack mechanism is often favoured by non-functioning (Von Bernstorff et al., 1999), down-regulation or loss (Walker et al., 1997) of the cancer cell Fas receptors. Moreover, it has been reported that treatment with antitumor drugs promotes the induction of Fas ligands on the cancer cells (Friesen et al., 1999).

There is a strong indication that treatment with macromolecular drug derivatives can overcome this. Rihova and co-workers found a strong expression of FasL on the SW620 human metastatic colorectal cancer cell line when it was exposed to doxorubicin or mitomycin C (MMC) (Rihova et al., 2001). However, when the cell line was exposed to polymeric derivatives of these drugs, no increase of the FasL was noticed on the SW620, even not when higher concentrations were used. The drug derivatives used in this experiment were MMC bound via a GFAL-spacer onto PEG-grafted poly(N^5-(2-hydroxyethyl-L-glutamine)) (PHEG) and doxorubicin coupled via a GFLG-spacer onto poly-(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) with or without anti-CD71 mAbs as targeting group. These results suggest that the expression of Fas ligands on cancer cells is different when they are exposed to free antitumor drugs or to their macromolecular derivatives. This is an important outcome that might indicate that polymeric prodrugs are able to protect the patient's immune system.

2.6. Polymeric prodrugs in clinical use or in clinical trial

The interdisciplinary research of the last decades has resulted in a number of polymer based products which are now on the market or have entered clinical trial.

One approach of particular note involving soluble macromolecular drug carriers is SMANCS. In the clinical formulation, neocarcinostatin (NCS) (molecular weight: 10,700) is conjugated to two chains of a styrene–maleic anhydride copolymer (SMA) (molecular weight average 1500, polydispersity <1.2) (Maeda et al., 1992;Maeda, 1991a,b). The SMA copolymer is itself derivatised with an alkyl group (usually butyl) which determines the overall hydrophobicity of the conjugate (Hirayama et al., 1986).

Aqueous SMANCS formulations have been tested in pilot studies in patients with solid tumors of the ovary, lung, stomach, adrenal gland and in the brain. Formulations based on SMANCS/Lipiodol have been shown to be efffective both as a diagnostic tool and for therapeutic use in solid tumors where the formulations are given arterially via a catheter. The prognosis of the patient receiving intra-arterial SMANCS/Lipiodol is a 90% chance of survival for at least 5 years after treatment, if the patient has no active liver cirrhosis and the tumor has not spread to more than two segments of the liver. With conventional therapy the survival time is about 6 months.

SMANCS is marketed in Japan by Yamanouchi for the treatment of hepatocellular carcinoma.

PEG-modified adenosine deaminase (ADAGEN®) and PEG–L-asparaginase (ONCASPAR®) were the first PEG modified enzymes that were on the market in the early 1090s (Delgado et al., 1992; Keating et al., 1993). PEG–ADA is used for the treatment of ADA-deficient Severe Combined Immunodeficiency Syndrome.

PEG–L-asparaginase is used to treat lymphocytic leukaemia and malignant lymphosarcoma. Both native enzymes have a short plasma half-life and PEG-modification resulted in a prolonged plasma clearance. Further more, the PEG-enzymes display a marked reduction in immunogenicity. Both products are in clincal use today.

PK1 and PK2 are both derivatives of HPMA copolymer with the antitumor agent doxorubicin linked onto

it via the peptidyl spacer Gly–Phe–Leu–Gly. PK2 also contains galactose as a targeting group to facilitate liver targeting.

PK1 is currently undergoing Phase II evaluation for treatment of breast, colon and non-small-cell lung cancer. Phase I results revealed that PK1 displayed greatly reduced toxicity with maintained antitumor efficacy compared with free doxorubicin (Vasey et al., 1999). The maximum tolerated dose of PK1 is about four times higher than the usual clinical dose of free doxorubicin. PK2 has entered Phase I clinical testing (Seymour et al., 2002).

The micelle forming conjugates of adriamycin with poly(ethylene glycol)–poly(aspartamide) block copolymers already showed excellent in vivo antitumor activities. These micellar systems are very attractive, since they can also be used to entrap a drug within their hydrophobic core as well as providing the opportunity for covalent conjugation. In 2001 these micellar aggregates have entered Phase I clinical trials (Nakanishi et al., 2001).

References

Abuchowski, A., Es, T.V., Palczuk, N.C., Davis, F.F., 1977. Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. J. Biol. Chem. 252, 578–3581.

Abuchowski, A., Kazo, G.M., Verhoest Jr., C.R., Van Es, T., Kafkewitz, D., Vian, A.T., Davis, F.F., 1984. Cancer Therapy with chemically modified enzymes. 1. A property of polyethylene glycol asparaginase conjugates. Cancer Bio. Chem. Biophys. 7, 175–186.

Anderson, D., Vansteenkiste, S., Schacht, E.H., Sen, S.V., Seymour, L.W., 1994. In vitro binding specificity of glycosylated dextrans to the asialoglycoprotein receptor of primary hepatocytes. Eur. J. Pharm. 3, 339–345.

Arap, W., Pasqualini, R., Ruoslahti, E., 1998. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 279, 377–380.

Ashwell, G., Harford, J., 1982. Carbohydrate-specific receptors of the liver. Ann. Rev. Biochem. 51, 531–554.

Bagshawe, K.D., Springer, C.J., Searle, F., Antoniw, P., Sharma, S.K., Melton, R.G., Sherwood, R.F., 1988. A cytotoxic agent can be generated selectively at cancer sites. Br. J. Cancer 58, 700–703.

Barry, B.W., 1983. Drug delivery systems. CHEMTECH 13, 38–44.

Bat, H.G., 1977. Polymeric drugs. Adv. Polym. Sci. 23, 25–53.

Bennett, M.W., O'Connell, J., O'Sullivan, G.C., Brady, C., Roche, D., Collins, J.K., Shanahan, F., 1998. The Fas counterattack in vivo: apoptotic depletion of tumor-infiltrating lymphocytes associated with Fas ligand expression by human esophageal carcinoma. J. Immunol. 160, 5669–5675.

Blakey, D.C., 1992. Drug targeting with monoclonal antibodies. Rev. Oncol. 5, 91–97.

Blout, E.R., Karlson, R., 1956. Polypeptides. III. The synthesis of high molecular weight poly-γ-benzyl-L-glutamates. J. Am. Chem. Soc. 78, 941–951.

Brooks, P.C., Montgomery, A.M.P., Rosenfeld, M., Reisfeld, R.A., Hu, T.H., Klier, G., Cheresh, D.A., 1994. Integrin $\alpha_v\beta_3$ antagonist promote tumor-regression by inducing apoptosis of angiogenic blood vessels. Cell 79, 1157–1164.

Chytry, V., Leibnitz, E., O'Hare, K., Scarlett, L., Duncan, R., 1987. Copolymers of 6-*o*-methacryloyl-D-galactose and *N*-(2-hydroxypropyl)methacryl-amide: targeting to liver after intravenous administration to rats. New Polym. Mater. 1, 21–28.

Coessens, V., Schacht, E., Domurado, D., 1996. Synthesis of polyglutamine and dextran conjugates of streptomycin with an acid-sensitive drug–carrier linkage. J. Control. Release 38, 141–150.

Daniel, P.T., Scholz, C., Westermann, J., Dorken, B., Pezzutto, A., 1998. Dendritic cells prevent CD95-mediated T lymphocyte death through costimulatory signals. Gene Ther. Cancer 28, 173–177.

De Marre, A., Seymour, L.W., Schacht, E., 1994a. Evaluation of the hydrolytic and enzymatic stability of macromolecular Mitomycin C derivatives. J. Control. Release 31, 89–97.

De Marre, A., Soyez, H., Schacht, E., 1994b. Synthesis of macromolecular mitomycin C derivatives. J. Control. Release 32, 129–137.

Delgado, C., Francis, G.E., Fisher, D., 1992. The uses and properties of PEG-linked proteins. Crit. Rev. Ther. Drug Carrier Syst. 9, 249–304.

De Winne, K., Seymour, L., Schacht, E., 2001. Macromolecular anti-tumor derivatives based on phenilene diamine mustard and mitomycine C. J. Control. Release 72, 246–246.

Donaruma, L.G., 1974. Synthetic biologically active polymers. Prog. Polym. Sci. 4, 1–25.

Duncan, R., 1987. Selective endocytosis of macromolecular drug carriers. In Robinson, J.R., Lee, V.H. (Eds.), Controlled Drug Delivery: Fundamentals and Applications, second ed. Marcel Dekker, New York, pp. 581–607.

Duncan, R., 2001. Polymer therapeutics. In: Cooper, E., (Ed.), Bussiness briefing. PharmaTech 2001, 178–184.

Duncan, R., Cable, R., Lloyd, H.C., Rejmanova, P., Kopecek, J., 1983a. Polymers containing enzymatically degradable bonds. 7a. Design of oligopeptide sidechains in poly(*N*-(2-hydroxypropyl) methacrylamide) copolymers to promote efficient degradation by lysosomal enzymes. Makromol. Chem. 184, 1997–2008.

Duncan, R., Kopecek, J., Lloyd J.B., 1983b. Development of *N*-(2-hydroxypropyl)-methacrylamide copolymers as carriers of therapeutic agents. In: Chielline, E., Guisti, P. (Eds.), Polymers in Medicine: Biomedical and Pharmacologicam Applications. Plenum Press, New York, pp. 97–114.

Duncan, R., Kopecek, J., Rejmanova, P., Lloyd, J.B., 1983c. Targeting of *N*-(2-hydroxypropyl)-methacrylamide copolymers

to liver by incorporation of galactose residues. Biochem. Biophys. Acta 755, 518–521.

Duncan, R., Seymour, L., Scarlett, L., Lloyd, J.B., Rejmanova, P., Kopecek, J., 1986. Fate of *N*-(2-hydroxypropyl)methacrylamide copolymers with pendant galactosamine residues after intravenous administration to rats. Biochim. Biophys. Acta 880, 62–71.

Eliceiri, B.P., Cheresh, D.A., 2001. Adhesion events in angiogenesis. Curr. Opin. Cell Biol. 13, 563–568.

Flanagan, P.A., Kopeckova, P., Kopecek, J., Duncan, R., 1989. Evaluation of antibody-*N*-(2-hydroxypropyl)-methacrylamide copolymer conjugates as targetable drug–carriers. 1. Binding, pinocytic uptake and intracellular distribution of transferrin and anti-transferrin receptor antibody-conjugates. Biochim. Biophys. Acta 993, 83–91.

Franssen, E.J.F., Moolenaar, F., De Zeeuw, D., Meijer, D.K.F., 1994. Drug targeting to the kidney with low-molecular weight proteins. Adv. Drug Del. Rev. 14, 67–88.

Friesen, C., Fulda, S., Debatin, K.-M., 1999. Cytotoxic drugs and the CD95 pathways. Leukemia 13, 1854–1858.

Friesen, C., Herr, I., Krammer, P.H., Debatin, K.-M., 1996. Involvement of the CD95 (Apo-1/Fas) receptor/ligand system in drug-induced apoptosis in leukemia cells. Nat. Med. 2, 574–577.

Gerola, A., Antoni, G., Benvenuti, F., Cocola, F., Neri, P., 1970. Poly-N5-(2-hydroxyethyl)-L-glutamine a new plasma expander. Adv. Exp. Med. Biol. 9, 329–338.

Ginestra, A., Monea, S., Seghezzi, G., Dolo, V., Nagase, H., Mignatti, P., Vittorelli, M.L., 1997. Urokinase plasminogen activator and gelatinases are associated with membrane vesicles shed by human HT1080 fibrosarcoma cells. J. Biol. Chem. 272, 17216–17222.

Goldstein, I.J., Hugues, R.C., Monsigny, M., Osawa, T., Sharon, N., 1980. What should be called a lectin? Nature 285, 66–66.

Hahne, M., Rinoldi, D., Schroter, M., Romero, P., Schreier, M., French, L.E., Schneider, L.P., Bornand, T., Fontana, A., Lienard, D., Cerottini, J.C., Tschopp, J., 1996. Melanoma cell expression of Fas(Apo-1/CD95) ligand: implications for tumor immune escope. Science 274, 1363–1366.

Hirayama, S., Sato, F., Oda, T., Maeda, H., 1986. Stability of high molecular weight anticancer agent SMANCS and its transfer from oil-phase to water-phase. Jpn. J. Antibiot 39, 815–822.

Hoste, K., Schacht, E., Seymour, L., 2000. New derivatives of polyglutamic acid as drug carrier systems. J. Control. Release 65, 367–374.

Humphries, M.J., Olden, K., Yamada, K.M., 1986. A synthetic peptide from fibronectin inhibits experimental metastasis of muring melanoma cells. Science 233, 467–470.

Jatzkewitz, H., 1955. Peptamin (glycyl-L-leucyl-mescaline) bound to blood plasma expander (polyvinylpyrrolidone) as a new depot form of a biologically active primary amine (mescaline). Z. Naturforsch. 10b, 27–31.

Kaneko, T., Willner, D., Monkovic, I., Knipe, J.O., Braslawsky, G.R., Greenfield, R.S., Dolatrai, M.V., 1991. New hydrazone derivatives of adriamycin and their immunoconjugates. A correlation between acid stability and cytotoxicity. Bioconjugate Chem. 2, 133–141.

Keating, M.J., Holmes, R., Lerner, S., Ho, D.H., 1993. L-Asparaginase and PEG–asparaginase—past, present and future. Leukemia Lymphoma 10, 153–157.

Keppler, D., Fondanèche, M.C., Dalet-Fumeron, V., Pagano, M., Burtin, P., 1988. Immunohistochemical and biochemical study of a cathepsin B-like proteinase in human colonic cancers. Cancer Res. 48, 6855–6862.

Kopecek, J., Bazilova, H., 1973. Poly(*N*-(hydroxypropyl)-methacrylamide)—1. Radical polymerisation and copolymerisation. Eur. Polym. J. 9, 7–14.

Kopecek, J., Kopeckova, P., Minko, T., Lu, Z.R., 2000. HMPA copolymer–anticancer drug conjugates: design, activity, and mechanism of action. Eur. J. Pharm. Biopharm. 50, 61–81.

Kopecek, J., Rejmanova, P., Duncan, R., Lloyd, J.B., 1985. Controlled release of drug model from *N*-(2-hydroxypropyl)methacrylamide copolymers. Ann. N.Y. Acad. Sci. 446, 93–103.

Kopecek, J., Chytry, V., 1981. Polymers containing enzymatically degradable bonds. 1. Chymotrypsin catalyzed hydrolysis of *p*-nitroanilides of phenylalanine and tyrosine attached to sidechains of copolymers of *N*-(2-hydroxypropyl)methacrylamide. Makromol. Chem. 182, 799–809.

Krammer, P.H., Dhein, J., Walczak, H., Behermann, I., Mariani, S., Matiba, B., Fath, M., Daniel, P.T., Knipping, E., Westendorp, M.O., Stricker, K., Baumler, C., Helbardt, S., Germer, M., Peter, M.E., Debatin, K.M., 1994. The role of APO-1-mediated apoptosis in the immune system. Immunol. Rev. 142, 175–191.

Kratz, F., Beyer, U., Collery, P., Lechenault, F., Cazabat, A., Schumacher, P., Falken, U., Unger, C., 1998. Preparation, characterization and in vitro efficacy of albumin conjugates of doxorubicin. Biol. Pharm. Bull. 21, 56–61.

Kratz, F., Beyer, U., Schütte, M.T., 1999. Drug–polymer conjugates containing acid-cleavable bonds carrier systems. Crit. Rev. Ther. Drug Carrier Syst. 16, 245–288.

Kricheldorf, K.R., 1987. α-Aminoacid-*N*-Carboxyanhydrides and Related Heterocycles. Springer-Verlag, Berlin, p. 24.

Lloyd, J.B., Duncan, R., Pratten, M.K., 1983. Soluble synthetic polymers as targetable agents for intracellular drug release. Br. Polym. J. 15, 158–159.

Maeda, H., 1991a. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. Adv. Drug Deliv. Rev. 6, 181–202.

Maeda, H., 1991b. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. Drug Deliv. Rev. 6, 181–202.

Maeda, H., Matsumura, Y., Kato, H., 1988. Purification and identification of (hydroxyprolyl^3) bradykinin in ascites fluid from a patient with gastric cancer. J. Biol. Chem. 263, 16051–16054.

Maeda, H., Sawa, T., Konno, T., 2001. Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. J. Control. Release 74, 47–61.

Maeda, H., Seymour, L.W., Miyamoto, Y., 1992. Conjugates of anticancer agents and polymer: advantages of macromolecular therapeutics in vivo. Bioconjugate Chem. 3, 351–362.

Maeda, H., Seymour, L.W., Miyamoto, Y., 1992. Conjugates of anticancer agents and polymer: advantages of macromolecular therapeutics in vivo. Bioconjugate Chem. 3, 351–362.

Maeda, M., Izuno, Y., Kawasaki, K., Kaneda, Y., Mu, Y., Tsutsumi, Y., Nakagawa, S., Mayumi, T., 1997. Amino acids and peptides. XXX. Preparation of Arg–Gly–Asp (RGD) hybrids with poly(ethylene glycol) analogs and their antimetastatic effect. Chem. Pharm. Bull. 45, 1788–1792.

Maher, S., Toomey, D., Condron, C., Bouchier-Hayes, D., 2002. Activation induced cell death: the controversial role of Fas and Fas ligand in immune privilege and tumour counterattack. Immunol. Cell Biol. 80, 131–137.

Martiny-Baron, G., Marme, D., 1995. VEGF-mediated tumor angiogenesis—a new target for cancer-therapy. Curr. Opin. Biotechnol. 6, 675–680.

Matsumoto, S., Yamamoto, A., Takakura, Y., Hashida, M., Sezaki, H., 1986. Cellular interaction and in vitro antitumour activity of mitomycin C-dextran conjugate. Cancer Res. 46, 4463–4468.

Matsumura, Y., Maeda, H., 1986. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumouritropic accumulation of proteins and antitumour agent SMANCS. Cancer Res. 46, 6387–6392.

Mego, J.L., Mc Queen, J.D., 1965. The uptake of labelled proteins by particulate fractions of tumor and normal tissue after injection into mice. Cancer Res. 25, 865–869.

Mitsiades, N., Poulaki, V., Kotoula, V., Leone, A., Tsokos, M., 1998. Fas ligand is present in tumors of the Ewing's sarcoma family and is cleaved into a soluble form by a metalloprotease. Am. J. Pathol. 153, 1947–1956.

Nagata, S., 1997. Apoptosis by death factor. Cell 88, 355–365.

Nakanishi, T., Fukushima, S., Okamoto, K., Suzuki, M., Matsumura, Y., Yokoyama, M., Okano, T., Sakurai, Y., Kataoka, K., 2001. Development of the polymer micelle carrier system for doxorubicin. J. Control. Release 74, 295–302.

Neri, P., Antoni, A., Benvenuti, F., Cocola, F., Gazzei, G., 1973. Synthesis of α,β-poly((2-hydroxyethyl)-D,L-aspartamide), a new plasma expander. J. Med. Chem. 16, 893–897.

Niehans, G.A., Brunner, T., Frizelle, S.P., Liston, J.C., Salerno, C.T., Knapp, D.J., Green, D.R., 1997. Human lung carcinomas express Fas ligand. Cancer Res. 57, 1007–1012.

O'Connell, J., Bennett, M.W., O'Sullivan, G.C., Collins, J.K., Shanahan, F., 1999. The Fas counterattack: cancer as a site of immune privilege. Immunol. Today 20, 46–52.

O'Connell, J., O'Sullivan, G.C., Collins, J.K., Shanahan, F., 1996. The Fas counterattack: Fas-mediated T cell killing by colon cancer cells expressing Fas ligand. J. Exp. Med. 184, 1075–1082.

O'Hare, K.B., Hume, I.C., Scarlett, L., Duncan, R., 1989. Evaluation of anticancer agents coupled to *N*-(2-hydroxypropyl)methacrylamide copolymers. Effect of galactose incorporation on interaction with hepatoma in vitro. Hepatology 10, 207–214.

Ogino, T., Inoue, M., Ando, Y., Arai, H., Morino, Y., 1988. Chemical modification of superoxide dismutase. Extension of plasma half-life of the enzyme through its reversible binding to circulating albumin. Int. J. Peptide Protein Res. 32, 153–159.

Okano, T., Yui, N., Yokoyama, M., Yoshida, R., 1994. Advances in Polymeric Systems for Drug Delivery. Gordon and Breach Science Publisher, Tokyo.

Osmak, M., Babic, D., Abramic, M., Vrhovec, I., Milicic, D., Skrk, J., 1997. Cathepsin D content in malignant tumours of corpus uteri. Eur. J. Cancer 33, 699–700.

Panarin, E.F., Ushakov, S.N., 1968. Synthesis of polymer salts and amidopenicillines (in Russian). Khim. Pharm. Zhur. 2, 28–31.

Pimm, M., Perkins, A., Strohalm, J., Ulbrich, K., Duncan, R., 1996. Gamma scintigraphy of a I-123-labelled *N*-(2-hydroxypropyl)methacrylamide copolymer–doxorubicin conjugate containing galactosamine following intravenous administration to nude mice bearing hepatic human colon carcinoma. J. Drug Target. 3, 385–390.

Putnam, D., Kopecek, J., 1995. Polymer conjugates with anticancer activity. Adv. Polym. Sci. 122, 55–123.

Ram, B.P., Tyle, P., 1987. Immunoconjugates: applications in targeted drug delivery for cancer therapy. Pharm. Res. 4, 181–188.

Rejmanova, P., Pohl, J., Baudys, M., Kostka, V., Kopecek, J., 1983. Polymers containing enzymatically degradable bonds. 8. Degradation of oligopeptide sequences in *N*-(2-hydroxypropyl)methacrylamide copolymers by bovine spleen cathepsin B. Makromol. Chem. 184, 2009–2020.

Restifo, N., 2000. Not so Fas: re-evaluating the mechanism of immune privilege and tumor escape. Nat. Med. 6, 493–495.

Rihova, B., Strohalm, J., Hoste, K., Jelinkova, M., Hovorka, O., Kovar, M., Plocova, D., Sirova, M., Stastny, M., Schacht, E., Ulbrich, K., 2001. Immunoprotective therapy with targeted anticancer drugs. Macromol. Symp. 172, 21–28.

Ringsdorf, H., 1975. Structure and properties of pharmacologically active polymers. J. Polym. Sci. Symp. 51, 135–153.

Rochefort, H., Capony, F., Garcia, M., 1990. Cathepsin D—a protease involved in breast-cancer metastasis. Cancer Metast. Rev. 9, 321–331.

Roseeuw, E., Coessens, V., Schacht, E., Vroman, B., Domurado, D., Marchal, G., 1999. Polymeric prodrugs of antibiotics with improved efficiency. J. Mater. Sci.-Mater. M. 10, 743–746.

Schwartz, A.L., 1984. The hepatic asialoglycoprotein receptor. CRC Crit. Rev. Biochem. 16, 207–233.

Senger, D.R., Galli, S.J., Dvorak, A.M., Perruzzi, C.A., Harvey, V.S., Dvorak, H.F., 1983. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219, 983–985.

Senter, P.D., Su, P.D.D., Katsuragi, T., Sakai, T., Cosand, W.L., Hellstrom, I., Hellstrom, K.E., 1991. Generation of 5-fluorouracil from 5-fluorocytosine by monoclonal antibody-cytosine deaminase conjugates. Bioconjugate Chem. 2, 447–451.

Seymour, L.W., 1994. Soluble polymers for lectin-mediated drug targeting. Adv. Drug Del. Rev. 14, 89–112.

Seymour, L.W., Flanagan, P.A., Al-Shamkhani, A., Subr, V., Ulbrich, K., Cassidy, J., Duncan, R., 1991. Synthetic polymers conjugated to monoclonal antibodies: vehicles for tumour-targeted drug delivery. Select. Cancer Ther. 7, 59–73.

Seymour, L.W., Ulbrich, K., Strohalm, J., Duncan, R., 1990. The pharmacokinetics of polymer-bound adriamycin. Biochem. Pharmacol. 39, 1125–1131.

Seymour, L.W., Duncan, R., Strohalm, J., Kopecek, J., 1987. Effect of molecular weight of *N*-(2-hydroxypropyl)methacrylamide copolymers on body distribution and rate of excretion after subcutaneous, intraperitoneal and intravenous administration to rats. J. Biomed. Mater. Res. 21, 1341–1358.

Seymour, L.W., Ferry, D.R., Anderson, D., Hesslewood, S., Julyan, P.J., Poyner, R., Doran, J., Young, A.M., Burtles, S., Kerr, D.J., 2002. Hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin. J. Clin. Oncol. 20, 1668–1676.

Seymour, L.W., Miyamoto, Y., Maeda, H., Brereton, M., Strohalm, J., Ulbrich, K., Duncan, R., 1995. Influence of molecular weight on passive tumour accumulation of a soluble macromolecular drug carrier. Eur. J. Cancer 31A, 766–770.

Sezaki, H., Hashida, M., 1984. Macromolecule–drug conjugates in targeted cancer chemotherapy. CRC Crit. Rev. Ther. Drug Carrier Syst. 1, 1–38.

Sharma, S.K., Bagshawe, K.D., Springer, C.J., Burge, P.J., Rogers, G.T., Boden, J.A., Antoniw, P., Melton, R.G., Sherwood, R.F., 1991. Antibody directed enzyme prodrug therapy (ADEPT): a three phase system. Disease Markers 9, 225–231.

Shen, W.C., Ryser, H.J.P., 1981. *Cis*-aconityl spacer between daunomycin and macromolecular carriers: a model of pH-sensitive linkage releasing drug from a lysosomotropic conjugate. Biochem. Biophys. Res. Commun. 102, 1048–1054.

Springer, C.J., Bagshawe, K.D., Sharma, S.K., Searle, F., Boden, J.A., Antoniw, P., Burke, P.J., Rogers, G.T., Sherwood, R.F., Melton, R.G., 1991. Ablation of human choriocarcinoma xenografts in nude mice by antibody-directed enzyme prodrug therapy (ADEPT) with three novel compounds. Eur. J. Cancer 27, 1361–1366.

Stetler-Stevenson, W.G., 1990. Type IV collagenases in tumor invasion and metastasis. Cancer Metast. Rev. 9, 289–303.

Strand, S., Hofmann, W.J., Hug, H., Muller, M., Otto, G., Strand, D., Marini, S.M., Stremmel, W., Krammer, P.H., Galle, P.R., 1996. Lymphocyte apoptosis induced by CD95 (APO-1/Fas) ligand-expressing tumor cells—a mechanism of immune evasion? Nat. Med. 2, 1361–1366.

Subr, V., Strohalm, J., Ulbrich, K., Duncan, R., Hume, Z., 1992. Polymers containing enzymatically degradable bonds, XII. Effect of spacer structure on the rate of release of daunomycin and adriamycin from poly(*N*-(2-hydroxypropyl)-methacrylamide) copolymer drug carriers in vitro and antitumour activity measured in vivo. J. Control. Release 18, 123–132.

Thistlethwaite, A.J., Leeper, D.B., Moylan, D.J., Nerlinger, R.E., 1985. pH distribution in human tumors. Int. J. Radiat. Oncol. 11, 1647–1652.

Trouet, A., Masquelier, M., Baurain, R., Deprez-De Campeneere, D., 1982. A covalent linkage between daunorubicin and protein that is stable in serum and reversible by lysosomal hydrolases, as required for a lysosomotropic drug–carrier conjugate: in vitro and in vivo studies. Proc. Natl. Acad. Sci. U.S.A. 79, 626–629.

Vansteenkiste, S., De Marre, A., Schacht, E., 1992. Synthesis of glycosylated dextrans. J. Bioact. Compat. Polym. 7, 4–14.

Vansteenkiste, S., Schacht, E., Duncan, R., Seymour, L., Pawluczyk, I., Baldwin, R., 1991. Fate of glycosylated dextrans after in vivo administration. J. Control. Release 16, 91–100.

Vasey, P.A., Kaye, S.B., Morrison, R., Twelves, C., Wilson, P., Duncan, R., Thomson, A.H., Murray, L.S., Hilditch, T.E., Murray, T., Burtles, S., Fraier, D., Frigerio, E., Cassidy, J., 1999. Phase I clinical and pharmacokinetic study of PK1 (*N*-(2-hydroxy-propyl)methacrylamide copolymer doxorubicin): first member of a new class of chemotherapeutic agents—drug–polymer conjugates. Clin. Cancer Res. 5, 83–94.

Vassalli, J.D., Pepper, M.S., 1994. Tumor biology—membrane proteases in focus. Nature 370, 14–15.

Vercauteren, R., Schacht, E., Duncan, R., 1992. Effect of the chemical modification of dextran on the degradation by rat liver lysosomal enzymes. J. Bioact. Biocomp. Polym. 7, 346–357.

Vert, M., 1986. Polyvalent polymeric drug carriers. CRC Crit. Rev. Ther. Drug Carrier Syst. 2, 291–327.

Von Bernstorff, W., Spanjaard, R.A., Chan, A.K., Lockhart, D.C., Sadanaga, N., Wood, I., Peiper, M., Goedegebuure, P.S., Eberlain, T.J., 1999. Pancreatic cancer cells can evade immune surveillance via nonfunctional Fas(APO-1/CD95) receptors and aberrant expression of functional Fas ligand. Surgery 125, 73–84.

Walker, P.R., Saas, P., Dietrich, P.-Y., 1997. Role of Fas ligand (CD95L) in immune escape: the tumor cell strikes back—commentary. J. Immunol. 15, 4521–4524.

Wawrzynczak, E.J., Derbyshire, E.J., 1992. Immunotoxins: the power and the glory. Immunol. Today 13, 381–383.

Weidner, N., Semple, J.P., Welch, W.R., Folkman, J., 1991. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. N. Engl. J. Med. 324, 1–8.

Yamaoka, T., Tabata, Y., Ikada, Y., 1994. Accumulation of poly(vinyl alcohol) at inflammatory site. ACS Symp. Ser. 545, 163–171.

Yancopoulos, G.D., Klagsbrun, M., Folkman, J., 1998. Vasculogenesis, angiogenesis, and growth factors: ephrins enter the fray at the border. Cell 93, 661–664.

Yokoyama, M., 1992. Block copolymers as drug carriers. Crit. Rev. Ther. Drug Carrier Syst. 9, 213–248.

Yokoyama, M., Miyauchi, M., Yamada, N., Okano, T., Sakurai, Y., Kataoka, K., Inoue, S., 1990. Characterization and anticancer activity of the micelle forming polymeric anti-cancer drug adriamycin-conjugated poly(ethylene glycol)–poly(aspartic acid) block copolymer. Cancer Res. 50, 1693–1700.

Yokoyama, M., Okano, T., Sakurai, Y., Ekimoto, H., Shibazaki, C., Kataoka, K., 1991. Toxicity and anti-tumour activity against solid tumours of micelle-forming polymeric anti-cancer drug and its extremely long circulation in blood. Cancer Res. 51, 3229–3236.