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(54) Polymeric prodrugs for beta-lactamase and uses thereof

(57) The instant invention relates to a method for the delivery of antitumor drugs to tumor cells by the administration of a tumor-selective antibody- β-lactamase conjugate that binds to tumor cells, and the additional administration of a novel polymeric cephalosporin prodrug that is converted at the tumor site, in the presence of the antibody-β-lactamase, to an active cytotoxic drug. According to a preferred embodiment of this invention, the polymeric cephalosporin prodrug contains a polyethylene glycol or a branched polyethylene glycol moiety. The methods, antibody-enzyme conjugate, prodrugs, pharmaceutical compositions, and combinations of this invention provide for enhanced selective killing of tumor cells and are thus useful in the treatment of cancers and other tumors.

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Description

The present invention relates generally to novel prodrugs and a method for delivering these prodrugs to a tumor cell site where they are converted to active cytotoxic agents. More particularly, the invention relates to polymeric cephalosporin prodrugs, which when administered with a tumor-secific-antibody-β-lactamase conjugate, are converted at the tumor site to active cytotoxic drugs.

Targeted drug delivery systems provide a mechanism for delivering cytotoxic agents directly to cancerous cells. The selective delivery of cytotoxic agents to tumor cells is desirable because systemic administration of these agents often kills normal cells within the body as well as the tumor cells sought to be eliminated. Antitumor drug delivery systems currently in use typically utilize a cytotoxic agent conjugated to a tumor-specific antibody to form an immunoconjugate. This immunoconjugate binds to tumor cells and thereby "delivers" the cytotoxic agent to the site of the tumor. The immunoconjugates utilized in these targeting systems include antibody-drug conjugates (see, e.g., Baldwin et al., Lancet, pp. 603-605, March 15, 1986) and antibody-toxin conjugates (see, e.g., Thorpe, in Monoclonal Antibodies '84: Biological and Clinical Applications, A. Oinchera et al., eds., pp 475-506, 1985).

Both polyclonal antibodies and monoclonal antibodies have been utilized in these immunoconjugates (see, e.g., Ohkawa et al., Cancer Immunol. Immunother. 23: 81, 1986; Rowland et al., Cancer Immunol. Immunother., 21: 183, 1986). Drugs used in these immunoconjugates include daunomycin (see, e.g., Gallego et al., Int. J. Cancer, 33: 737, 1984; Arnon et al., Immunological Rev., 62: 5, 1982; mexotrexate (Endo et al., Cancer Research, 47: 1076, 1987), mitomycin C (Ohkawa et al., supra), and vindesine (Rowland et al., supra). Toxins used in the antibody-toxin conjugates include bacterial toxins such as ricin (see e.g., Moolten et al., Immunol. Rev., 62: 47, 1982).

Despite the amount of research directed towards the use of immunoconjugates for therapeutic purposes, several limitations involved in these delivery approaches have become apparent (see, e.g., Embleton, Biochem. Society Transactions, 14: 393, 615th Meeting, Belfast, 1986). For example, the large amount of drug required to be delivered to the target tumor cell to effect killing of the cell is often unattainable because of limitations imposed by the number of tumor-associated antigens on the surface of the cells and the number of drug molecules that can be attached to any given antibody molecule. This limitation has led to the use of more potent cytotoxic agents such as plant toxins in these conjugates and to the development of polymer-bound antibody-drug conjugates having very high drug multiplicity ratios (see, e.g., Thorpe, supra, pp. 475-506, and Baldwin et al., in Monoclonal Antibodies and Cancer Therapy, pp. 215-231, Alan R. Liss, Inc., 1985). However, even with the large drug loading ratios or with the use of potent toxins, many immunoconjugates still display suboptimal cytotoxic activity and are unable to effect complete killing at doses where all available antigenic sites are saturated.

It has also been recognized that the cytotoxic activity of an immunoconjugate is often dependent on its uptake, mediated by the antibody component of the conjugate into the tumor cell (see, e.g., J.M. Lambert et al., J. Biol. Chem., 260: 12035, 1985). This internalization is crucial when using an antibody-drug conjugate in which the drug has an intracellular site of action or when using antibody-toxin conjugates. However, the vast majority of tumor-associated antigens and thus the antibody-drug or antibody-toxin conjugates bound to those antigens, are not internalized. Those conjugates that are internalized are often transported to the lysosome of the cell where the drug or toxin is degraded (see Vitetta et al., Science, 238: 1098, 1987). Accordingly, although an antibody-drug or antibody toxin conjugate may have excellent tumor-binding characteristics, the conjugate may nonetheless have a limited cytotoxic utility due to an inability to reach its site of action within the cell.

In addition, it is well established that tumor cell populations are often heterogeneous with respect to antigen expression (see, e.g., Albino et al., J. Exp. Med., 154: 1764, 1981). Furthermore, it has been demonstrated that antigen-positive tumor cells may give rise to antigen-negative progeny (see, e.g., Yeh et al., J. Immunol, 126: 1312, 1981). Thus, in any population of tumor cells, there will be a certain number of cells that do not possess the antigen for which a particular immunoconjugate is specific. The immunoconjugate will therefore not be able to bind to these cells and mediate their killing.

Due to these drawbacks, the currently utilized antitumor drug or toxin delivery systems have had a limited amount of success, especially when used for in vivo treatment.

In addition to the immunoconjugates discussed above, antibody-enzyme conjugates have been studied in vitro in combination with a second untargeted enzyme for the conversion of iodide or arsphenamine to their toxic forms in order to amplify antibody-mediated cytotoxicity (see, e.g., Parker et al., Proc. Natl. Acad. Sci. USA, 72: 338, 1975; Philpott et al., Cancer Research, 34: 2159, 1974).

According to these in vitro studies, the enzyme, glucose oxidase, is attached to an antibody and used in combination with an untargeted peroxidase enzyme to convert iodide or arsphenamine to cytotoxic iodine or arsenical, respectively. This approach, therefore, requires not only the targeting of glucose oxidase to tumor cells with antibody, but also the presence at the tumor site of two other untargeted events. The likelihood that all three of these agents will be present in vivo at the tumor site at the same time is small.

Canadian Patent No. 1,216,791, discloses the conjugation to an antibody of an enzyme capable of liberating ammonium ions from substrates. The ammonium ions are then said to potentiate the cytotoxic action of certain immunotoxins targeted to the tumor site.

European Patent Application No. 84302218.7 discloses a method for treating a diseased cell population such as a tumor wherein an antibody is used to target a non-metabolizable antigen to tumor cells. The antigen accumulates within at least a percentage of the tumor cells, which are then lysed to release the antigen into a ubiquitous fibronectin capturing matrix formed at the tumor site. An iodine-containing ligand which is specific for and will bind to the antigen affixed to the matrix is administered. The cytotoxic iodine acts to kill the tumor cells at that site. Also suggested is the use of an antibody-conjugate to target enzyme to a tumor site and the addition of a non-lethal substrate which the enzyme can convert to a cytotoxic material (see European Application No. 84302218.7, pp. 34-35). However, nowhere in the application is there any disclosure of how one is perform this embodiment. Similarly, Hellstrom et al., in Controlled Drug Delivery (2d ed.), Robinson and Lee (eds.) p. 639, 1987, suggest that "drugs which would be nontoxic until activated by an agent (e.g., an enzyme) localized to a tumor may be another approach...."

U.S. Patent No. 4,975,278, hereby incorporated by reference in its entirety, provides a method for delivering cytotoxic agents to tumor cells by the combined use of antibody-enzyme conjugates and prodrugs. According to this invention, an enzyme that is capable of converting a poorly or non-cytotoxic prodrug into an active cytotoxic drug is conjugated to a tumor-specific antibody. This antibody-enzyme conjugate is administered to a tumor-bearing mammalian host and binds, due to the antibody specificity, to the surface of those tumor cells which possess the tumor antigen for which the antibody is specific. The prodrug is then administered to the host and is converted at the tumor site by the action of the antibody-bound enzyme into a more active cytotoxic drug.

Nitrogen mustards have long been recognized as cytotoxic agents (See, e.g., Stock, in Drug Design, E. J., Ariens, ed., Vol. II, pp. 532-571, Academic Press, New York, 1971.) Benn, et al., J. Chem. Soc., 2365 (1961) prepared a variety of amides, including urethanes and ureas, from N,N-di-2'-chloroethyl-para-phenylenediamine that are useful for reactions with various functional groups that are of potential value for the attachment of nitrogen mustards to a wide variety of other units. The attachment of the electron-attracting urethane group deactivates the highly toxic nitrogen mustard. Reactivation of the nitrogen mustard at the tumor site may occur if the urethane is decomposed by fission of the ester or peptide linkage.

Mobashery, et al. (J. Am. Chem. Soc., 108:1685, 1986) teaches the use of β-lactamases resident in bacteria resistant to the β-lactam antibiotics, to hydrolyze cephalosporin-toxophore derivatives to effect the release of the toxophore within the bacterium.

Mobashery et al., (J. Biol. Chem., 261: 7879, 1986) synthesized an antibacterial agent consisting of the antibiotic peptide βCl-LAla-βCl-LAla linked through a C_{10} ester to the cephem nucleus of cephalosporin. The hydrolytic cleavage of the β-lactam ring by β-lactamase resident in the bacterium releases the heteroatom-linked C10 substituent.

A general discussion of the chemistry of the cephalosporins is provided by Abraham, Quarterly reviews - Chemical Society, 21:231, 1967, and Abraham et al., in Cephalosporins and Penicillins: Chemistry and Biology, E.H. Flynn, ed., Academic Press, N.Y., 1972, pp 1-26.

U.S. Patent No. 3,484,437 teaches derivatives of cephalosporanic acid formed by the reaction of a deacylated cephalosporin salt with isocyanates to form carbamates.

U.S. Patent No. 3,355,452 teaches the 0-desacetyl-O-carbamoyl-7-acylamino-cephalosporanic acid derivatives of 7-amino-cephalosporanic acid, where the 7-N-acyl group is a carboxylic acid radical and the CO group is bonded to a carbon atom.

There has been a great deal of investigation concerning the use of polymers as carriers of anticancer drugs (Maeda, H., et al. (1992), Bioconjugate Chem. 3, 351-362; Duncan, R. (1992), Anticancer Drugs 3, 175-210). The molecular weight carriers can lead to reductions in systemic toxicity, longer retention time in the body, alterations in biological distribution, and in improvements in therapeutic efficacy. Polyethylene glycol (PEG[1], Zalipsky, S,. et al., (1983) Eur. Polym. J. 12, 1177-1183; Ouchi, T., et al., (1992), Drug Design Discovery 9, 93-105); Caliceti, P., et al., (1993), Il Farmaco 48, 919-932; Panarin, E. F., et al., (1989), J. Controlled Rel. 10, 119-129), PEG copolymers (Poiani, G. J., et al., (1994), Bioconjugate Chem. 5, 621-630), dextran (Munechika, K., et al., (1994), Biol. Pharm. Bull. 17, 1193-1198), hydroxypropylmethacrylamide (Seymore, L. W., et al., (1994), Br. J. Cancer 70, 636-641), and poly(styrene-co-maleic acid) (Maeda, H, (1992), J. Controlled Rel., 19, 315-324) are but a few examples of polymers that have been used to deliver anticancer drugs and other biologically active molecules to target issues.

In most cases, release of active anticancer drugs from the polymer support is mediated by simple aqueous hydrolysis or by proteolytic or esterase enzymes (Maeda, H., et al. (1992), Bioconjugate Chem. 3, 351-362; Duncan, R. (1992), Anticancer Drugs 3, 175-210). Since the conditions for these reactions are not preferentially confined to tumor tissues, some nonspecific drug release is inevitable. Consequently, there may be advantages in developing strategies for drug release that exploit some of the physiological and biochemical differences between neoplastic and normal tissues. Such differences may either be inherent, or established by targeting enzymes to tumor cell surfaces in the form of mAb-enzyme conjugates that recognize tumor associated antigens. This targeting strategy, which has been successfully applied to the activation of a number of low molecular weight anticancer prodrugs (Bagshawe, K. D. (1994), Clin.

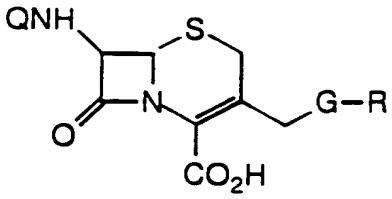
Pharmacokinet. 27, 368-376; Deonarain, M. P., et al. (1994), Br. J. Cancer 70, 786-794; Senter, P. D., et al., (1993) Bioconjugate Chem. 4, 3-9), should also be applicable to the release of active drugs that are covalently bound to polymer supports.

Many of the enzymes utilized for prodrug activation carry out reactions not normally occurring in mammalian systems. For example, cephalosporin containing prodrugs undergo drug elimination when hydrolyzed by β-lactams (Vrudhula, V. M., et al., (1993), Bioconjugate Chem. 4, 334-340; Meyer, D. L., et al., (1993) Cancer Res. 53, 3956-3963).

Heretofore, polymeric cephalosporin prodrugs have been unknown.

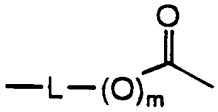
The present invention is based on the discovery of novel polymeric cephalosporin-related prodrugs, capable of conversion to antitumor agents at the tumor site using a β-lactamase-antibody conjugate. The antibody is directed against a tumor antigen present on the surface of the specific tumor type targeted.

The present invention provides polymeric cephalosporin prodrugs of the general formula (I)



(I)

wherein Q is a pharmaceutically acceptable polymeric moiety; G is -NH- or is of the formula

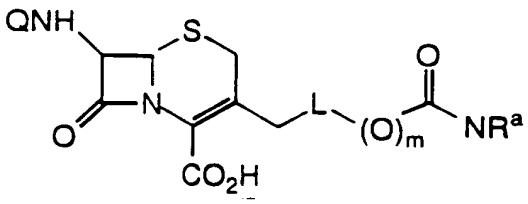


L is a direct bond or -S-$(CH_2)_n$-; R is an agent capable of exerting a cytotoxic effect on tumor cells when released from said cephalosporin-prodrug; n is 2, 3, or 4; and m is 0 or 1 with the proviso that when L is a direct bond, m is 1; or a pharmaceutically acceptable salt thereof.

It is preferred that the polymeric moiety, i.e., "Q" is the residue of a polyethylene glycol or a branched polyethylene glycol.

The cytotoxic compound is one having at least one functional group amenable to chemical modification to provide the cephalosporin prodrug. Generally, such functional groups are selected from amino, carboxyl, and hydroxyl groups such that the linkage between the cytotoxic agent and the cephalosporin component is of the carbamate, amide, ester, and carbonate types.

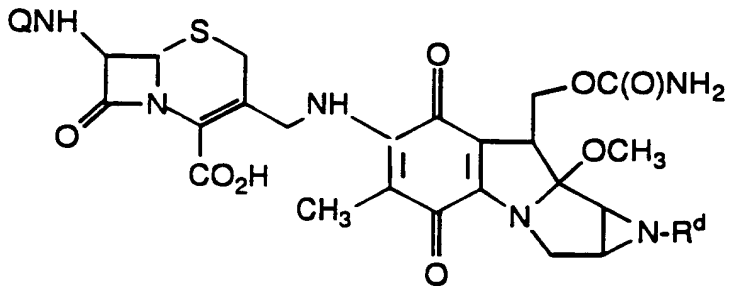
In one aspect, the present invention provides as one subclass of compounds of formula (I) cephalosporin prodrugs of the general formula (II) in which the cytotoxic agent is linked to the cephalosporin nucleus via carbamate or amide group



(II)

wherein Q, L, and m are as defined under formula (I); and NR^a is a nitrogen containing cytotoxic drug; or a pharmaceutically acceptable salt thereof.

In another aspect the present invention provides a cephalosporin-cytotoxic agent prodrug linked via an amine group such as a cephalosporin-mitomycin prodrug having the formula (IIa)



(IIa)

wherein Q is as defined above under formula (I) and R^d is hydrogen or C_{1-3} alkyl.

Another embodiment of the subject invention is directed to a method for delivering a cytotoxic agent to tumor cells by administering a pharmaceutically effective amount of at least one antibody-β-lactamase conjugate comprising an antibody reactive with an antigen on the surface of the tumor cells. A pharmaceutically effective amount of a polymeric cephalosporin prodrug is also administered, where the polymeric cephalosporin prodrug comprises a polymeric moiety linked in the cephalosporin which is in turn linked to the cytotoxic agent.

In an alternative embodiment, the present invention is directed to a method of delivering a cytotoxic agent to tumor cells wherein the antigen binding region of an antibody reactive with a tumor-associated antigen is linked to at least a functionally active part of β-lactamase, and is administered with a pharmaceutically effective amount of a polymeric cephalosporin prodrug.

In another embodiment, the subject invention is directed to a method of treating mammalian tumors which includes the step of administering to a mammal a pharmaceutically effective amount of at least one antibody-β-lactamase conjugate and a pharmaceutically effective amount of at least one polymeric cephalosporin prodrug.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Figure 1. Mechanism of drug release by β-lactamase.

Figure 2. HPLC analyses of PEG-AC-Dox samples. A: M-PEG-AC-Dox. B: B-PEG-AC-Dox. C: M-PEG-AC-Dox + bL (solid line), Dox (dotted line). D: B-PEG-AC-Dox + bL (solid line), doxorubicin (dotted line). See the example section for the meaning of the abbreviations.

Figure 3. Cytotoxic effects of PEG-AC-Dox samples ±mAb-bL conjugates on H2981 lung adenocarcinoma cells as determined by the incorporation of [^{3}H] thymidine into DNA compared to untreated control cells. A: Cells were exposed to L6-bL, washed and then treated with M-PEG-AC-Dox for 1 hour. The cytotoxic effects were determined aproximately 24 hours later. B: Cells were exposed to conjugates, washed, and then treated with B-PEG-AC-Dox for 2 hours.

Figure 4 Pharmacokinetics of PEG-AC-Dox samples and doxorubicin in nude mice bearing subcutaneous 3677 human tumor xenografts. Tumor and blood samples were extracted, and the amount of prodrug and drug was quantified by HPLC. A: Blood levels of M-PEG-AC-Dox and B-PEG-AC-Dox after intravenous prodrug injection (52 μmol/kg). The results are compared with doxorubicin levels in animals receiving 15.5 μmol/kg doxorubicin. B: Tumor levels of M-PEG-AC-Dox, B-PEG-AC-Dox, and doxorubicin after intravenous injection (52 μmol polymer/kg, 15.5 μmol free Dox/kg).

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of synthetic organic chemistry, protein chemistry, molecular biology, microbiology, and recombinant DNA technology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Scopes, R.K., Protein Purification Principles and Practices, 2d ed. (Springer-Verlag, 1987), Methods in Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.), Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989, Handbook of Experimental Immunology, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds, 1986, Blackwell Scientific Publications); House, Modern Synthetic Reactions, 2nd ed., Benjamin /Cummings, Menlo Park, Cal., 1972.

All patents, patent applications, and publications mentioned herein, whether supra or infra, are hereby incorporated by reference in their entirety.

A. Definitions

In defining the present invention, the following terms will be employed, and are intended to be defined as indicated below.

DOCKET ALARM

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