POLYMER CARRIERS FOR TARGETED DRUG DELIVERY AND CONTROLLED DRUG RELEASE

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Abstract

Nondegradable *N*-(2-Hydroxypropyl)methacrylamide (HPMA) copolymers or biodegradable poly(ethylene glycol) (PEG) multiblock copolymers conjugated with antibodies were designed as water-soluble carriers of anticancer drugs facilitating site-specific therapy. High-molecular-weight polymer carriers were used for passive targeting to solid tumors while conjugates with specific polyclonal or monoclonal antibodies were designed for specific delivery of anti-cancer drug doxorubicin to tumor cells or model tumors inoculated in mice. Doxorubicin conjugates targeted with B1 monoclonal antibody (mAb) were shown to possess strictly tumor-specific binding capacity to target BCL1 cells *in vitro* and superior *in vivo* activity to free doxorubicin or non-targeted polymer drug in the treatment of established BCL1 leukemia in mice. Likewise, the use of conjugates targeted with anti EL4 mAb resulted in large amount of long-term survivors after treatment of mice bearing mouse EL4 lymphoma. Preliminary evaluation of doxorubicin conjugate in human demonstrated that the polymer drugs rank among the most promising candidates for successful application in human cancer chemotherapy.

Introduction

Water-soluble conjugates of synthetic copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) with anticancer drugs and antibodies provide a potential drug delivery system facilitating specific drug delivery to model tumors in mice [1, 2]. In the system, an anti-cancer drug is attached to the polymer through a biodegradable spacer susceptible to enzymatic hydrolysis enabling intracellular drug release at a controlled rate, and a specific antibody is attached as a homing device, recognising specific receptors expressed on the surface of the target cells. Water-soluble drug carriers based on nondegradable copolymers of *N*-(2-hydroxypropyl)methacrylamide (PHPMA), or biodegradable multiblock poly(ethylene glycol)s (PEG) polymers were designed as lysosomotropic drug delivery systems. Various structures of the carriers and polymer drugs have been synthesised and studied. In the classic structure, anti-cancer drug doxorubicin (DOX) and a targeting antibody are attached to the hydrophilic PHPMA- or PEG-based backbone via enzymatically degradable oligopeptide sequences randomly distributed along the polymer chain. Such conjugates provide a potential and powerful drug delivery system facilitating specific drug delivery to tumor cells or model tumors inoculated in mice [3]. Despite its significant anti-cancer activity, the system has some drawbacks, such as not well-defined branched structure, high molecular weight and broad distribution of molecular weights. These drawbacks are minimised in a star structure of the conjugate. In the star system, a number of semitelechelic PHPMA chains, bearing DOX attached through biodegradable GFLG spacers, are linked to the central antibody molecule via amide bonds formed by the reaction of terminal reactive ester groups of the polymer with -amino groups of lysine residues in the antibody. Both classic and star systems enable drug release only in secondary lysosomes after contact with lysosomal enzymes. Biological activity of all the DOX conjugates depends on the detailed structure of the conjugate and the ability of the system to release active drug in the target cells. A prerequisite for biological activity of the classic and star conjugates is the presence of lysosomal enzymes at the polymer drug target.

In the third system under study - hydrazone conjugates (HC), DOX is attached to the biodegradable PEG or nondegradable PHPMA backbone via a spacer containing hydrazone group. The hydrazone conjugates are stable under physiological conditions (blood circulation, pH 7.4), but hydrolytically degradable in mildly acidic environment (e.g., in endosomes and lysosomes, pH ~ 5 - 6). The systems can circulate in the blood stream for a long time and are specifically activated (DOX is released) in the target cells, including those not exhibiting enzymatic activity.

Results and Discussion

Polymer carriers were used for conjugation with DOX and selected monoclonal and polyclonal antibodies differing in their specificity for antigen (B1 mAb, anti-Thy1,2 mAb, anti-EL4 mAb, IgG).

Lysosomotropic conjugates. In addition to classic systems, the star conjugates differing in the degree of antibody substitution and the length of the polymer chain were synthesised. All the antibody conjugates of star structure had lower molecular weights and a significantly narrower molecular weight distribution than the conjugates of classic structure. The rate of release of DOX from the classic conjugates incubated in the presence of lysosomal enzyme cathepsin B strongly depended on the structure of the oligopeptide spacer, being the highest for the conjugate containing the GFLG spacer. DOX release from the star conjugate was almost faster by a factor of two than from

those of classic structure. The star conjugates showed a lower binding activity to cancer cells *in vitro*, but their cytostatic activity measured by [^{3}H]thymidine incorporation was higher by a factor of three than that observed with classic conjugates. Cytostatic activity of non-targeted and IgG-modified (with irrelevant immunoglobulin) PHPMA conjugates was more than hundred times lower compared with the star conjugates targeted with specific monoclonal antibody.

Both classic and star types of antibody-targeted conjugates shoved a considerably stronger anti-tumor *in vivo* activity than non-targeted PHPMA-DOX conjugates or free doxorubicin, but the star conjugates had a remarkably higher anti-tumor effect than the classic systems. A single intravenous injection of 100 mg of doxorubicin in the form of the star conjugate on day 11 completely cured 5 out of 9 experimental animals whereas the classic structure of the targeted conjugate administered in the same way only increased the survival of experimental mice to 138 % relative to control. These results show that the star structure of the antibody-targeted PHPMA-DOX conjugate is highly suitable for targeted drug delivery, possessing better characteristics, a higher cytostatic activity *in vitro* and a stronger anti-tumor potential *in vivo* than the classic conjugates undergoing clinical evaluation at present [4, 5].

Hydrazone conjugates. Release of DOX from the conjugates incubated in a buffer at pH 5 was faster by a factor of ten than that at pH 7.4. The effect of pH on DOX release was more pronounced for PEG-based conjugates. The rate of drug release depended on the structure and length of the spacer (based on *cis*-aconitic acid, glycine, ß-alanine, 4-aminobenzoic acid, 6-aminohexanoic acid or oligopeptides). At pH 5, the highest rate was obtained for the conjugate containing the 6-aminohexanoic acid-hydrazone spacer and the slowest for the conjugates containing the *cis*-aconityl spacer. The presence of lysosomal enzyme cathepsin B in incubation media increased the rate of DOX release from the conjugate with the GFLG spacer while the DOX release from the conjugates with amino acid or diglycine spacers remained unchanged [6]. Cytotoxicity of the conjugates for treated cells (T-splenocytes, EL-4 lymphoma cells) depended on the detailed structure of the spacer and the used antibody. The hydrazone conjugates were more cytotoxic than those with ester bonds and the cytotoxicity of the antibody-targeted conjugates was comparable with that of free DOX (IC_{50} < 0.1 μg/mL). *In vivo* anti-tumor activity of the conjugates compared with free DOX was significantly higher, exhibiting extensive inhibition of tumor growth and 40 - 60 % of long-term survivors (there were no survivors after treatment of mice with free DOX). The efficiency of the antibody-targeted conjugates was the highest.

Conclusion

Application of conjugates of cancerostatics with synthetic water-soluble polymers and antibodies as site-specific anti-cancer drugs has been studied. Biological tests, considerable anti-tumor activity in mice and preliminary evaluation in human demonstrate that the polymer drug rank among the most promising candidates for successful application in human cancer chemotherapy.

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