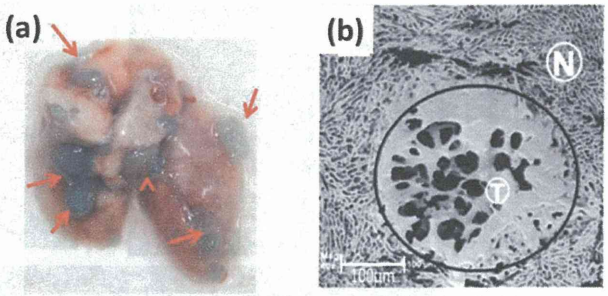

Figure 2 The EPR effect in metastatic tumors. (a) Metastatic tumors in the lung, originating from subcutaneously implanted colon 26 tumor, were visualized by staining with Evans blue-albumin. This result is similar to the primary tumor staining seen in Figure 1a and d. (b) Metastatic tumor (T) in the liver originating from the spleen was visualized via scanning electron microscopy. The tumor (MoCR) was implanted in the spleen of the CBA mouse; see text for details Used by permission from Daruwalla et al. [76].

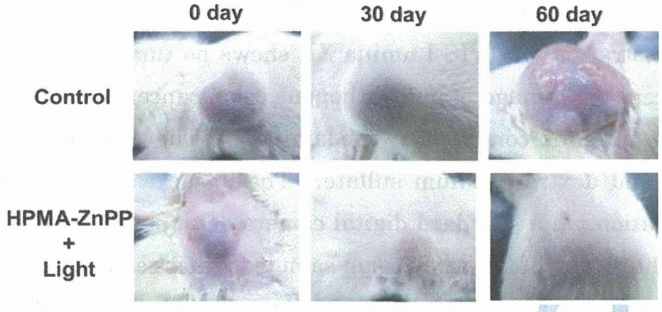

Figure 3 Photodynamic therapy in DMBA-induced breast cancer. A rat bearing the autochthonous breast tumor received no drug (control) or 15 mg/kg PHPMA-ZnPP i.v., and then tumors were irradiated with xenon light at 24 and 48 h after PHPMA-ZnPP administration and were observed at the times indicated. Complete tumor eradication was seen on day 60. The control is a similar tumor-bearing rat that did not receive ZnPP polymer but had the same dose of light irradiation. The control tumor continued to grow, as seen in human breast cancer.

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Declaration of interest

The authors state no conflict of interest and have received no payment of this manuscript.

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Improved pharmacokinetics and antitumor activity of new dendrimer-derived poly(*N*-(2-hydroxypropyl)methacrylamide) conjugates of pirarubicin

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Keywords: HPMA polymer conjugate, pirarubicin (THP), acid-cleavable linkage, EPR effect, dendrimer conjugated THP

Abbreviations: THP: 4'-O-tetrahydropyranyl doxorubicin; DOX: doxorubicin; SP: star polymer; LP: linear polymer; AUC: area under the curve; AOM: azoxymethan; DSS: dextran sodium sulfate; HPMA: *N*-(2-hydroxyproyl)methacrylamide; EPR: enhanced permeability and retention; PEG: polyethylene glycol; PAMAM: polyamido amine; ABIC: 4,4′-azobis(4-cyanovaleric acid); DIPC: *N,N′*-diisopropylcarbodiimide; EDPA: *N*-ethyldiisopropylamine; TNBSA: 2,4,6-trinitrobenzene-1-sulfonic acid; MTT: 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide

Novelty and impact of the work.

This article describes comparison of two different types of antitumor polymer-conjugated drugs; LP (linear polymer)-THP and SP (spherical polymer, dendrimer)-THP conjugates, respectively, and clarified tissue distribution, therapeutic efficacy, and toxicity. Excellent tumor selective accumulation was observed after i.v. administration of both polymeric drug-conjugates. Both showed far superior therapeutic effect, and least toxicity, than parental low MW free THP (pirarubicin). Especially, SP-THP showed remarkable therapeutic efficacy against not only implanted tumor model but also chemical- induced autochthonous tumor in the colon, which warrant further development.

Abstract

Previously we showed that linear poly(*N*-(2-hydroxypropyl)methacrylamide) conjugates of pirarubicin (THP), LP-THP, with MW about 39 kDa, exhibited far better tumor accumulation and therapeutic effect than that of parental free THP. To improve the pharmacokinetics of LP-THP further, high-MW conjugate of poly(amido amine) (PAMAM) dendrimer grafted with semitelechelic HPMA copolymer (PHPMA) was synthesized [star polymer (SP); 400 kDa] and conjugated with THP via hydrazone bond-containing spacer (SP-THP). Here we describe the synthesis of the SP-THP conjugate and evaluation of its antitumor action in *in vitro* and *in vivo* system. SP-THP consists of 2nd generation dendrimer in the core, of which surface amino groups were grafted with LP-THP. THP was conjugated to SP to form SP-THP via acid cleavable hydrazone bonding, which responds to acidic milieu of tumor tissue. As a consequence, it would release free THP, by active therapeutic principle, at the lysosomes and endosomes of tumor cells. SP-THP exhibits larger hydrodynamic diameter (25.9 nm) in aqueous solution than that of LP-THP (8.6 nm) as observed by light scattering and size exclusion chromatography. Because of the larger size, the tumor $AUC_{5h\text{-}72h}$ of SP-THP was 3.3 times higher than that of LP-THP. More importantly, released free THP was retained selectively in the tumor tissue for at least up to 72h after administration of SP-THP. Tumor level of THP was 10 – 30 times higher than in the normal tissue, resulting in much lower side effect compared to conventional free THP. In *in vivo* antitumor study, S-180 tumor-bearing mice, and chemically with AOM / DSS-induced colon tumor-bearing mice were used to compare the therapeutic efficacy of SP-THP and LP-THP. SP-THP exhibited superior antitumor effect to LP-THP against both S-180 and AOM / DSS-induced colon tumor.

Introduction

4'-O-tetrahydropyranyl doxorubicin (Pirarubicin®, or THP) is an anthracycline antibiotic used for treatment of various cancers in such organs as breast, head and neck, cervix, and lymphoma, etc [1]. An intrinsic problem of low-MW anticancer drugs is also applicable to THP (MW 628); its body distribution is indiscriminate in all normal tissues and organs before tumor delivery. Thus adverse effects such as bone-marrow suppression, cardiac toxicity as well as others limit the usage of higher dose of THP in clinical setting. Thus improvement of pharmacokinetics, especially tumor selective delivery is the prime requirement.

Poly(*N*-(2-hydroxypropyl)methacrylamide (PHPMA) is highly water-soluble biocompatible macromolecule, namely nontoxic and non-immunogenic. [2, 3]. After intravenous injection, high-MW PHPMA (more than 40 kDa) is retained in the systemic circulation for longer time (> 24h) at significant concentration, thus it preferentially accumulates in the tumor tissue by enhanced permeability and retention (EPR) effect [4]. By conjugating low-MW antitumor drugs to the PHPMA, the tumor accumulation of the antitumor drug can be enhanced and the therapeutic response for solid tumor improved.

To improve the tumor accumulation of PHPMA drug conjugates, molecular size of PHPMA may be increased; either by branching or grafting HPMA copolymers or by their self-assembly to form high-MW micellar structures. However, the synthesis of explicitly branched side chains or graft polymers in comb structure is relatively difficult to control; high polydispersity index and lower reproducibility become a concern. On the other hand, dendrimer is nearly monodisperse, and its surface can be freely and explicitly modified, e.g. by attaching semitelechelic