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Indium-based and iodine-based labeling of HPMA copolymer–epirubicin conjugates: Impact of structure on the in vivo fate

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ABSTRACT

Recently, we developed 2nd generation backbone degradable *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-drug conjugates which contain enzymatically cleavable sequences (GFLG) in both polymeric backbone and side-chains. This design allows using polymeric carriers with molecular weights above renal threshold without impairing their biocompatibility, thereby leading to significant improvement in therapeutic efficacy. For example, 2nd generation HPMA copolymer-epirubicin (EPI) conjugates (2P-EPI) demonstrated complete tumor regression in the treatment of mice bearing ovarian carcinoma. To obtain a better understanding of the in vivo fate of this system, we developed a dual-labeling strategy to simultaneously investigate the pharmacokinetics and biodistribution of the polymer carrier and drug EPI. First, we synthesized two different types of dual-radiolabeled conjugates, including 1) ^{111}In-2P-EPI-^{125}I (polymeric carrier 2P was radiolabeled with ^{111}In and drug EPI with ^{125}I), and 2) ^{125}I-2P-EPI-^{111}In (polymeric carrier 2P was radiolabeled with ^{125}I and drug EPI with ^{111}In). Then, we compared the pharmacokinetics and biodistribution of these two dual-labeled conjugates in female nude mice bearing A2780 human ovarian carcinoma. There was no significant difference in the blood circulation between polymeric carrier and payload; the carriers (^{111}In-2P and ^{125}I-2P) showed similar retention of radioactivity in both tumor and major organs except kidney. However, compared to ^{111}In-labeled payload EPI, ^{125}I-labeled EPI showed lower radioactivity in normal organs and tumor at 48 h and 144 h after intravenous administration of conjugates. This may be due to different drug release rates resulting from steric hindrance to the formation of enzyme-substrate complex as indicated by cleavage experiments with lysosomal enzymes (Tritosomes). A slower release rate of EPI(DTPA)^{111}In than EPI(Tyr)^{125}I was observed. It may be also due to in vivo catabolism and subsequent iodine loss as literature reported. Nevertheless, tumor-to-tissue uptake ratios of both radionuclides were comparable, indicating that drug-labeling strategy does not affect the tumor targeting ability of HPMA copolymer conjugates.

1. Introduction

Conjugation of anticancer drugs to water-soluble polymers offers a possibility to improve their solubility, decrease adverse effects, modify pharmacokinetics, favorably change their biodistribution, and improve therapeutic efficacy by enhanced permeability and retention (EPR) effect [1–4]. Numerous synthetic polymers have been used such as poly(-ethylene glycol) (PEG) [5,6], [*N*-(2-hydroxypropyl)methacrylamide] (HPMA) copolymers [7–11], poly(amino acids) [12], polyoxazoline [13], poly(malic acid) [14], etc. Among the various polymeric carriers, the most commonly used is PEG, which has been approved by FDA for clinical application and is commercial available in a wide range of molar masses, end-functionalities as well as different architectures. However, the use of PEG can be problematic in some instances, with recent results indicating PEG-containing therapeutics can elicit complement activation, rapid clearance after repeated injections, and may suffer from peroxidation [15,16]. Moreover, the inability to effectively functionalize the polyether backbone mitigates the utility of many PEG drug delivery systems [17]. HPMA copolymers have comparable biocompatibility and advantages over PEG on non-immunogenicity and established bioconjugation strategies. Its favorable properties have been validated by diverse preclinical and clinical studies [18–22]. Recently, higher molecular weight (Mw) biodegradable HPMA copolymer-drug conjugates have been designed with enzymatically degradable tetrapeptide sequences (GFLG) in both polymer backbone and side chains to prolong plasma circulation and enhance tumor accumulation while preserving biocompatibility [23–28].

The knowledge of the pharmacokinetics and biodistribution of macromolecular therapeutics is a prerequisite for the understanding of the mechanism of their action and ultimate translation into clinical use.

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The fate of HPMA copolymers after administration to animals has been intensively studied. Initially, drug concentration in various organs was determined by direct extraction of drug from lyophilized tissue samples followed by HPLC analysis or fluorescence assay [29,30]. This approach needs large groups of animals and tedious work; it was replaced by radiolabeling strategy, which has high sensitivity and was widely applied in preclinical studies and clinical investigations [31–33]. Among different radionuclides single-step iodination (^{124}I, ^{125}I and ^{131}I) and two-step radiometal labeling (^{90}Y, ^{111}In and ^{177}Lu, etc.) are often performed. Iodination has been frequently used due to low cost and simple radiochemistry. For example, it was reported that radioiodination of drugs (daunomycin and doxorubicin) was achieved by mixing drug (or conjugate) solution with iodide in an iodogen reaction vial under ambient condition for a few minutes [19,34]. In the majority cases, however, tyrosine moiety was typically incorporated into polymer carrier via copolymerization followed by iodine labeling [35–41]. In these cases, the radioactive signals were correlated to the polymer carrier rather than drug. To examine the circulation and accumulation of real drug molecules, ^{14}C-labeled drug might be an option. Nevertheless, such isotope-labeled drugs are expensive; the synthesis of conjugates will be complicated because ^{14}C has a long half-life and will cause large level of irradiation. Recently, dual-labeling strategies have been developed in which one probe aims to track the polymer carrier, while the other one monitor the fate of drug (modified or model drug) [26,42–43]. For example, dual-fluorescent conjugates were studied using Fluorescence resonance energy transfer (FRET) as a tool to track chain scission of the conjugates and drug release from the carrier [28], or using noninvasive multispectral optical imaging to real time monitor the distribution and tumor accumulation of polymer carrier and a cleavable model drug [44]. Recently we designed dual-isotope-labeled 2nd generation HPMA copolymer-drug model conjugate, whose HPMA copolymer backbone was labeled with ^{125}I, whereas ^{111}In-DTPA complex was bound at GFLG side-chain termini and served as the drug model [26].

We have reported the pharmacokinetics and therapeutic efficacy of 2nd generation diblock HPMA copolymer-epirubicin (EPI) conjugates (2P-EPI) in the treatment of experimental ovarian cancer [28]. Notably, treatment with 2P-EPI resulted in complete tumor remission and long-term inhibition of tumorigenesis (>100 days), whereas the tumor recurrence was observed in mice treated with the 1st generation HPMA copolymer-EPI conjugate (P-EPI, with Mw < 50 kDa). To demonstrate the different pharmacologies between these two generations of conjugates, we designed and synthesized a series of dual radiolabeled HPMA copolymer-EPI conjugates in which ^{125}I and ^{111}In were used to label polymer carrier and drug (EPI), respectively, or vice versa. This paper is devoted to the study of pharmacokinetics and biodistribution of 2nd generation HPMA copolymer-EPI conjugates in nude mice. In one approach, we labeled the polymer carrier with ^{125}I and used DTPA-^{111}In to modify the EPI structure. In the second design, we used DTPA-^{111}In to label the polymer carrier and ^{125}I for drug modification. The issues we addressed are: A) How does modification of the conjugate structure influence its fate? Is there a difference between the two labeling designs? B) How does the modification of drug structure impact the formation of the enzyme-substrate complex, rate of enzymatic drug release and the biodistribution of the drug? C) How does the data obtained differ from the behavior of the unlabeled conjugate that would be used as the macromolecular therapeutics?

2. Experimental section

2.1. Abbreviations

APMA	*N*-(3-aminopropyl)methacrylamide hydrochloride
Boc-GFLG-OMe	methyl9-benzyl-2,2-dimethyl-4,7,10,13-tetraoxo-12-propyl-3-oxa-5,8,11,14-tetraazahexadecan-16-oate
Boc-GFLG-NH_2	*tert*-butyl(14-amino-4-benzyl-7-isobutyl-2,5,8,11-tetraoxo-3,6,9,12-tetraazatetradecyl)carbamate
CTA	chain transfer agent (4-cyanopentanoic acid dithiobenzoate)
CTA-GFLG-CTA	10-benzyl-2,25-dicyano-13-isobutyl-5,8,11,14,17,22-hexaoxo-6,9,12,15,18,21-hexaazahexacosane-2,25-diyl dibenzodithioate
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DMAP	4-(dimethylamino) pyridine
EPI	epirubicin
Fmoc-Abu(N_3)-OH	(S)-2-(Fmoc-amino)-4-azidobutanoic acid
HPMA	*N*-(2-hydroxypropyl)methacrylamide
HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxidhexafluorophosphate
HOBt	1-hydroxybenzotriazole
^{111}In-P-EPI-^{125}I	frist generation HPMA copolymer-EPI conjugate with ^{111}In-DTPA labeled polymer backbone and ^{125}I-Tyr labeled EPI
^{111}In-2P-EPI-^{125}I	second generation HPMA copolymer-EPI conjugate with ^{111}In-DTPA labeled polymer backbone and ^{125}I-Tyr labeled EPI
^{125}I-2P-EPI-^{111}In	second generation HPMA copolymer-EPI conjugate with ^{125}I-Tyr labeled polymer backbone and ^{111}In-DTPA labeled EPI
MA-Tyr-NH_2	*N*-methacryloyltyrosinamide
MA-GFLG-OH	*N*-methacryloylglycylphenylalanylleucylglycine
MA-GFLG-Abu(N_3)-OH	*N*-(2-((4-azido-1-(((2S,3R,4S,6R)-3-hydroxy-2-methyl-6-((3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-1-yl)oxy) tetrahydro-2H-pyran-4-yl)amino)-1-oxobutan-2-yl)amino)-2-oxoethyl)-2-(2-(2-methacrylamidoacetamido)-3-phenylpropanamido)-4-methylpentanamide
MA-GFLG-Abu(N_3)-EPI	*N*-(2-((4-azido-1-(((2S,3R,4S,6R)-3-hydroxy-2-methyl-6-((3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-1-yl) oxy)tetrahydro-2H-pyran-4-yl)amino)-1-oxobutan-2-yl)amino)-2-oxoethyl)-2-(2-(2-methacrylamidoacetamido)-3-phenylpropanamido)-4-methylpentanamide
MA-GFLG-Abu(Tyr)-EPI	2-(4-(1-(12-benzyl-3-(((2S,3R,4S,6R)-3-hydroxy-2-methyl-6-((3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-1-yl) oxy)tetrahydro-2H-pyran-4-yl)carbamoyl)-9-isobutyl-18-methyl-5,8,11,14,17-pentaoxo-4,7,10,13,16-pentaazanonadec-18-en-1-yl)-1H-1,2,3-triazol-5-yl)butanamido)-3-(4-hydroxyphenyl)propanoic acid
MA-GFLG-NHBoc	*tert*-butyl(11-benzyl-8-isobutyl-17-methyl-4,7,10,13,16-pentaoxo-3,6,9,12,15-pentaazaoctadec-17-en-1-yl)carbamate
MA-GG-EPI	*N*-(2-((2-(((2S,3R,4S,6R)-3-hydroxy-2-methyl-6-((3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-1-yl)oxy)tetrahydro-2H-pyran-4-yl)amino)-2-oxoethyl)amino)-2-oxoethyl)methacrylamide
NH_2-GFLG-NH_2	2-(2-(2-aminoacetamido)-3-phenylpropanamido)-*N*-(2-((2-aminoethyl)amino)-2-oxoethyl)-4-methylpentanamide
p-SCN-Bn-DTPA	2,2′-((1-((2-((2-carboxyethyl)(carboxymethyl)amino)ethyl)(carboxymethyl)amino)-3-(4-thiocyanatophenyl)propan-2-yl)azanediyl)diacetic acid
P-EPI	first generation HPMA and MA-GFLG-EPI copolymer conjugate
P-EPI-Tyr	first generation HPMA and MA-GFLG-Abu(Tyr)-EPI copolymer conjugate
P-EPI-N_3	first generation HPMA, APMA and MA-GFLG-Abu(N_3)-EPI copolymer conjugate
P-DTPA-EPI(Tyr)	first generation HPMA copolymer-EPI conjugate with DTPA pendent on polymer backbone and tyrosine moiety attachment with EPI
2P-EPI-N_3	second generation HPMA, APMA and MA-GFLG-Abu(N_3)-EPI copolymer conjugate
2P-DTPA-EPI(Tyr)	second generation HPMA copolymer-EPI conjugate with DTPA pendent on polymer back bone and tyrosine moiety attachment with EPI

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