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Effect of physicochemical modification on the biodistribution and tumor accumulation of HPMA copolymers

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Abstract

Copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) are prototypic and well-characterized polymeric drug carriers that are being broadly implemented in the delivery of anticancer therapeutics. To better predict the in vivo potential of the copolymers and to describe the biodistributional consequences of functionalization, 13 physicochemically different HPMA copolymers were synthesized, varying in molecular weight and in the nature and amount of functional groups introduced. Upon radiolabeling, the copolymers were injected i.v., and their circulation kinetics, tissue distribution and tumor accumulation were monitored in rats bearing subcutaneous Dunning AT1 tumors. It was found that increasing the average molecular weight of HPMA copolymers resulted in prolonged circulation times and in increased tumor concentrations. Conjugation of carboxyl and hydrazide groups, as well as introduction of spacer, drug and peptide moieties reduced the long-circulating properties of the copolymers and as a result, lower levels were found in tumors and in all organs other than kidney. Interestingly, however, in spite of the reduced (absolute) tumor concentrations, hardly any reduction in the relative levels localizing to tumors was found. Tumor-to-organ ratios were comparable to unmodified control for the majority of chemically modified copolymers, indicating that functionalization does not necessarily affect the tumor targeting ability of the copolymers and suggesting that HPMA copolymer-based drug delivery systems may prove to be attractive tools for more effectively treating various forms of advanced solid malignancy.

Keywords: HPMA; EPR; Drug delivery; Tumor targeting; Polymer therapeutics

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1. Introduction

Copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) are prototypic and well-characterized drug carriers that hold significant promise for implementation in anticancer therapy [1–4]. With their long-circulating properties, HPMA copolymers are able to localize to tumors relatively effectively by means of the so-called enhanced permeability and retention (EPR) effect [5]. EPR relies on the notion that tumor vasculature tends to be significantly more leaky than normal, continuous endothelium [6]. Long-circulating macromolecular drug carriers use this enhanced vascular permeability to extravasate into the tumor interstitium, and because of the lack of a functional lymphatic drainage system within solid tumors, they tend to accumulate there (passively) over time [2–6].

Currently, several HPMA-based chemotherapeutic agents are being evaluated clinically. PK1, an HPMA copolymer in which doxorubicin is coupled to the polymeric backbone by means of an enzymatically cleavable tetrapeptide spacer, was the first conjugate to enter phase I trials [7–9]. Based on the relatively promising results obtained for PK1, a few years later, PK2 was designed, in which galactose moieties were included to actively and more specifically target hepatocytes [10,11]. In parallel, a number of other HPMA copolymer-based anticancer agents were designed, carrying both classical chemotherapeutics, like cisplatinum [12–14] and paclitaxel [15], as well as more recently discovered drugs, like the heat shock protein inhibitor geldanamycin [16,17] and the angiogenesis inhibitor TNP-470 [18,19]. In addition, HPMA copolymers have been shown to be able to improve the tumor-targeted delivery of proteins, like ribonucleases [20] and β-lactamase [21], and to allow for the design of polymer-based imaging agents, in which tracers like 131-iodine [11], 99-technetium [22] and gadolinium [23] are used to visualize tumors, metastases and tumor vasculature.

The conjugation of most, if not of all, of the abovementioned agents to HPMA copolymers is expected to have significant impact on the physicochemical properties of the copolymers. Up to now, however, hardly any study has directly delineated how the functionalization of HPMA copolymers affects their biodistribution and their tumor targeting ability. Hypothesizing that parental (i.e. chemically and functionally unmodified) HPMA copolymers reside in the most optimal random coil conformation, that they thus possess the most optimal long-circulating properties, and that they are therefore more effective in targeting solid tumors than HPMA copolymers carrying drugs, spacers and tracers, we set out to investigate the effects of functionalization by synthesizing 13 different HPMA copolymers (see Table 1). In four sequentially performed sets of experiments, the copolymers were then radiolabeled and injected i.v., and their kinetics, their tissue distribution and their tumor accumulation were monitored in Copenhagen rats bearing subcutaneously transplanted Dunning AT1 tumors

Table 1
Identities and physicochemical characteristics of the 13 HPMA copolymers synthesized

No.	Polymer identity	pHPMA (*x*)	Group introduced (*y*)	$TyrNH_2$ (*z*)	Weight (kDa)	Dispersity (M_w/M_n)
I	poly(HPMA)	99.6 mol%	–	0.4 mol%	23.4	1.4
II	poly(HPMA)	99.2 mol%	–	0.8 mol%	30.5	1.3
III	poly(HPMA)	99.7 mol%	–	0.3 mol%	64.5	1.2
IV	p(HPMA)-COOH	96.3 mol%	3.4 mol% -OH (→COOH)	0.3 mol%	33.6	1.5
V	p(HPMA)-GG-$NHNH_2$	95.9 mol%	3.1 mol% -GlyGly-$NHNH_2$	1.0 mol%	29.0	2.0
VI	p(HPMA)-COOH	91.7 mol%	8.0 mol% -OH (→COOH)	0.3 mol%	21.0	1.6
VII	p(HPMA)-GG-$NHNH_2$	91.3 mol%	7.6 mol% -GlyGly-$NHNH_2$	1.1 mol%	19.0	1.8
VIII	p(HPMA)-GG-Dox	92.3 mol%	7.2 mol% -GlyGly-Dox	0.5 mol%	30.8	1.2
IX	p(HPMA)-GFLG-OH	94.3 mol%	5.1 mol% -Gly-DL-PheLeuGly-OH	0.6 mol%	25.5	1.5
X	p(HPMA)-GFLG-Dox	93.2 mol%	6.3 mol% -Gly-DL-PheLeuGly-Dox	0.5 mol%	29.5	1.3
XI	p(HPMA)-GG-PHSCN	96.5 mol%	2.5 mol% -GlyGly-PHSCN	1.0 mol%	31.0	1.5
XII	p(HPMA)-GG-AHX-PHSCN	96.5 mol%	2.5 mol% -GlyGly-AHX-PHSCN	1.0 mol%	31.0	1.5
XIII	p(HPMA)-GG-PEG-PHSCN	96.5 mol%	2.5 mol% -GlyGly-PEG_{500}-PHSCN	1.0 mol%	31.0	1.5