International Journal of Pharmaceutics 476 (2014) 164–168

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Note

Prolonged local retention of subcutaneously injected polymers monitored by noninvasive SPECT imaging

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ARTICLE INFO

Article history:
Received 1 July 2014
Received in revised form 19 September 2014
Accepted 28 September 2014
Available online 1 October 2014

Keywords:
SPECT imaging
Subcutaneous injection
Polymer
Dendrimer
Poly(glutamic acid)

ABSTRACT

Polymers are widely applied to drug delivery systems because polymers are generally excreted from the body more slowly than small molecules. Subcutaneous injection is one plausible means of administration. In this study, the *in vivo* behaviors of subcutaneously injected polymers, linear poly (glutamic acid) (Poly-Glu), acetylated dendrimer (Ac-den) and collagen peptide-conjugated dendrimer (CP-den), were investigated. Single photon emission computed tomography (SPECT) imaging was used to noninvasively monitor the *in vivo* behaviors. Diethylenetriaminepentaacetic acid (DTPA) was conjugated to these polymers, which were labeled with radioactive ^{111}In. These ^{111}In-DTPA-bearing polymers (Poly-Glu–DTPA, Ac-den–DTPA and CP-den–DTPA) and unconjugated DTPA were subcutaneously injected into tumor-bearing mice, which were subjected to SPECT imaging. These ^{111}In-DTPA-bearing polymers were largely retained at the injection site for at least 1 day, whereas the unconjugated DTPA was rapidly cleared from the whole body through excretion. Poly-Glu–DTPA and Ac-den–DTPA were partly accumulated in the kidney (and the liver), but the CP-den–DTPA was not. However, these ^{111}In-DTPA-bearing polymers were accumulated in the liver and the kidney following intravenous administration. These results indicate that the subcutaneously injected polymers did not largely gain substantial access to the systemic circulation, which is useful for a depot of drug around the injection site.

Drug delivery systems (DDSs) represent an emerging and interesting technology for the delivery of drug molecules to a specific target site of interest. This technology is particularly important in cancer chemotherapy because of the severeside effects generally associated with anticancer drugs as a consequence of their off-target effects. Polymeric drug carriers have been identified as an attractive solution in this context, because the cellular internalization and the biodistribution can be readily controlled. Many kinds of polymers have been applied to delivery of anticancer drug such as doxorubicin (Dox) and paclitaxel (Haag and Kratz, 2006; Larson and Ghandehari, 2012; Vicent and Duncan, 2006; Fox et al., 2009; Gaspar and Duncan, 2009). These polymers have been administered in different ways, including intravenous, intraperitoneal and subcutaneous injections (Gaspar and Duncan, 2009). Although lots of reports have appeared in the literature concerning the biodistribution of intravenously injected polymers (Vicent and Duncan, 2006; Fox et al., 2009; Gaspar and Duncan, 2009), detailed *in vivo* studies of subcutaneously injected polymers have been conducted for only three different types of synthetic polymer to date, including polyethylene glycols, polyvinyl alcohols and polylactides (Yamaoka et al., 1995; Bridges et al., 2000). Further studies for the design of suitable polymeric drug carriers for subcutaneous administration are indispensable. Subcutaneously injected polymers penetrate into tissue and subsequently enter the systemic circulation (Yamaoka et al., 1995; Bridges et al., 2000), which is possibly dependent in the molecular weight and backbone of the polymer.

A wide range of imaging technologies have been developed to allow for the noninvasive *in vivo* monitoring, including fluorescence imaging and nuclear medicine imaging such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) (Shimon and David, 2006; O'Farrell et al., 2013; Kobayashi et al., 2011). Schadlich et al. reported the synthesis of a fluorescent dye-conjugated polyvinyl alcohol. It was subcutaneously injected into mice and monitored by fluorescence imaging (Schadlich et al., 2011). One of the limitations of fluorescence imaging, however, is the insufficient penetration of light into tissue. It can therefore be difficult to analyze fluorescent materials in a quantitative manner at the whole-body level. Nuclear medicine imaging (PET and SPECT) is applicable to whole-body quantitative analysis (Shimon and David, 2006; O'Farrell et al.,

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http://dx.doi.org/10.1016/j.ijpharm.2014.09.053
0378-5173/

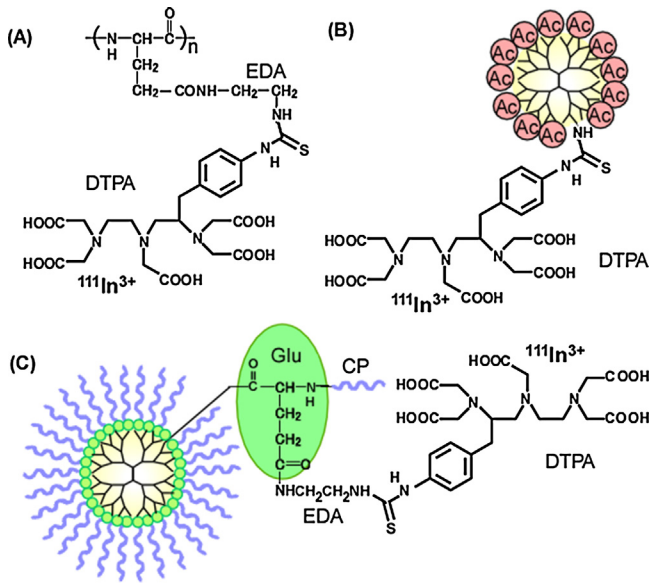

Fig. 1. Polymer imaging agents. (A) ^{111}In-labeded poly-Glu with DTPA (poly-Glu–DTPA), (B) ^{111}In-labeded acetylated (Ac) dendrimer with DTPA (Ac-den–DTPA) and

2013; Kobayashi et al., 2011). Because SPECT radionuclides generally have longer half-lives than PET ones, SPECT imaging is suitable for monitoring the long-term *in vivo* behaviors of subcutaneously injected polymers.

In the current study, SPECT imaging has been used to monitor the *in vivo* behaviors of subcutaneously injected polymers. Poly (glutamic acid) (poly-Glu), an acetylated polyamidoamine (PAMAM) dendrimer (Ac-den) and a collagen peptide-conjugated dendrimer (CP-den) were used. Dendrimers are highly branched macromolecules, whose structure is different from linear polymers. And, it was reported that CP-den is a collagen-mimetic (Suehiro et al., 2010; Kojima et al., 2011). The radiolabeled polymer agents were prepared by conjugating diethylenetriaminepentaacetic acid (DTPA), followed by the chelation of these polymers with radioactive $^{111}In^{3+}$ (Fig. 1). These were injected subcutaneously into mice, and their *in vivo* behaviors were monitored using SPECT and CT imaging. DTPA was injected subcutaneously as a control. Additionally, these radioactive polymers were injected intravenously into mice to compare the *in vivo* behaviors.

DTPA-conjugated polymers (poly-Glu–DTPA, Ac-den–DTPA and CP-den–DTPA) were synthesized from CP (2 kDa)-conjugated PAMAM dendrimer (G4), PAMAM dendrimer (G4) and poly-Glu (7.5 kDa), and labeled with radioactive 111-indium, according to our previously reported methods with modifications (Kojima et al., 2010, 2013a,b). Details of these procedures have been provided in the supplementary information. The cytotoxicities of the polymer agents with cold indium ions towards human breast cancer cells (MDA-MB-231 cells) were determined, as previously described in the literature (Kojima et al., 2013a,b,b). The SPECT and CT imaging of the polymers following their subcutaneous and intravenous injection into tumor-bearing mice was performed, as described in the supplementary information. All of experimental procedures involving the animals were carried out in accordance with the policies of the Animal Care and Use Committee of the Hamamatsu University School of Medicine (Shizuoka, Japan) and were approved by the local Animal Care and Use Committee.

A poly-Glu and CP-dens bearing Dox *via* a hydrazone bond (poly-Glu–Dox and CP-den–Dox) were previously synthesized (Kojima et al., 2013a,b,b). In the current study, the pH-degradable hydrazone-linked Dox was replaced with non-degradable ethylenediamine (EDA)-linked DTPA. As a control, Ac-den–DTPA was also synthesized. *p*-Isothiocyanate benzyl–DTPA (*p*-SCN-Bn–DTPA) was conjugated to the amine termini of the polymers, and the resulting compounds were characterized by UV–vis spectrometry. *p*-SCN-Bn–DTPA gave an absorption peak at 270 nm, which appeared following the conjugation of the chelate to the polymers. The binding number of the DTPA was estimated from the standard curve for *p*-SCN-Bn–DTPA at 270 nm (supplementary information). A summary of these polymer agents is shown in Table 1. The molecular weight of the CP-den–DTPA was larger than that of the poly-Glu–DTPA and Ac-den–DTPA. The cytotoxicities of the polymer agents were determined in MDA-MB-231 cells prior to their administration into mice (Table 1). These polymers did not exhibit any significant cytotoxicity.

Indium-111, a radioactive isotope of indium, was chelated to poly-Glu–DTPA, Ac-den–DTPA and CP-den–DTPA. Resulting polymer agents were injected subcutaneously into the area surrounding tumor tissues in tumor-bearing mice. DTPA was injected as a control (Fig. 2). Poly-Glu–DTPA was observed primarily at the injection site, though radioactivity in the kidney and the liver increased slightly after 12 h. CP-den–DTPA was observed at the injection site after 1 day, but not in other tissues. Even after 4 days, the localization of CP-den–DTPA was unchanged (supplementary information). In contrast, Ac-den–DTPA was observed at the injection site after 30 min, but it gradually decreased. Instead, the radioactivity in the kidney increased. The radioactivity of DTPA decreased rapidly at the injection site and increased in the bladder even after 30 min. Radioactivity almost disappeared from the whole body after 8 h. These observations suggested that agents of molecular weight >38 kDa were largely retained at the injection site for at least 1 day.

These polymer agents were also injected intravenously into tumor-bearing mice (Fig. 3). Poly-Glu–DTPA was detected primarily in the kidney and the bladder after 30 min. Small amounts of Poly-Glu–DTPA accumulated gradually in the liver. Ac-den–DTPA was detected primarily in the kidney and the bladder after 30 min, and was excluded from the body. In contrast, CP-den–DTPA accumulated in the liver. The *in vivo* behaviors of intravenously injected polymers were considerably different from those injected subcutaneously. It has been reported that intravenously injected

Table 1
Summary of the SPECT imaging agents used in this study.

Sample	Reactive group	Bound DTPA[c]	Molecular weight[d]	Cytotoxicity[e]
Poly-Glu–DTPA	50[b]	54	38 kDa	73% ± 13%
Ac-den–DTPA	64	20	27 kDa	82% ± 6%
CP-den–DTPA[a]	64	23	151 kDa	83% ± 4%
DTPA	–	1	0.393 kDa	NA

[a] Reactivity of collagen peptide to the dendrimer was 89%.
[b] Polymerization degree.
[c] Estimated from UV–vis spectrometry.
[d] Calculated.
[e] Polymer solutions (10 μM) were exposed to cells for 72 h.