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Influence of dendrimer generation and polyethylene glycol length on the biodistribution of PEGylated dendrimers

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ABSTRACT

Dendrimers are a potential drug carrier. Because modification with polyethylene glycol (PEG) is known to improve the blood retention, PEGylated dendrimers have been studied as a useful drug carrier. In this study, three types of PEGylated L-lysine-bearing polyamidoamine dendrimers (PEG2k-Lys-PAMAM (G4), PEG5k-Lys-PAMAM (G4), PEG2k-Lys-PAMAM (G5)) were synthesized, which are composed of a dendrimer of different generations (generations 4 and 5) and PEG chains with different molecular weights (2k and 5k). An acetylated L-lysine-bearing dendrimer was also synthesized as a non-PEGylated dendrimer. Bifunctional diethylenetriaminepentaacetic acid (pSCN-benzyl-DTPA) was bound to the epsilon -amino group of lysine in a dendrimer, to be labeled with radioactive indium-111. These PEGylayed dendrimers showed longer blood retention and lower accumulation in other normal organs such as the kidneys than the non-PEGylated dendrimer. The PEGylated dendrimers with the higher generation and the longer PEG led the greater blood retention.

Dendrimers are unique polymers, which have monodispersed molecular weight, well-defined morphology and interior to hold drug molecules. Many researchers have investigated to apply dendrimers to drug delivery. PEGylation is effective for drug delivery because of the escape from the recognition by the reticuloendothelial system (RES). (Lee et al., 2005; D'Emanuele and Attwood, 2005; Gajbhiye et al., 2007; Wolinsky and Grinstaff, 2008). Different types of PEGylated dendrimers using polyamidoamine (PAMAM) dendrimers, poly(lysine) dendrimers, polyester dendrimers, polyol dendrimers, and triazine dendrimers have already reported (Gajbhiye et al., 2007; Wolinsky and Grinstaff, 2008; Kim et al., 2008; Singh et al., 2008; Guillaudeu et al., 2008; Kaminskas et al., 2008; Okuda et al., 2006a,b; Lim et al., 2008; Kobayashi et al., 2001a). PAMAM dendrimer is one of the most classical dendrimers (Sovenson and Tomalia, 2005). PEG-modified PAMAM dendrimers were synthesized as a drug carrier by a number of groups (Gajbhiye et al., 2007; Kim et al., 2008; Singh et al., 2008; Kojima et al., 2000). Although the biodistribution of the PEGylated PAMAM dendrimers was reported (Gajbhiye et al., 2007; Wolinsky and Grinstaff, 2008; Kobayashi et al., 2001a), the systematical analysis of the biodistribution of the PEGylated PAMAM dendrimers with different generations and different PEG lengths has not been investigated much.

In this study, we investigated the comprehensive influence of the generation of the PAMAM dendrimer and the molecular weight of PEG in PEGylated PAMAM dendrimers on the biodistribution. We have synthesized PEG-modified PAMAM dendrimers at virtually every terminal amino group, which maintain the monodispersity of the dendrimer platform. These dendrimers could encapsulate various materials such as anticancer drugs and photosensitizers (Kojima et al., 2000, 2007). Additionally, the PEGylated PAMAM dendrimers having glutamic acid were also synthesized, to covalently attach drug molecules (Kono et al., 2008). In this study, various PEGylated PAMAM dendrimers having lysine residues were synthesized using PAMAM dendrimers of generation 4 and 5 and PEG with molecular weight of 2k and 5k (PEG2k-Lys-PAMAM (G4), PEG5k-Lys-PAMAM (G4) and PEG2k-Lys-PAMAM (G5)). As a non-PEGylated dendrimers, acetylated lysine-dendrimer (Ac-Lys-PAMAM) was also prepared. These ε-amino groups of lysine were reacted with a bifunctional 2-(4-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid (pSCN-benzyl-DTPA), which keeps their symmetrical structure, to be labeled with radioactive indium-111 (Fig. 1). The biodistribution of these ^{111}In-labeled dendrimers in mice was analyzed and compared.

PEG-Lys-PAMAM dendrimer (PEG2k-Lys-PAMAM (G4)), using ethylenediamine core PAMAM dendrimer of generation 4 (Sigma–Aldrich Corp. (St. Louis, MO)) and PEG of molecular weight 2k (Sigma–Aldrich Corp.), was already synthesized as described in our previous report (Haba et al., 2005). PEG5k-Lys-PAMAM

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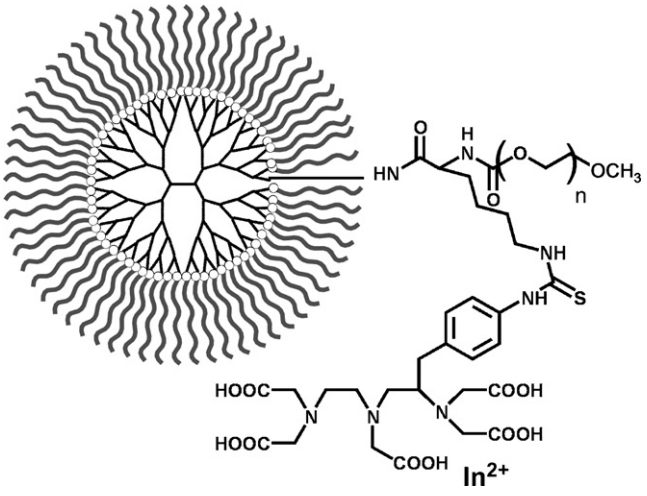

Fig. 1. Structure of PEGylated dendrimers with indium–DTPA.

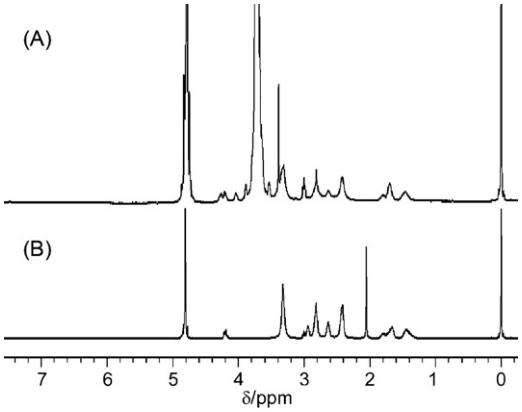

Fig. 2. ^{1}H NMR spectra of (A) PEG2k-Lys-PAMAM (G5) and (B) Ac-Lys-PAMAM (G4). The spectra of PEG5k-Lys-PAMAM (G4) and PEG2k-Lys-PAMAM (G4) were similar to (A).

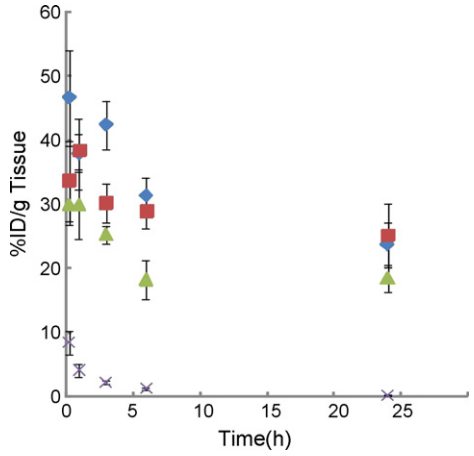

Fig. 3. Plasma concentrations of PEG2k-Lys-PAMAM (G5) (diamond), PEG5k-Lys-PAMAM (G4) (square), PEG2k-Lys-PAMAM (G4) (triangle) and Ac-Lys-PAMAM (G4) (cross).

(G4) and PEG2k-Lys-PAMAM (G5) were also prepared by the same procedure except the starting materials. Acetylated lysine (Ac-Lys)-attached dendrimer was also prepared. First, acetylation of Lys(Z) (Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan)) was performed according to our previous report (Kojima et al., 2009). The modification of PAMAM dendrimer (G4) with Ac-Lys(Z) and the deprotection of Z group were performed, as described in our previous report (Haba et al., 2005). Chelate conjugation and radiolabel were performed, as previously described (Kobayashi et al., 2001a,b).

All animal experiment procedures were carried out in compliance with the Guide for the Care and Use of Laboratory Animal Resources (1996), National Research Council, and approved by the local Animal Care and Use Committee. 6–8-Week-old female athymic nude mice weighing 16–21 g (National Cancer Institute, Frederick, MD) were injected intravenously with approximately 1.5 μCi of ^{111}In-labeled dendrimers (about 2 μg in 200 μl of phosphate buffer saline (PBS)). After 0.25, 1, 3, 6 and 24 h, mice were sacrificed via CO_2 inhalation, and blood and major organs were harvested, wet-weighed, and counted in a γ-scintillation counter ($n = 5$ per time point). All data were decay corrected.

We synthesized three kinds of PEGylated dendrimers, PEG2k-Lys-PAMAM (G5), PEG5k-Lys-PAMAM (G4) and PEG2k-Lys-PAMAM (G4). First, Boc-Lys(Z) (Peptide Institute (Osaka, Japan)) was reacted with PAMAM dendrimer of generation 4 or 5, followed by the deprotection of Boc group. Then, the amino reactive PEG monomethyl ether (PEG monomethyl ether 4-nitrophenyl carbonate), which was synthesized by reacting PEG monomethyl ether with 4-nitrophenyl chloroformate (Kojima et al., 2000), was attached to dendrimer (Haba et al., 2005). The typical ^{1}H NMR spectrum was shown in Fig. 2(A). From ^{1}H NMR spectra, it was shown that essentially all amino groups of the PAMAM dendrimer were modified with Lys and PEG, as listed in Table 1. Ac-Lys-PAMAM (G4) was also synthesized as a control. The binding numbers of Lys to the dendrimer were also estimated from ^{1}H NMR (Fig. 2(B)). Again, essentially all amino groups of the dendrimer were modified with Lys.

To label these dendrimers with radioactive indium, bifunctional DTPA was conjugated to the dendrimer. DTPA with p-isothiocyanatobenzyl group was attached to the ε-amino group of lysine in these four kinds of dendrimers after the deprotection of Z group (Table 1). Single peaks were observed in gel permeation chromatography (GPC) profiles of these DTPA-conjugated dendrimers (data not shown). The molecular weights of these den-

Table 1
Three types of PEGylated dendrimers and non-PEGylated dendrimer.

Dendrimer	PEG molecular weight (Da)	Dendrimer Generation	Terminal group	Bound number			Molecular weight (kDa) with/without DTPA[d]
				Lys[a]	PEG[b]	DTPA[c] (reactivity)	
PEG2k-Lys-PAMAM (G5)	2k	G5	128	115	110	35 (27%)	284/262
PEG5k-Lys-PAMAM (G4)	5k	G4	64	64	60	19 (30%)	334/321
PEG2k-Lys-PAMAM (G4)	2k	G4	64	64	63	22 (34%)	162/147
Ac-Lys-PAMAM (G4)	–	G4	64	64	-	17 (26%)	36/25

[a] Estimated from the ^{1}H NMR spectra of Boc-Lys(Z)- or Ac-Lys(Z)-bearing dendrimers.
[b] Estimated from the ^{1}H NMR spectra of PEG-Lys(Z)-bearing dendrimers.
[c] Estimated from the UV absorption at 262 nm of PEG-Lys(DTPA)- or Ac-Lys(DTPA)-bearing dendrimers ($\varepsilon = 1.6 \times 10^{4}$ M^{-1} cm^{-1}).
[d] Calculated from the molecular weights of PEG-Lys-bearing and Ac-Lys-bearing dendrimers. The binding numbers of PEG and Lys in this table were used. The molecular weight of urethane moiety is included in that of PEG.

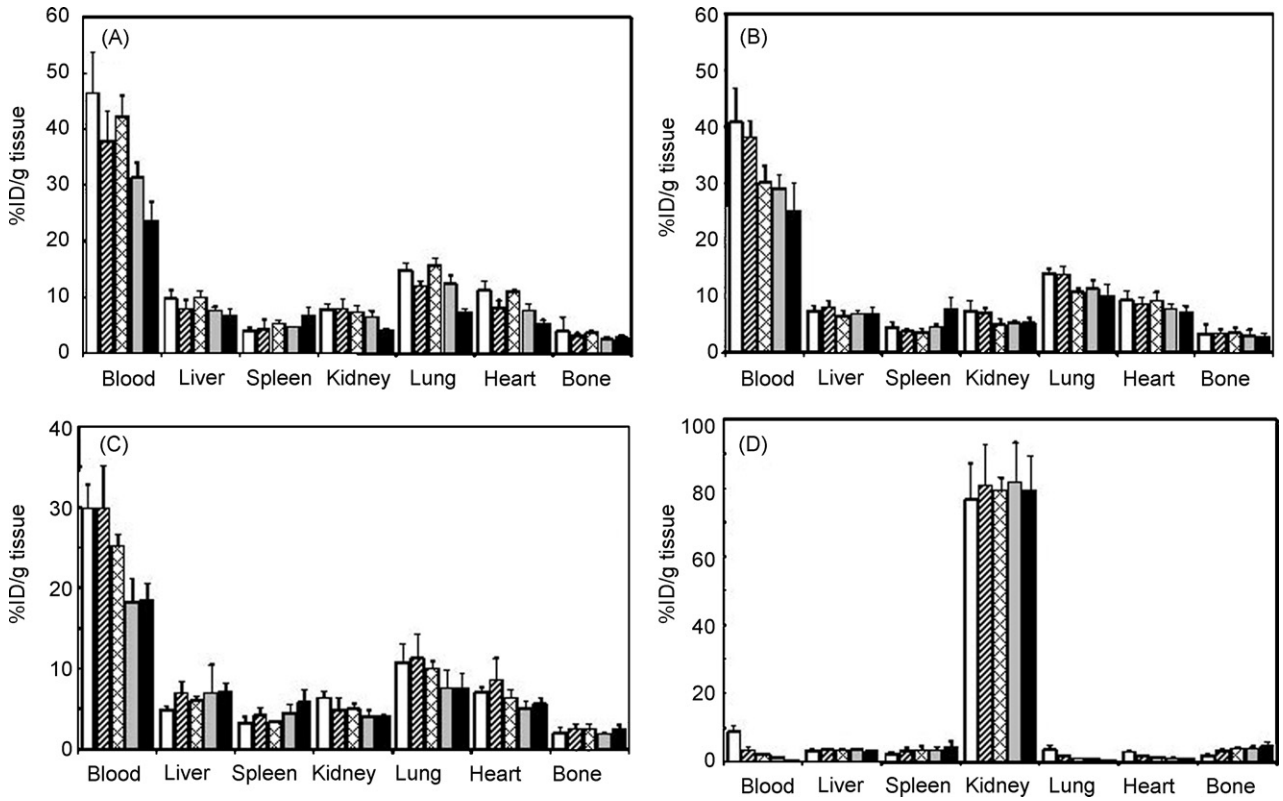

Fig. 4. Biodistributions of (A) PEG2k-Lys-PAMAM (G5), (B) PEG5k-Lys-PAMAM (G4), (C) PEG2k-Lys-PAMAM (G4) and (D) Ac-Lys-PAMAM (G4). The %ID/g tissue after 0.25 h (white bars), 1 h (hatched bars), 3 h (cross-hatched bars), 6 h (grey bars) and 24 h (black bars) is shown.

drimers were also listed in Table 1. Those of PEG2k-Lys-PAMAM (G5) and PEG5k-Lys-PAMAM (G4) are similar, which are larger than PEG2k-Lys-PAMAM (G4). Ac-Lys-PAMAM (G4) is much smaller than the PEGylated dendrimers.

Four kinds of chelate-conjugated dendrimers were radiolabeled with ^{111}In. To remove any non-incorporated free indium, reaction mixtures were purified by ultrafiltration. All radiolabeled dendrimers were analyzed by HPLC, in which radiochemical purity was found to be more than 97%. These radiolabeled dendrimers were injected into the tail vein of mice. The plasma concentrations of these dendrimers are shown in Fig. 3. In the case of Ac-Lys-PAMAM (G4), only less than 10% ID/g was remained after 15 min and almost all cleared after 24 h. In contrast, PEGylated dendrimers showed higher retention in blood. PEG2k-Lys-PAMAM (G5) and PEG5k-Lys-PAMAM (G4) exhibited longer blood circulation than PEG2k-Lys-PAMAM (G4). The molecular weights of PEG2k-Lys-PAMAM (G5) and PEG5k-Lys-PAMAM (G4) are about 300 kDa, which are around twice that of PEG2k-Lys-PAMAM (G4). Therefore, this might due to the higher molecular weight of these dendrimers. The biodistribution of these dendrimers are shown in Fig. 4. The acetylated dendrimer was largely observed in kidney. On the other hand, PEGylated dendrimers were mostly detected in blood even after 24 h. Only small amounts of dendrimers were in other tissues as far as we investigated. Because the whole body radioactivities of PEGylated and non-PEGylated dendrimers were almost unchanged after 24 h, the excretion of these dendrimers may be negligible in our experiments.

This PEG effect was consistent with the previous reports of some dendrimers such as PAMAM dendrimers, poly-L-lysine-dendrimers and triazine dendrimers (Guillaudeu et al., 2008; Kaminskas et al., 2008; Okuda et al., 2006a,b; Lim et al., 2008; Kobayashi et al., 2001a). Our experiments show that PEG2k-Lys-PAMAM (G5) and PEG5k-Lys-PAMAM (G4) exhibited longer blood circulation than PEG2k-Lys-PAMAM (G4). This may be due to the higher molecular weight of the PEGylated dendrimers. Although the PAMAM dendrimer of G9 is relatively similar to PEG2k-Lys-PAMAM (G5) and PEG2k-Lys-PAMAM (G5) in molecular weight, the PAMAM dendrimer (G9) were rapidly cleared from blood (Kobayashi et al., 2001b). This suggests that our PEGylated dendrimers have a long circulation property not only due to the almost completely PEGylated surface but also due to the high molecular weight.

In conclusion, we have synthesized and characterized PEGylated lysine-bearing PAMAM dendrimers with different generations of dendrimer and PEG lengths. The chelate agent, DTPA, was conjugated to the side chain of lysine, and labeled with radioactive indium. The biodistribution of PEGylated dendrimers were very different from the non-PEGylated dendrimer. PEGylation induced the long blood circulation and avoided the accumulation in normal organs including the kidneys and the liver. It has been reported that nanoparticles with long blood circulation can accumulate at the tumor tissues by EPR effects (Lee et al., 2005; Gajbhiye et al., 2007; Wolinsky and Grinstaff, 2008; Greenwald et al., 2000; Guillaudeu et al., 2008; Okuda et al., 2006a; Lim et al., 2008). Therefore, the PEGylated dendrimers can be a potential drug carrier for cancer chemotherapy.

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