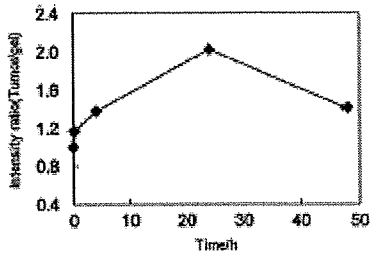

Fig. 7. Relative signal intensities of tumor area at defined time (0 h, 4 h, 24 h, 48 h) after the injection of the polymeric micelle MRI contrast agent.

carrier may not penetrate into muscle as do low-"molecular weight" drugs that are released from the carrier.

Wang Y et al. reported that poly[N-(2-hydroxypropyl)methacrylamide] (PHPMA) [14] gadolinium-conjugates exhibited size-dependent tumor accumulation. They stated that a large molecular weight of PHPMA (121 kDa) gadolinium conjugate exhibited the best tumor accumulation at 7 days after injection. Although, Bogdanov A et al. reported another example of successful passive targeting to solid tumors with a graft copolymer of poly(ethylene glycol) featuring poly(L-lysine) [9]. This contrast agent exhibited tumor targeting with a long blood-circulation time ($t_{1/2}$ = 36 h); however, this long-circulation property in blood indicates that the contrast agent cannot excrete smoothly from the body owing to the polymer's very large molecular weight (690 kDa). The researchers synthesized different molecular weights of similar polymers to compare the polymers' biodistribution [10] and found that the polymers accumulated at solid tumors in a "molecular-weight"-dependent manner. This molecular-weight dependency indicated that smaller molecular weights of polymers can be excreted through the kidneys.

These above-mentioned polymers exhibited better tumor accumulation, corresponded to larger molecular weights of the polymers. However, the excretion of the contrast agent, especially in the case of the macromolecular contrast agent, is a serious matter for the development of diagnostic agents.

Therefore, we checked the kidneys' excretion of our polymeric micelle contrast agent. In urine, 20.8 ± 7.8% of the polymeric micelle was found 48 h after the injection. This result indicates that the polymeric micelle was excreted through the kidney filtration. Since the size of the polymeric micelle contrast agent was 50–250 nm as shown in Fig. 3(a), the polymeric micelles cannot pass through the kidney filtration. Therefore, these polymers that formed in urine appears to have passed through the kidney filtration in a dissociated polymer form, since the average molecular weight of this block copolymer is only 15,000. This is an excellent property of the polymeric micelle MRI contrast agent; namely, this agent exhibits long circulation in blood in a micelle form, while this agent can be excreted through the kidneys in a dissociated polymer form.

Furthermore, the obtained polymeric micelle MRI contrast agent delivered a larger amount to solid tumors than did previously reported macromolecular MRI contrast agents that can be also excreted from the kidneys (agents such as PHPMA gadolinium-conjugate [14] and graft copolymer of poly(ethylene glycol) with poly (L-lysine) [10]).

In order to estimate possible acute toxicity, we injected the 4-fold of the volume of the contrast agent into the mouse tail vein, and observed the body weight change over the course of 16 days. There was no significant difference in comparison to the control (less than ± 10%). Although we have to conduct further experiments to obtain toxicity-related information of greater exactness, these preliminary results indicate that this polymeric micelle can dissociate and be excreted from the kidneys, and that this tumor targeting results a passive targeting mechanism (the EPR effect). In a future study, we would like to optimize the pharmacokinetics and the dissociation behavior of the polymeric micelles by controlling the composition of the block copolymers.

3.4. MR imaging at tumor tissue

We took an MR image of the tumor-bearing mouse after the injection of the polymeric micelle contrast agent. Fig. 6 shows T_1-weighted MR images of tumor tissues before and after 24 h at an injection dose of 0.05 mmol Gd/kg. After the injection of the polymeric micelle, MR images exhibited a significant signal enhancement at the kidneys. This signal enhancement at the kidneys indicates that kidneys excreted the contrast agent, as shown in Fig. 6(c). However, even 24 h after the injection, an intense signal was observed in the heart and aorta areas. This indicates that a considerable amount of the contrast agent was circulating in the bloodstream, as described in pharmacokinetic results. The relative signal intensity at axial slices of the tumor tissues underwent a 2.0-fold increase after 24 h, as compared with the signal before the injection. The signal intensity of the tumor area had gradually increased by 24 h and had slightly decreased by 48 h, as shown in Fig. 7. This behavior of the signal intensities is similar to the doxorubicin concentration delivered by the polymeric micelle carrier system. All these results indicate that the enhancement of MR signals in the tumor area rested on the successful passive accumulation of the MRI contrast agent at solid tumors.

4. Conclusion

We prepared polymeric micelle MRI contrast agents using poly (ethylene glycol)-*b*-poly(L-lysine) block copolymers. A reaction of poly(ethylene glycol)-*b*-poly(L-lysine) with a DOTA derivative resulted in a quantitative DOTA conjugation regarding the lysine residues of the block copolymer, and the obtained block copolymer formed a polymeric micelle. This micellar structure was maintained after a partial chelation of the DOTA moiety with gadolinium ions. The biodistribution and the excretion of the polymeric micelle was evaluated in colon 26-bearing CDF_1 female mice. Selective accumulation of the polymeric micelle at the tumor tissues was observed 24 h after the injection. The contrast agent's accumulation substantially enhanced the signal intensity of the MR images at the tumor. This polymeric micelle MRI contrast agent will be a useful diagnostic tool, particularly in combination with a polymeric micelle-based drug-targeting system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jconrel.2009.01.010.

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