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International Journal of Pharmaceutics 357 (2008) 318–322
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Note

Block copolymer design for stable encapsulation of *N*-(4-hydroxyphenyl)retinamide into polymeric micelles in mice

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Received 5 October 2007; received in revised form 22 January 2008; accepted 24 January 2008
Available online 3 February 2008

Abstract

For stable encapsulation of *N*-(4-hydroxyphenyl)retinamide (4-HPR) into polymeric micelles, four types of block copolymers were synthesized with different esterified functional groups: heptyl (C7), nonyl (C9), benzyl (Bz), and phenylpropyl (C3Ph). The stability of 4-HPR encapsulated polymeric micelles was evaluated by measuring the blood concentration of 4-HPR in mice. After intravenous administration of 4-HPR and 4-HPR encapsulated PEG liposomes, the blood concentration of 4-HPR was about 2.8% and 2.2% of the dose/mL, suggesting the rapid release of 4-HPR from PEG liposomes. In contrast, the blood concentration of 4-HPR after intravenous administration of all 4-HPR encapsulated polymeric micelles studied was much higher (about 22–34% of the dose/mL). Among them, the polymeric micelles prepared by block copolymers (Bz) showed the highest blood concentration of 4-HPR. As far as the effects of the level of Bz groups in the block copolymers are concerned, the blood concentration of 4-HPR was enhanced by Bz groups at a level of 72% and 77%, but not by Bz groups at a level of 43% and 51%. These results suggest that 4-HPR is stably encapsulated in polymeric micelles prepared by block copolymers (Bz) but a level of over 72% of Bz groups is needed. These findings will be of value in the future use, design, and development of polymeric micelles for *in vivo* application of 4-HPR.

Keywords: 4-HPR; Fenretinide; Polymeric micelle; Controlled release; Drug delivery systems

N-(4-Hydroxyphenyl)retinamide (4-HPR, fenretinide) is a synthetic retinoid which shows high anti-tumor activity against a variety of malignant cells (Formelli et al., 1996). Although oral administration of 4-HPR has been used in clinical trials so far, its bioavailability is very limited because of its low membrane permeability (Kokate et al., 2006). In addition, intravenous 4-HPR is rapidly eliminated from body (Swanson et al., 1980; Hultin et al., 1986). Therefore, 4-HPR cannot exert a high enough anti-tumor activity because its low blood concentration (Formelli et al., 1993). Raffaghello et al. and Takahashi et al. have reported that 4-HPR encapsulated liposomes containing monoclonal antibody or sterylglucoside mixture exert anti-tumor activities when given intravenously. Therefore, the development of a targeting carrier for 4-HPR is needed in order to obtain potent *in vivo* anti-tumor activity (Raffaghello et al., 2003; Takahashi et al., 2003).

Polymeric micelles prepared by block copolymers, which are composed of both hydrophilic and hydrophobic segments, have been reported to be suitable drug carriers for lipophilic drugs (Kataoka et al., 2001; Gaucher et al., 2005). Recently, we have reported the efficient encapsulation of hydrophobic drugs in polymeric micelles by optimizing the hydrophobic segments with esterified functional groups of poly(ethylene glycol)–poly(aspartate ester) block copolymers (Yokoyama et al., 2004; Kawakami et al., 2005; Watanabe et al., 2006; Chansri et al., 2008). These observations prompted us to investigate the potential use of polymeric micelle formulations by optimizing the hydrophobic segments with esterified functional groups to enhance the blood retention of 4-HPR following intravenous administration. Here, four types of poly(ethylene glycol)–poly(aspartate ester) block copolymers with different esterified functional groups were synthesized to optimize the

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doi:10.1016/j.ijpharm.2008.01.044

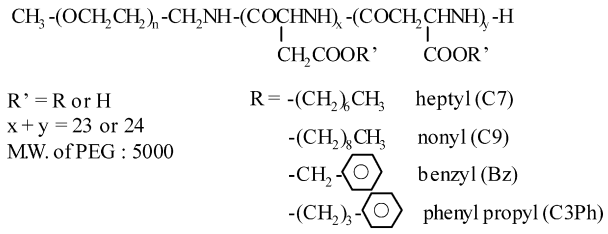

Fig. 1. Schematic illustration of synthesized block copolymers.

stable encapsulation of 4-HPR in the inner core of polymeric micelles (Fig. 1).

Since the *in vivo* drug release from particulate carriers is much higher than that from *in vitro* drug release (Takino et al., 1993; Shabbits et al., 2002), *in vivo* evaluation is important for the development of polymeric micelles for 4-HPR encapsulation. Therefore, the blood concentration of 4-HPR following intravenous administration of 4-HPR encapsulated polymeric micelles into the tail vein of mice was measured using HPLC. Poly(ethylene glycol)–poly(aspartate ester) block copolymers were obtained by an esterification reaction between a bromide compound and the aspartic acid residue of poly(ethylene glycol)–poly(aspartic acid) block copolymers (Yokoyama et al., 2004). Block copolymers with heptyl, nonyl, benzyl and phenyl propyl groups are abbreviated as C7, C9, Bz and C3Ph, respectively. The esterification level determined by ^{1}H NMR is expressed as a % value following the block copolymer abbreviation. 4-HPR encapsulated polymeric micelles were prepared by a conventional evaporation method modified as described in our previous report (Kawakami et al., 2005). The ratio of block copolymers/4-HPR for their preparation was fixed as 2.5 (weight ratio). As a control, poly(ethylene glycol) modified liposomes (PEG liposomes) were selected to evaluate the potentials of novel polymeric micelle formulations because of their wide use in cancer chemotherapy (Torchilin, 2005).

The physicochemical properties, such as the particle size and zeta potential of the macromolecules, are determining factors for their biodistribution (Takakura and Hashida, 1996). For escape

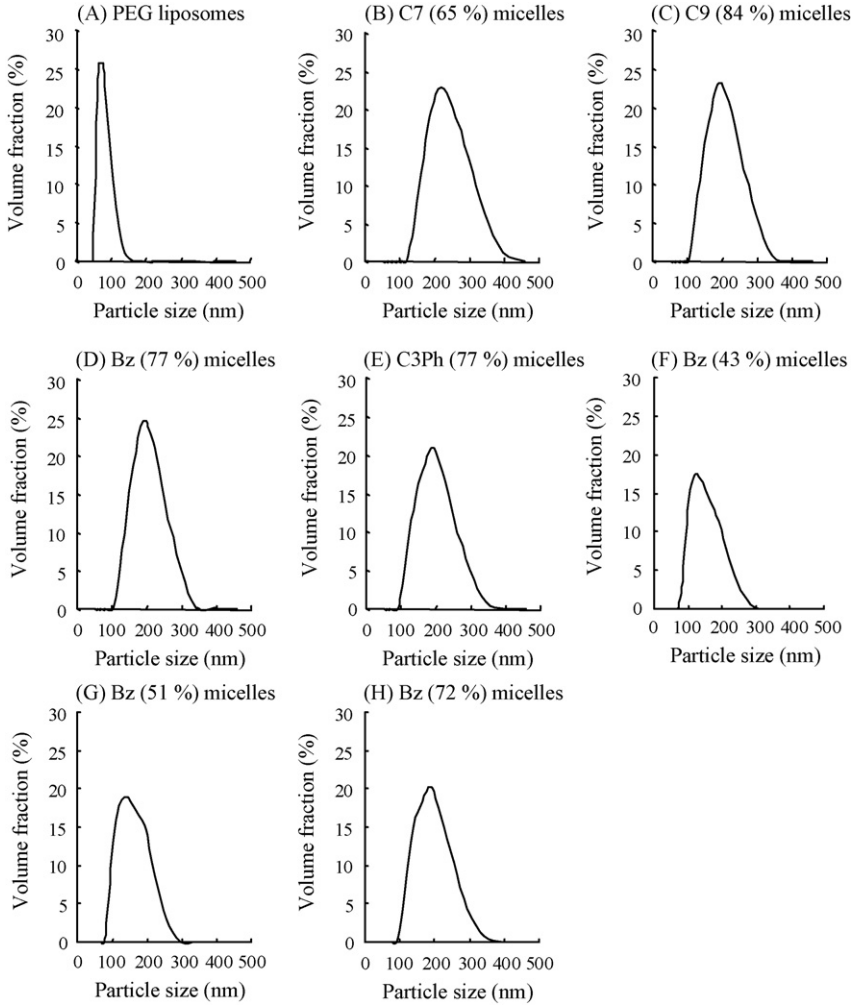

Fig. 2. Size distribution of 4-HPR encapsulated PEG liposomes (A) and polymeric micelles with several types and levels of esterified functional groups (B–H). 4-HPR encapsulated PEG liposomes were composed of hydrogenated soybean phosphatidyl choline (HSPC), cholesterol, distearoylphosphatidylethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (PEG-DSPE), and 4-HPR (33.3:16.7:1.67:1, molar ratio). The ratio of block copolymers/4-HPR for 4-HPR encapsulated polymeric micelles was fixed as 2.5 (weight ratio). They were prepared by a conventional evaporation method modified as described in our previous report (Kawakami et al., 2005).

Table 1
Zeta potential of 4-HPR encapsulated PEG liposomes (A) and polymeric micelles with several types and levels of esterified functional groups (B–H)

Carrier	Zeta potential (mV)
(A) PEG liposomes	−2.7 ± 1.4
(B) C7 (65%) micelles	−1.7 ± 0.7
(C) C9 (84%) micelles	−2.6 ± 1.0
(D) Bz (77%) micelles	−1.4 ± 1.2
(E) C3Ph (77%) micelles	−0.8 ± 0.8
(F) Bz (43%) micelles	−10.7 ± 1.8
(G)Bz (51%) micelles	−3.1 ± 0.9
(H) Bz (72%) micelles	−2.3 ± 3.2

Each value represents the means ± S.D. (n = 3–4).

of uptake by the reticuloendothelial system and long-term blood retention in the systemic circulation, it is needed that the particle size and zeta potential of PEG modified particulates (liposomes and polymeric micelles) are about <200 nm and weak anion (Oku and Namba, 1994; Nishiyama et al., 2003). Therefore, the mean particle size and zeta potential of 4-HPR encapsulated polymeric micelles were measured using Zetasizer Nano Series (Malven Instruments Ltd., Worcestershire, UK). As shown in Fig. 2 and Table 1, the mean particle size and zeta potential of all 4-HPR encapsulated polymeric micelles ranged from 142 to 225 nm and from −10.7 to −0.8 mV respectively, not dramatically changed irrespective of types and levels of esterified functional groups. The mean particle size and zeta potential of 4-HPR encapsulated PEG liposomes were about 76 nm and −2.7 mV. Thus, 4-HPR encapsulated polymeric micelles and PEG liposomes can avoid uptake by the reticuloendothelial system and enhance the blood retention of 4-HPR after intravenous administration.

After intravenous administration of 4-HPR itself (dissolved in polyoxyethylene hydrogenated castor oil (HCO-60), which was a solubilizing agent) and 4-HPR encapsulated PEG liposomes at 1 h, the blood concentration of 4-HPR was about 2.8% and 2.2% of the dose/mL (Fig. 3), suggesting the rapid release of 4-HPR from PEG liposomes. From the reports of Swanson et al. (1980) and Hultin et al. (1986), the blood concentration of 4-HPR at 1 h after intravenous injection was calculated to be about 3% of the dose/mL, supporting our result. In contrast, the blood concentration of 4-HPR was about 22–34% of the dose/mL after intravenous administration of 4-HPR encapsulated polymeric micelles, suggesting the stable encapsulation of 4-HPR by these types of polymeric micelles. Among them, the polymeric micelles prepared by block copolymers (Bz (77%)) exhibited the highest blood concentration of 4-HPR. Recently, Kataoka et al. reported that doxorubicin can be stably encapsulated in polymeric micelles prepared by poly(ethylene glycol)–poly(β-benzyl-L-aspartate) block copolymers through π–π stacking (Kataoka et al., 2000). Therefore, 4-HPR, which possesses a benzene ring, might be also stably encapsulated in polymeric micelles prepared by block copolymers (Bz (77%)) through this π–π stacking. As far as the effects of the level of Bz groups in the block copolymers are concerned, the blood concentration of 4-HPR was enhanced by Bz groups at a level of 72% and 77%, but not by Bz groups at a level of 43% and 51% (Fig. 4). These results suggest that 4-HPR is stably encapsulated in polymeric micelles prepared by block copolymers (Bz) although a level of over 72% of Bz groups in block copolymers is needed.

Furthermore, long-term biodistribution of 4-HPR after intravenous injection of 4-HPR encapsulated polymeric micelles prepared by block copolymers (Bz (77%)) was evaluated. After intravenous injection of 4-HPR itself (dissolved in HCO-60), 4-HPR was rapidly eliminated from blood circulation and highly distributed in liver (Fig. 5). In addition, all of tissue to blood concentration ratio (K_p) of 4-HPR were increased as time passed, especially K_p value on kidney at 8 h was most high compared with other tissues (Fig. 6). These trends were supported by other report with different intravenous formulation of 4-HPR (Swanson et al., 1980). On the other hand, 4-HPR encapsulated polymeric micelles prepared by block copolymers (Bz (77%)) showed much higher blood concentration of 4-HPR for more than 8 h and lower liver distribution of 4-HPR until 1 h compared with 4-HPR itself (Fig. 5). These results indicated that, 4-HPR encapsulated polymeric micelles prepared by block copolymers (Bz (77%)) showed prolonged circulation of 4-HPR for stable encapsulation of 4-HPR and escape of initial uptake by liver. The mean area under the curve ($AUC_{0–8\,h}$) in blood of 4-HPR itself

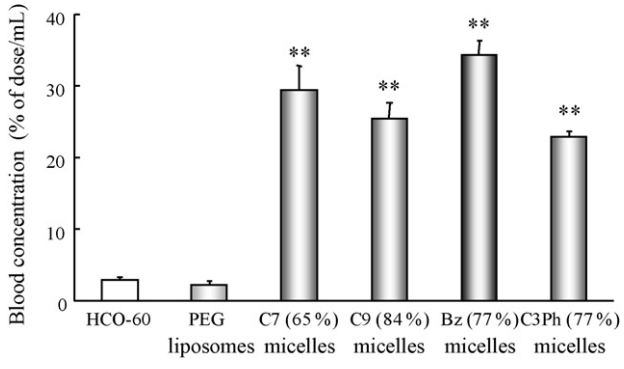

Fig. 3. Blood concentration of 4-HPR itself (dissolved in HCO-60), 4-HPR encapsulated in PEG liposomes, and in polymeric micelles with several types of esterified functional groups at 1 h after intravenous injection into mice at a dose of 5 mg/kg as 4-HPR. Each value represents the means ± S.D. (n = 3–4). **P < 0.01, compared with HCO-60 groups.

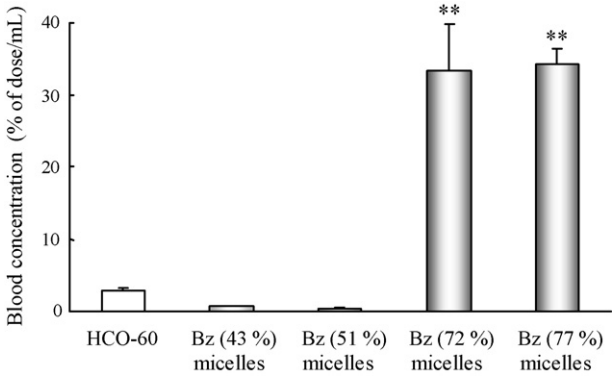

Fig. 4. Effect of level of Bz groups in block copolymers on blood concentration of 4-HPR encapsulated in Bz micelles at 1 h after intravenous injection into mice at a dose of 5 mg/kg as 4-HPR. Each value represents the means ± S.D. (n = 3–4). **P < 0.01, compared with HCO-60 groups.

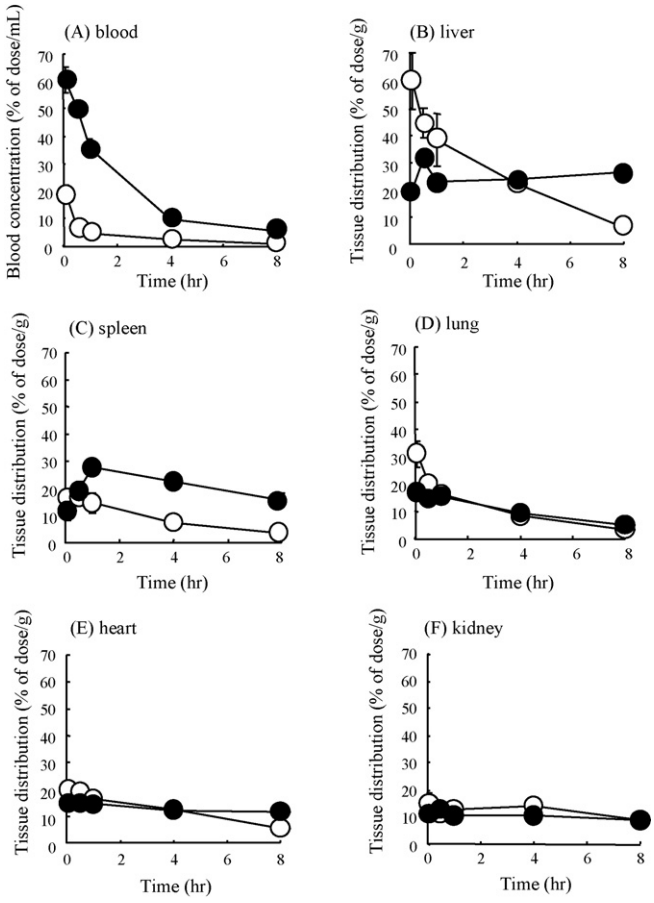

Fig. 5. Blood concentration (A) or tissue accumulation (liver (B), spleen (C), lung (D), heart (E), kidney (F)) of 4-HPR itself (dissolved in HCO-60) (◯) and 4-HPR encapsulated in Bz (77%) micelles (●) after intravenous injection into mice at a dose of 5 mg/kg as 4-HPR. Each value represents the mean ± S.D. (n = 3–4).

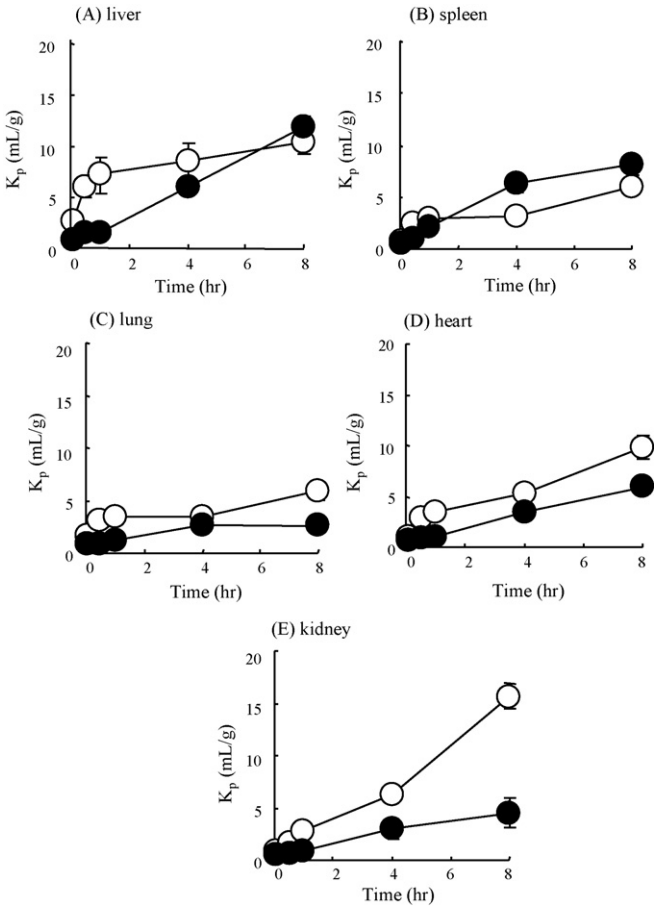

Fig. 6. Tissue to blood concentration ratio (K_p) of 4-HPR in liver (A), spleen (B), lung (C), heart (D), and kidney (E) of 4-HPR itself (dissolved in HCO-60) (◯) and 4-HPR encapsulated in Bz (77%) micelles (●) after intravenous injection into mice at a dose of 5 mg/kg as 4-HPR. Each value represents the mean ± S.D. (n = 3–4).

and 4-HPR encapsulated in Bz (77%) micelles calculated by the linear trapezoidal rule was 26.4 and 148 (% of dose × h/mL) and its relative AUC ratio was 5.6. Furthermore, K_p in lung, heart, and kidney after intravenous injection of 4-HPR encapsulated polymeric micelles prepared by block copolymers (Bz (77%)) were lower than that of 4-HPR itself (Fig. 6), suggesting that, 4-HPR encapsulated polymeric micelles prepared by block copolymers (Bz (77%)) escaped the transition of 4-HPR from blood to these tissues.

In this study, enhanced blood retention of 4-HPR was achieved by using polymeric micelles prepared by poly(ethylene glycol)–poly(aspartate ester) block copolymers, which were optimized hydrophobic segments with esterified functional groups. In particular, 4-HPR encapsulated polymeric micelles prepared by block copolymers (Bz (77%)) had the highest blood concentration of 4-HPR, which was about 106 μM (34% of the dose/mL) at a dose of 5 mg/kg. As far as the pharmacological effects of 4-HPR on cancer cells are concerned, Kalemkerian et al. have reported that 4-HPR efficiently inhibited the growth of small-cell lung cancer cell line and its IC_{50} values ranged from 0.1 to 3.0 μM (Kalemkerian et al., 1995). The neovascularization formed by solid tumors exhibits some unique features, such as hypervasculature, leaky capillaries and poor lymphatic clearance. These characteristics cause the accumulation of macromolecules in tumors for a long period, known as enhanced permeability and retention (EPR) effects (Matsumura and Maeda, 1986). The spaces in the blood endothelium formed by solid tumors are reported to range from 300 to 4700 nm (Yuan et al., 1995; Hashizume et al., 2000). Since the mean diameter of 4-HPR encapsulated polymeric micelles prepared by block copolymers (Bz (77%)) is about 175 nm, and maximally 342 nm, these may be small enough to pass through the endothelium of solid tumors. These observations led us to believe that 4-HPR encapsulated polymeric micelles prepared by block copolymers (Bz (77%)) could be effective carrier systems for use in future cancer therapy.

In conclusion, poly(ethylene glycol)–poly(aspartate ester) block copolymers with heptyl, nonyl, benzyl and phenyl propyl groups (abbreviated as C7, C9, Bz and C3Ph) were synthesized for stable encapsulation of 4-HPR. It is suggested that 4-HPR is stably encapsulated in polymeric micelles prepared by block copolymers (Bz) although a level of over 72% of Bz groups in the block copolymers is needed for stable encapsulation of 4-HPR. The information we have obtained in this study will be of

value for the future use, design, and development of polymeric micelles involving the *in vivo* application of 4-HPR.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research and the Program for Promoting the Establishment of Strategic Research Centers, Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by the Health and Labour Sciences Research Grants for Research on Advanced Medical Technology from the Ministry of Health, Labour and Welfare of Japan. Yokoyama M. and Yamamoto T. acknowledge support by the Program for Promoting the Establishment of Strategic Research Centers, Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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