Evaluation of Carboxymethylpullulan as a Novel Carrier for Targeting Immune Tissues

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Purpose. To demonstrate the potential of carboxymethylpullulan (CMPul) as a carrier for targeting immune tissues, and to find whether immune tissues could be set as the target of an immunosuppressant to treat autoimmune diseases.

Methods. The biodistribution of CMPul was investigated to evaluate its potency as a carrier for targeting immune tissues. Furthermore, an immunosuppressant-CMPul conjugate was prepared and its suppressive effect on rat adjuvant arthritis was examined.

Results. The disappearance rate of ^{3}H-labeled CMPul from the blood circulation was much slower than that of ^{3}H-labeled pullulan (Pul) after intravenous injection to normal rats. The concentration of ^{3}H-labeled CMPul in the spleen and lymph nodes was much higher than that of ^{3}H-labeled Pul at 24 hours after the injection, whereas the concentration of ^{3}H-labeled CMPul in the liver was significantly lower than that of ^{3}H-labeled Pul. A similar targeting property of ^{3}H-labeled CMPul for these immune tissues was observed in arthritic rats. A conjugate composed of a novel immunosuppressant PA-48153C and CMPul showed a suppressive effect on rat adjuvant arthritis judging from a reduction of the arthritic index and spleen weight and an increase of body weight.

Conclusions. CMPul is expected to be a promising carrier for targeting immune tissues with an immunosuppressant to enable treatment of autoimmune diseases.

KEY WORDS: carboxymethylpullulan; targeting; immune tissues; immunosuppressant; conjugate; adjuvant arthritis.

INTRODUCTION

Pul is an α-1,6-linked linear, nonionic polymer of maltotriose and has been extensively used as an additive in the food industry. This polysaccharide has many advantages as a drug carrier, such as high water solubility, easiness of chemical modification, and lack of immunogenicity. Pul is especially promising as a polymeric carrier for drugs targeted to the liver because it has an affinity for this organ (1). On the other hand, CMPul, readily prepared from Pul, has a weakly negative charge, is retained in the blood circulation (2), and is expected to accumulate in a tumor because of passive targeting (2,3). Therefore, CMPul is a promising carrier for a passive-targeting DDS. For example, the antitumor activity of the doxorubicin-CMPul conjugate was found to be more potent than doxorubicin alone, indicating enhancement of the therapeutic index (2). In addition, the concentration of CMPul in the spleen was found to be higher than that in the liver after intravenous injection to mice, suggesting that CMPul would be a suitable carrier for targeting the spleen (4). However, the detailed biodistribution of CMPul is yet to be reported.

Recently, Papisov et al. (5,6) reported that both dextran-coated monocrystalline iron oxide nanoparticles (5) and dextran-grafted poly-l-lysine (6) significantly accumulated in the lymph nodes but not in the liver after intravenous administration. These results suggest that some polysaccharides may be suitable carriers for targeting the lymph nodes as well as the spleen, and may have the potential for therapeutic application, including chemotherapy and immunomodulation.

In the present study, we investigated the biodistribution of CMPul to evaluate its potency as a carrier for targeting immune tissues such as the spleen and lymph nodes. Furthermore, to find whether immune tissues could be set as the target of an immunosuppressant to treat autoimmune diseases, we prepared an immunosuppressant-CMPul conjugate and examined its suppressive effect on rat adjuvant arthritis.

MATERIALS AND METHODS

Animals

Female Lewis rats were purchased from Charles River Japan, Inc. (Yokohama, Japan).

Induction and Assessment of Adjuvant Arthritis

Female Lewis rats (7 weeks old) were injected intradermally into the right hind paw with 0.5 mg of *Mycobacterium butyricum* (Difco Laboratories, Detroit, MI, USA) in 50 μl of liquid paraffin (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The arthritis was induced not only in the right hind paw but also in the other paws at and after 13 days after the adjuvant treatment. The weights of the popliteal lymph nodes and spleen were higher in arthritic rats than that in normal rats. To determine the arthritic index, the severity of the arthritis in each of the four paws was graded visually from 0 to 3 using a method adapted from that previously described for mice (7). The arthritic index of a diseased rat was the sum of the grades for the four paws, with the maximum possible index per rat being 12.

Tissue Distribution Experiment

Pul was labeled with ^{3}H-dimethylsulfate. CMPul (degree of substitution of the carboxymethyl group: 0.6) was prepared by Nogusa's method (2) from pullulan (apparent MW: 150,000), which was purchased from Hayashibara Biochemical Laboratories (Okayama, Japan). CMPul was labeled with [2-^{3}H] glycine as described previously (4). ^{3}H-labeled Pul or ^{3}H-labeled CM-Pul was administered intravenously to normal Lewis rats (7 weeks old) at a dose of 1 mg/kg. Radioactivity in the plasma of each rat was measured at 5 min, 0.5, 1, 2, 4, 6, and 24 hours after administration. The rats were sacrificed, then blood, plasma, cervical lymph nodes, mesenteric lymph nodes, liver, kidney, spleen, and lung were sampled, rinsed

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ABBREVIATIONS: AUC, area under the concentration-time curve; CMPul, carboxymethylpullulan; DDS, drug delivery system; DMF, *N, N′*-dimethyl formamide; EEDQ, 1-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline; Pul, pullulan

with saline (except blood and plasma), and weighed at 1 day after administration. The amount of ^3H radioactivity was measured with a liquid scintillation counter (Tri-carb 2000 CA, Packard Instrument Company, Meriden, CT, USA) using a liquid scintillation cocktail (Pico-Fluor 40, Packard Instrument Company). In addition, the tissue distribution of ^3H-labeled CM-Pul in Lewis rats with adjuvant arthritis was investigated as follows. At 18 days after injection of *Mycobacterium butyricum,* ^3H-labeled CM-Pul was administered intravenously to the rats at a dose of 1 mg/kg. The monitoring of ^3H radioactivity in plasma was carried out as described above. ^3H radioactivity in the tissues was measured at 1 and 7 days after administration.

The tissue distribution data were evaluated using a tissue uptake rate index calculated in terms of clearance as follows (3):

$$CL_{in} = T(t1)/AUC_{0-t1} \quad (1)$$

where CL_{in} (ml/hr/g) is the tissue uptake rate index (clearance) from the plasma to the tissue, T(t1) (% of dose/g) is the amount of radioactivity in the tissue at time t1, and AUC_{0-t10} (% of dose · hr/ml) is the area under the plasma concentration-time curve up to time t1.

Synthesis of Immunosuppressant-CMPul Conjugate

PA-48153C, a novel immunosuppressant (8,9), was used in this study. PA-48153C (**1**) was coupled with 8-azido-3,6,9,12-pentaoxa-heptadecanoyl chloride (**3**) in toluene-pyridine to afford **4** (91%). Compound **4** was converted in two steps [b) $PPh_3/B_{oc2}O$/THF (52%) and c) TFA, (98%)] to **5,** which has an amino group, and then condensation of amine **5** with CMPul using 1-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in *N, N′*-dimethyl formamide (DMF)-H_2O at room temperature gave PA-48153C-CMPul [**2**, degree of substitution (ds) of PA-48153C: 0.04] (Fig. 1). The ds was based on the integration of the ^1H NMR signals attributed to the glucose residue's anomeric proton of CMPul (5.30–5.90 and 4.90–5.30 ppm) relative to that of 17,18,19-CH_3 signals of PA-48153C at 0.70–1.00 ppm. Based on the ds, the contents of PA-48153C, CMPul, and the spacer portion were 5.6% (w/w), 89.8% (w/w), and 4.6% (w/w), respectively.

Treatment of Rat Adjuvant Arthritis with Immunosuppressant-CMPul Conjugate

Female Lewis rats with adjuvant arthritis were subdivided into groups with equal mean severity of arthritis, and started on i.v. treatment with PA-48153C-CMPul and PA-48153C alone [in saline containing 0.65% (w/w) Cremophor EL (Nacalai Tesque, Inc., Kyoto, Japan)] at 13 days after injection of adjuvant. The doses of PA-48153C-CMPul and PA-48153C were 2,8 and 2,4 mg PA-48153C equivalents/kg, respectively. According to the CMPul content (89.8%) of PA-48153C-CMPul, the doses of these conjugates as CMPul were 32 and 128 mg/kg, respectively. This treatment was performed daily for 5 days and was repeated for a further 4 days after an interval of 3 days. The arthritic index and body weight change for each group were measured throughout the experimental period. In addition, the weights of the spleen and popliteal lymph nodes were measured at 1 day after the final i.v. treatment.

Determination of Tissue Concentration of PA-45153C in Rats After Intravenous Administration

To clarify the suppressive effect of targeted PA-48153C on rat adjuvant arthritis, PA-48153C concentration in tissues, especially in the spleen and lymph nodes was determined after intravenous administration of PA-48153C-CMPul or PA-48153C alone (4 mg PA-48153C equivalent/kg) to normal female Lewis rats (9 weeks old). The rats were sacrificed, then plasma, spleen, and mesenteric lymph nodes were sampled at 0.5, 6, and 24 hours after administration. The plasma and tissues were stored at –20°C and –80°C, respectively, until HPLC analysis. The frozen tissues were homogenized with nine volumes of cold 1/15 M phosphate buffer (pH 7) using a tissue homogenizer (Polytron, Kinematica AG, Littau/Luzern, Switzerland) at 0°C. PA-48153C in the plasma and tissue homogenates was extracted with acetonitrile and subjected to HPLC analysis. HPLC conditions for determination of PA-48153C were as follows: Cosmosil 5C18 (4.6 × 150 mm, Nacalai) for plasma, Develosil ODS-UG-5 (4.6 × 150 mm, Nomura Chemical Co., Seto, Japan) for the others, mobile phase of 0.1% CF_3COOH-MeCN (40:60, v/v), flow rate of 1 ml/min, detection of 210 nm.

Determination of *In Vitro* Release of Immunosuppressant from Conjugate in Rat Plasma

To examine the sensitivity of PA-48153C-CMPul to hydrolysis by esterase in the blood, it (20 μg PA-48153C equivalent/ml) was incubated at 37°C in plasma from normal female Lewis rats (9 weeks old). After aliquots were sampled at vari-

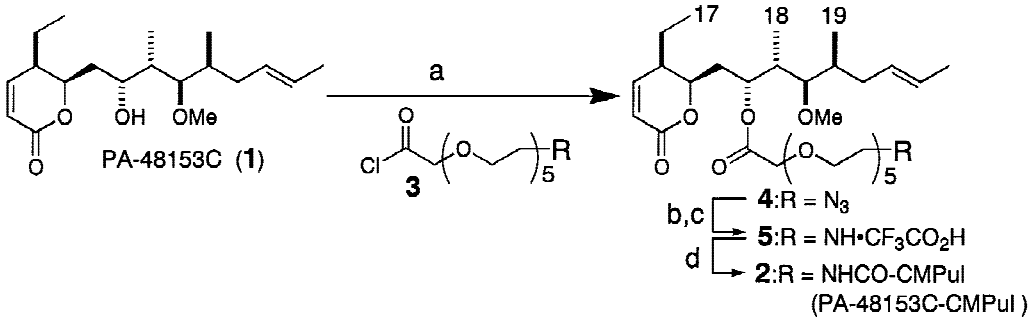

Fig. 1. Synthesis of PA-48153C-CMPul conjugate. (a) **3**/Pyridine-toluene (91%), (b) PPh_3/Boc_2O/THF (52%), (c) TFA (98%), (d) CMPul/EEDQ / DMF-H_2O.

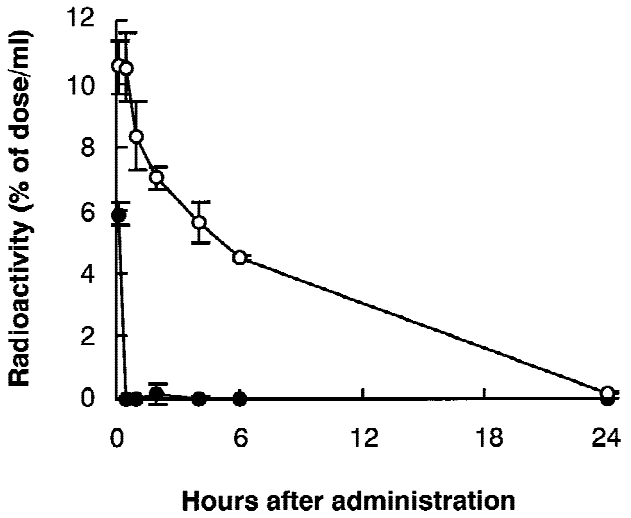

Fig. 2. Radioactivity-time profile of ^{3}H-Pul or -CMPul in plasma after intravenous administration to normal Lewis rats at a dose of 1 mg/kg. Data represent the mean ± S.D. (n = 3). ●, Pul; ○, CMPul.

ous time intervals, the PA-48153C released was determined by HPLC as described above.

Statistical Analysis

The data was evaluated using Tukey's test or Dunnet's test except for the tissue AUC (area under the concentration-time curve) values for PA-48153. This $AUC_{0-24\ hours}$ in each tissue was calculated and statistically compared as described previously (10).

RESULTS AND DISCUSSION

Tissue Distribution of Pul and CMPul in Normal Rats After Intravenous Injection

As shown in Fig. 2, ^{3}H-labeled Pul rapidly disappeared from the blood circulation, with complete elimination within 30 min after injection. In contrast, the disappearance rate of ^{3}H-labeled CMPul, with its detection in plasma even at 24 hours after injection, was much slower. The plasma $AUC_{0-24\ hours}$ of ^{3}H-labeled CMPul was approximately 30-fold higher than that of ^{3}H-labeled Pul (P <0.01)(Table I).

Figure 3 shows the results of tissue distribution of ^{3}H-labeled Pul and ^{3}H-labeled CMPul at 24 hours after injection. As reported previously (1), ^{3}H-labeled Pul was distributed in the liver to a higher extent than in any other tissue, indicating that Pul has a high inherent affinity for the liver. On the other hand, the tissue concentration of ^{3}H-labeled CMPul was significantly higher than that of ^{3}H-labeled Pul except for the concentration in the liver ($P < 0.01$ or 0.05).

Interestingly, the concentration of ^{3}H-labeled CMPul in the spleen and lymph nodes was much higher than that of ^{3}H-labeled Pul, whereas the concentration of ^{3}H-labeled CMPul in the liver was significantly lower than that of ^{3}H-labeled Pul ($P < 0.01$). The uptake rate indices of ^{3}H-labeled CMPul for the spleen, mesenteric lymph nodes and cervical lymph nodes were much greater than that for the liver, in contrast to those for ^{3}H-labeled Pul (Table I). These findings suggest that CMPul has a high affinity for the spleen and lymph nodes, but not for the liver.

Yamaoka et al. (1) reported that the remarkable affinity of Pul for the liver was the reason for its short half-life period in the blood circulation. Because the liver has discontinuous endothelial capillaries that enable macromolecules to penetrate through vascular walls, circulating macromolecules in blood can come into free contact with the surface of hepatocytes with negative charges (11). Therefore, the liver is thought to be the most important tissue for the systemic disposition of cationic and neutral macromolecules (11).

In addition, Pul may be also taken up by the liver via the asialoglycoprotein receptor, as occurs with dextran (12). The moderate anionic charge on CMPul decreases the interaction with the liver and may prevent the recognition of its sugar residues by the asialoglycoprotein receptor. These results suggest that CMPul with weakly negative charges may be distributed in the liver at lower concentrations than Pul because of ionic repulsion to negatively charged liver cells, resulting in prolonged retention of CMPul in the blood circulation.

Our previous studies indicated that CMPul might be incorporated into the marginal zone macrophages in the spleen by their scavenger receptors (4,13). Investigation of the mechanism of selective accumulation of CMPul in the lymph nodes is now in progress, suggesting that CMPul may be in-

Table I. AUC and Tissue Uptake Rates for Radioactive Polysaccharides in Lewis Rats[a]

Polysaccharide	AUC[b] (% of dose · hr/ml)	Tissue uptake rate index[c] (μl/hr/g)					
		Spleen	Liver	MLN[d]	CLN[e]	Lung	Kidney
^{3}H-Pul[f]	2.5 ± 0.7	1305 ± 300	3155 ± 858	1544 ± 151	851 ± 696	9.9 ± 7.1	31.1 ± 19.4
^{3}H-CMPul[f]	81.7 ± 1.1**	385 ± 28.2**	27.8 ± 3.3**	638 ± 46.8**	83.0 ± 17.4**	3.4 ± 0.7	2.4 ± 0.1*
^{3}H-CMPul[g]	49.6 ± 2.0##	623 ± 100	25.7 ± 2.3	457 ± 12.9	64.0 ± 6.8	2.5 ± 0.5	3.4 ± 0.9

[a] Data represent the mean ± S.D. (n = 3). *$P < 0.05$, **$P < 0.01$: Significantly different from ^{3}H-Pul-administered normal rats (by Tukey's test). ## $P < 0.01$: Significantly different from ^{3}H-CMPul-administered normal rats (by Tukey's test).
[b] Area under the plasma concentration-time curve up to 24 hours after injection (% of dose · hr/ml).
[c] Uptake rate expressed in terms of clearance (μl/hr/g).
[d] Mesenteric lymph nodes.
[e] Cervical lymph nodes.
[f] Values were calculated from the data at 24 hours after injection in normal rats.
[g] Values were calculated from the data at 24 hours after injection in arthritic rats.

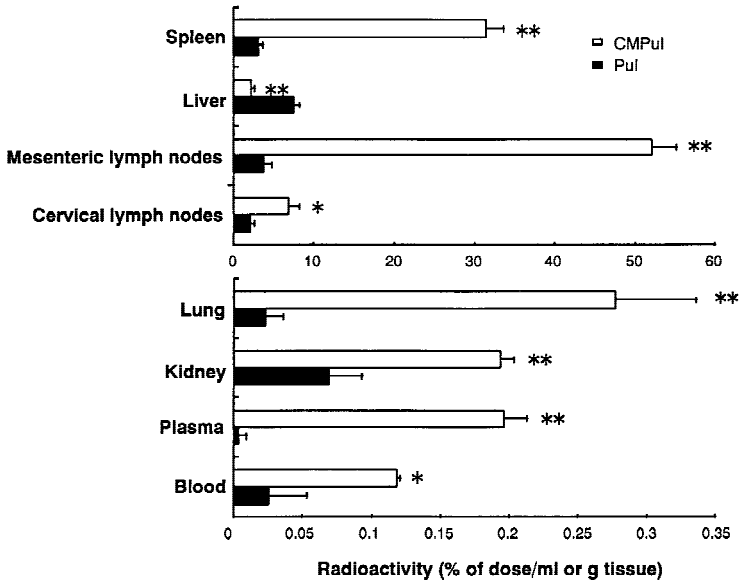

Fig. 3. Radioactivity in tissues at 1 day after intravenous administration of ^{3}H-Pul or -CMPul to normal Lewis rats at a dose of 1 mg/kg. Data represent the mean ± S.D. (n = 3). *P < 0.05, **P < 0.01: Significantly different from ^{3}H-Pul-administered group (by Tukey's test).

corporated into some macrophages in the lymph nodes in a similar manner as for the spleen (data not shown).

Tissue Distribution of CMPul in Normal Rats and Rats with Adjuvant Arthritis After Intravenous Injection

To evaluate the potential of CMPul as a carrier for targeting immune tissues of animals with autoimmune diseases, the tissue distribution profile of ^{3}H-CMPul in arthritic rats was compared with that in normal rats. As shown in Fig. 4, the disappearance rate of ^{3}H-labeled CMPul from the blood circulation was slightly faster in arthritic rats than that in normal rats. The plasma $AUC_{0-4\ hours}$ of ^{3}H-labeled CMPul in normal rats was approximately 1.6-fold higher than that in arthritic rats (P < 0.01). Also, the tissue concentration of ^{3}H-labeled CMPul was significantly higher in normal rats than in arthritic rats, except the concentrations in the spleen and kidney (Fig. 5). The weight of the popliteal lymph nodes, but not of the mesenteric and cervical lymph nodes, was much higher in arthritic rats than in normal rats because of swelling in both hind paws, which made it as high as the weight of the mesenteric or cervical lymph nodes. In addition, the concentration of ^{3}H-labeled CMPul in the popliteal lymph nodes was approximately 11% of the dose/g tissue of arthritic rats (data not shown).

These results suggest that the lower accumulation of ^{3}H-labeled CMPul in the mesenteric or cervical lymph nodes of arthritic rats may result from its high accumulation in popliteal lymph nodes, and that CMPul may also have a targeting potency for the spleen and lymph nodes in arthritic rats. In fact, Table I shows that the uptake rate indices of ^{3}H-labeled CMPul for these immune tissues were not significantly different between normal rats and arthritic rats. The tissue distribution profile of ^{3}H-labeled CMPul in arthritic rats was maintained even at 7 days after injection similarly to that at 1 day after injection (data not shown), demonstrating the potentiality of CMPul serving as a carrier for targeting immune tissues of animals with chronic autoimmune diseases, such as rheumatoid arthritis.

Suppressive Effect of PA-48153C-CMPul Conjugate on Rat Adjuvant Arthritis

To clarify whether the strategy of targeting an immunosuppressant to the immune tissues is feasible for the treatment of autoimmune diseases, the suppressive effect of PA-48153C-CMPul conjugate on rat adjuvant arthritis was investigated. As shown in Fig. 6A, only arthritic rats treated with 8 mg PA-48153C equivalent/kg of PA-48153C-CMPul showed a significant reduction of the arthritic index as compared with rats in the control group (treated with vehicle) at 20, 22, and

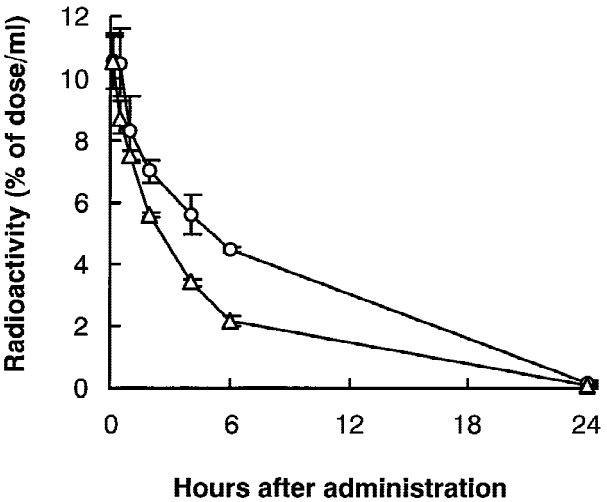

Fig. 4. Radioactivity-time profile of ^{3}H-CMPul in plasma after intravenous administration to normal Lewis rats or Lewis rats with adjuvant arthritis at a dose of 1 mg/kg. Data represent the mean ± S.D. (n = 3). ○, normal rats; △, arthritic rats.

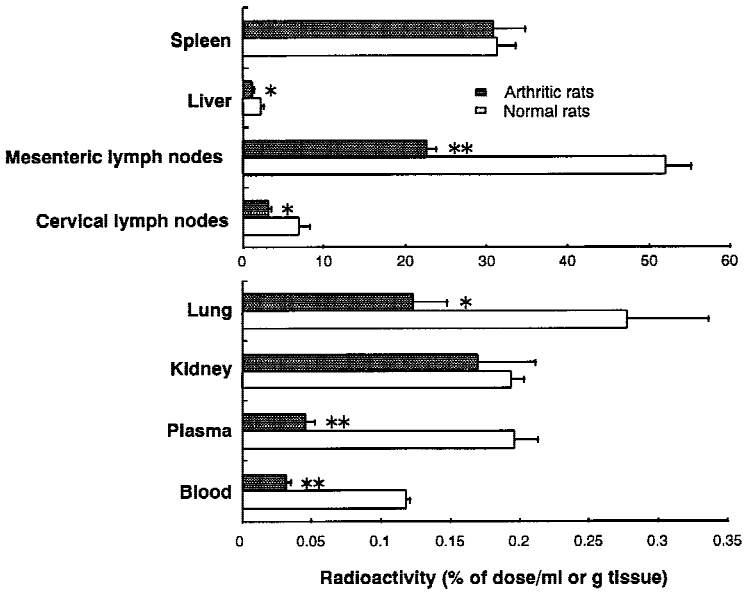

Fig. 5. Radioactivity in tissues at 1 day after intravenous administration of ^{3}H-CMPul to normal Lewis rats or Lewis rats with adjuvant arthritis at a dose of 1 mg/kg. Data represent the mean ± S.D. (n = 3). *$P < 0.05$, **$P < 0.01$: Significantly different from normal rats (by Tukey's test).

24 days after adjuvant treatment ($P < 0.01$). In addition, the spleen weight of these rats was significantly lower than that of the control group on day 24 ($P < 0.01$), showing alleviation of arthritic severity only in rats treated with 8 mg PA-48153C equivalent /kg of PA-48153C-CMPul (Fig. 6C).

Although the treatment of arthritic rats with 2 mg PA-48153C equivalent /kg of PA-48153C-CMPul induced only a slight reduction of the arthritic index (Fig. 6A), it led to a significant increase of the body weight of these rats as compared with the control group ($P < 0.05$ on day 17, $P < 0.01$ on days 20, 21, 22, 23, and 24) (Fig. 6B). This suggests that the PA-48153C-CMPul conjugate may also alleviate the severity of arthritis in these diseased rats even at the dose of 2 mg PA-48153C equivalent/kg. In contrast, PA-48153C alone did

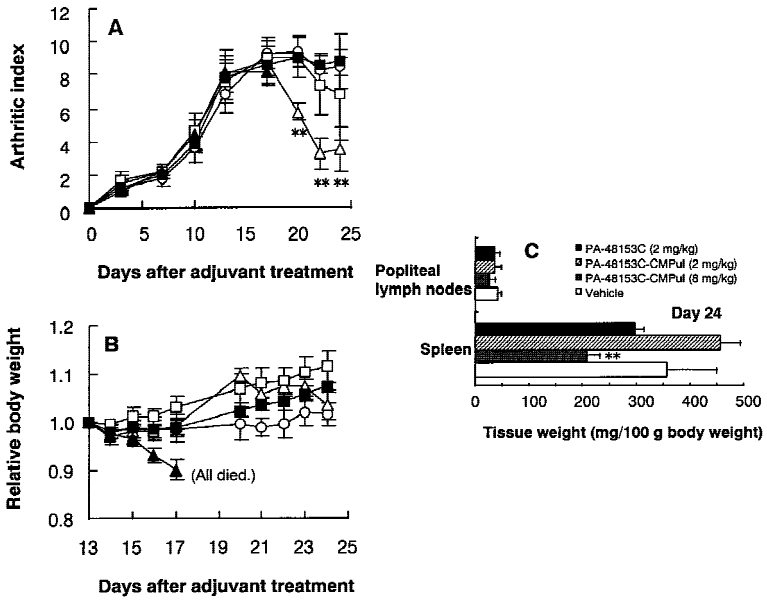

Fig. 6. Effect of PA-48153C-CMPul or PA-48153C alone on rat adjuvant arthritis. Arthritic index (A), relative body weight (B), and tissue weight on day 24 (C) were evaluated. Intravenous administration was performed at 13, 14, 15, 16, 17, 20, 21, 22, and 23 days after adjuvant treatment. Data represent the mean ± S.D. (n = 4–5). ○, vehicle; △, PA-48153C-CMPul (8 mg PA-48153C equivalent/kg); □, PA-48153C-CMPul (2 mg PA-48153C equivalent/kg); ▲, PA-48153C (4 mg/kg); ■, PA-48153C (2 mg/kg). **$P < 0.01$: Significantly different from vehicle-treated group (by Dunnet's test).

not alleviate the severity of arthritis in the rats but displayed a strong toxicity at the dose of 4 mg/kg, resulting in death of the treated rats (Fig. 6B).

Tissue Distribution of PA-48153C in Rats After Intravenous Administration of PA-48153C Alone or PA-48153C-CMPul Conjugate

Figure 7 shows the concentration-time profiles of PA-48153C in plasma, spleen and mesenteric lymph nodes after intravenous administration of PA-48153C alone or PA-48153C-CMPul conjugate. The disappearance rate of PA-48153C from the blood circulation was much faster for PA-48153C alone than that for PA-48153C-CMPul. The plasma $AUC_{0-24\ hours}$ of PA-48153C for PA-48153C-CMPul was approximately 15-fold higher than that for PA-48153C alone ($P < 0.01$). Although the spleen $AUC_{0-24\ hours}$ of PA-48153C for PA-48153C-CMPul was not significantly different from that for PA-48153C alone ($P = 0.08$), it was approximately 5-fold higher than that for PA-48153C alone. These results demonstrate that the conjugation of PA-48153C with CMPul did not only lead to prolonged retention of PA-48153C concentration in the blood circulation, but also to targeting of PA-48153C to the spleen. The suppressive effect of PA-48153C-CMPul conjugate on rat adjuvant arthritis (Fig. 6) is thought to result from the improved biodistribution of PA-48153C due to conjugation with CMPul.

On the other hand, the concentration-time profile of PA-48153C in mesenteric lymph nodes after intravenous administration of PA-48153C-CMPul was similar to that for PA-48153C alone, indicating no targeting potency of CMPul for lymph nodes. This may have led to the absence of weight change of popliteal lymph nodes in arthritic rats treated with PA-48153C-CMPul (Fig. 6).

This unexpected result might be attributed to the following two possibilities. One is a decreased accumulation of PA-48153C-CMPul in lymph nodes after intravenous administration. According to our preliminary results with mice, when ^{3}H-CMPul (1 mg/kg) was intravenously coadministered with 100-fold cold CMPul (100 mg/kg) to mice, the concentration of ^{3}H-CMPul in the spleen fell from 34.86 ± 5.59 to 3.05 ± 0.30 (% of dose/g) at 24 hours after injection as compared with the administration of ^{3}H-CMPul (1 mg/kg) alone (unpublished data). This suggests that the distribution of CMPul in the immune tissues is affected by the dose of CMPul. It is thought that the concentration of PA-48153C-CMPul (2 and 8 mg PA-48153C equivalent/kg) in the immune tissues was not as high as expected according to the data for tissue distribution and tissue uptake rate indices for ^{3}H-CMPul alone (Figs. 3 and 5, Table I) because the doses of these conjugates as CMPul were 32 and 128 mg/kg, respectively. Therefore, lowering the CMPul content of a conjugate by increasing the content of the immunosuppressant should be important for further improving the suppressive effect of the conjugate. These findings point to the need for the selection of a suitable drug, which has a stronger suppressive effect, and the improvement of its conjugation with CMPul.

The other possible explanation is an insufficient release of PA-48153C from the conjugate due to lack of a suitable esterase for cleavage in the lymph nodes. Figure 8 shows the *in vitro* release of PA-48153C from PA-48153C-CMPul in the

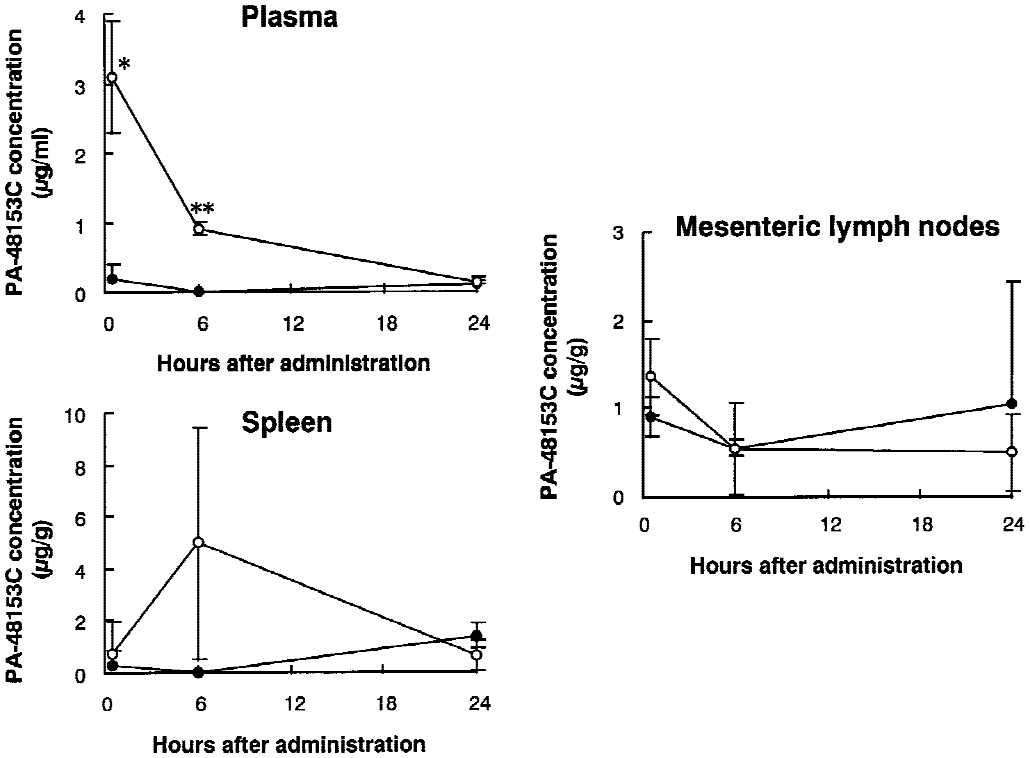

Fig. 7. Tissue concentration-time profile of free PA-48153C after intravenous administration of PA-48153C alone or PA-48153C-CMPul to normal Lewis rats at a dose of 4 mg/kg as the concentration of PA-48153C. Data represent the mean ± S.D. (n = 3). ●, PA-48153C; ○, PA-48153C-CMPul. *$P < 0.05$, **$P < 0.01$: Significantly different from PA-48153C-treated group (by Tukey's test).

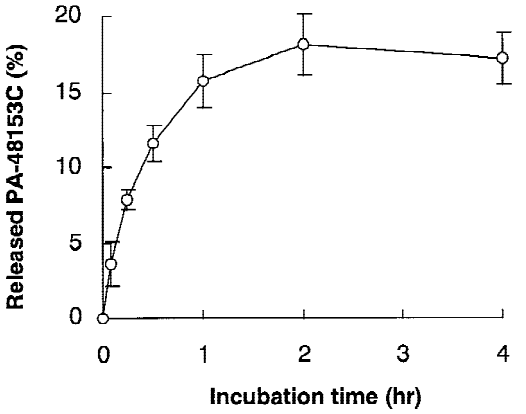

Fig. 8. *In vitro* Release of PA-48153C from PA-48153C-CMPul in Rat Plasma. Data represent the mean ± S.D. (n = 3).

plasma of Lewis rats. This result indicates that the release of free PA-48153C was less than 20% of the conjugate, and that a large portion of the conjugate could be distributed in the lymph nodes without hydrolysis by esterase in the blood. CMPul is thought to be incorporated into some macrophages in the spleen and lymph nodes, suggesting that a conjugate bound through a lysosomotropic oligopeptide spacer may be promising as a DDS targeting immune tissues. The use of such a conjugate should improve the strategy of targeting an immunosuppressant to the immune tissues in the treatment of autoimmune diseases.

In conclusion, this study suggests that CMPul may be a promising carrier for targeting immune tissues and that the targeting of an immunosuppressant to immune tissues may be useful for the treatment of autoimmune diseases, such as rheumatoid arthritis.

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