Targeted and Sustained Delivery of Aclarubicin to Lymphatics by Lactic Acid-Oligomer Microsphere in Rat

Hiroshi YOSHIKAWA,*,[a] Yasuhiro NAKAO,[a] Kanji TAKADA,[a] Shozo MURANISHI,[a] Ryoichi WADA,[b] Yasuhiko TABATA,[b] Suong-Hyu HYON[b] and Yoshito IKADA[b]

Department of Biopharmaceutics, Kyoto Pharmaceutical University,[a] Misasagi, Yamashina, Kyoto 607, Japan and Research Center for Medical Polymers and Biomaterials, Kyoto University,[b] Syogoin, Sakyo, Kyoto 606, Japan. *Received July 27, 1988*

We examined targeted delivery of an anticancer drug, aclarubicin (ACR), to the lymphatic system in rats by encapsulation of the drug in microsphere (MS) prepared from nontoxic and biodegradable L-lactic acid-oligomer with an average molecular weight ($\bar{M}w$) of 3600. ACR was released at an almost constant rate from two kinds of ACR-MSs having different size (1—5 μm and less than 1 μm) over 20 d in phosphate-buffered saline at 37 °C. The intraperitoneal administration of both ACR-MSs (dose of ACR; 5 mg/kg) to rats sustained an almost constant ACR level (300—400 and 400—600 ng/ml) in the lymph of the thoracic duct during over 10 d, and the ACR level in the blood was extremely low, although intraperitoneal injection of ACR alone gave lower level of ACR in the lymph than in the blood level within 12 h.

Keywords targeted delivery; aclarubicin; selective lymphatic delivery; sustained release; lactic acid-oligomer; microsphere

The unsuccessful treatment of malignant tumor is frequently due to the occurrence of lymphatic metastasis, which leads to the death of the host even after successful treatment of the primary tumor.[1] Therefore, in cancer chemotherapy, a sufficient supply of anticancer drug to the lymphatics seems desirable for preventing or suppressing lymph node metastasis. Some attempts at direct injection of drugs into the regional lymphatic vessels have been performed,[2-4] but these treatments were restricted owing to surgical difficulties. Therefore, a drug delivery system able to selectively and efficiently distribute an anticancer drug into the lymphatic system is required. The lymphatics are known to play an important role in the removal of macromolecules from the interstitial space, and a variety of particles have been observed passing through the lymphatic vessels but the blood capillaries[5,6] due to the difference of permeability.[7,8] These observations suggest the feasibility of targeting anticancer drugs by the use of large molecular weight substances or particles. The purpose of the present study was to assess the feasibility of employing nontoxic and biodegradable microsphere (MS) as a selectively lymphotrophic carrier.

As a biocompatible and biodegradable material which can form MS, poly(lactic acid)[poly(LA)] is well known. The use of MS composed of poly(LA) with a molecular weight of over 10^4 has been investigated for the controlled release of drugs which require especially long-term release, such as narcotic antagonists,[9,10] or hormones.[11] However, for anticancer drugs, targeting with controlled release for a moderate period is more important than extremely long-term release such as several months or a year, which might result in side effects on normal cells. Therefore, as a material of MS for the release of an anticancer drug in this work, we selected LA-oligomer with a molecular weight below 10^4, because we found recently that such LA-oligomer are useful for continuous release of antibiotics over several weeks.[12]

Most studies using biodegradable MS have been aimed at the control of drug release, but little attention has been paid to targeting. As far as we know, there has been no report on the targeting of a drug into the lymphatic system with the aid of biodegradable MS. In this paper, we describe the targeted delivery of an anticancer drug to the lymphatics with the aid of LA-oligomer MS.

Experimental

Materials A 90% (w/v) aqueous solution of L-LA (C.V. Chemie Combinatie, Netherland) was subjected to condensation polymerization under reduced pressure to obtain oligomeric LA (LA-oligomer).[13] The LA-oligomer was separated from the polymerization products by repeated precipitation with methanol from solutions in methylene chloride, followed by drying under reduced pressure. Its weight-average molecular weight ($\bar{M}w$) was determined by the method of gel permeation chromatography using standard polystyrene (Nacalai Tesque, Inc., Kyoto, Japan) and gel permeation chromatograph [HLC 802UR; column, 7.5 mm × 100 cm, column packing, G2000—5000 (Toso Co., Tokyo, Japan)] with tetrahydrofuran as eluent (1.7 ml/min). The oligomer used in this work had $\bar{M}w$ of approximate 3600. The anticancer drug aclarubicin (ACR), internal standard aclacinomycin B and standard preparations of metabolites (MA144M1 and -N1) were kindly supplied by Sanraku Inc., Tokyo, Japan. All other chemicals and solvents were of reagent grade, commercially obtained.

Preparation of MS Loaded with ACR The MSs containing ACR (ACR-MS) were prepared by the solvent evaporation method.[14] A given amount of ACR and 1 g of LA-oligomer were dissolved in 10 ml of methylene chloride. The resulting solution was then emulsified in 100 ml of 2% (w/v) poly(vinyl alcohol) aqueous solution by sonication at room temperature. The emulsion was agitated with a magnetic stirrer under protection from light. Stirring was continued for 10 h under atmospheric pressure at room temperature, until the methylene chloride solvent had completely evaporated. The ACR-MSs were collected by centrifugation, washed three times with cold distilled water, and lyophilized.

Characterization of ACR-MS The size of ACR-MS was determined by scanning electron microscopy (SEM, Model S-450, Hitachi Ltd., Tokyo, Japan). To determine the ACR content in MS, a weighed amount of ACR-MS was dissolved in 1 ml of methylene chloride and the solution was diluted to 100 ml with methanol.

***In Vitro* Release Studies** Each of ACR-MS (1 mg) was suspended in 5 ml of phosphate-buffered saline (PBS) of pH 7.4 and the resulting suspension was immersed in a shaker bath kept at 37 °C. The supernatant was periodically removed, the same amount of fresh medium being added. The level of ACR released from ACR-MS was measured.

Administration to Rats Male Wistar rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) weighing 350—400 g were anesthetized intraperitoneally with pentobarbital. ACR dissolved in PBS of pH 7.4 or ACR-MS suspended in PBS of pH 7.4 was administered to rats (dose of ACR, 5 mg/kg) by intraperitoneal injection. Blood (300 μl) was taken by cardiac puncture and the lymph of the thoracic duct was collected according to a modification of Bollman's method,[15] using the same rats. The ACR and metabolites levels in the blood and lymph samples were determined.

Assay of Drug The high-performance liquid chromatographic method

used for the measurement of ACR and its metabolites was a slight modification[15] of that described by Ogasawara *et al.*[16] The reversed-phase column (Cosmosil 5C18 packed column, 4.6 mm i.d. × 250 mm, Nacalai Tesque, Inc., Kyoto, Japan) was eluted with acetonitrile–0.04 M ammonium formate buffered solution (60 : 40, v/v, pH 5.0). A mixture of 0.5 ml of sample, 0.5 ml of 0.2 M phosphate buffered solution (pH 7.0) and 1.5 ml of ethyl acetate was vigorously shaken for 10 min and centrifuged. The aqueous layer was reextracted with 1.5 ml of ethyl acetate and the ethyl acetate layers were combined and evaporated. The residue was dissolved in 0.5 ml of methanol solution containing internal standard and this solution was also evaporated to dryness. The residue was redissolved in 0.1 ml of the mobile phase and then injected into the column. ACR and its glycoside-type metabolites (MA144M1 and -N1) were measured by the standard method with the use of a fluorescence detector (RF-530, Shimadzu Co., Kyoto, Japan) at the excitation wavelength of 435 nm and emission wavelength of 505 nm. Aclacinomycin B, which is not one of the mammalian metabolites, was used as an internal standard and MA144M1 and -N1 were used as standard preparations of metabolites.

Results and Discussion

Characterization of ACR-MS ACR was entrapped with high yield (90—95% recovery) in LA-oligomer MS probably because of the lipophilicity of ACR and MS; the content of ACR in MS was about 100 mg/g for both MSs as mentioned later. The ACR-MSs had spherical shape, but their sizes were dependent on the condition of the preparation. The diameter of ACR-MS was less than 1 μm in the case of preparation by sonication at 60 W whereas ACR-MS of 1—5 μm were formed by sonication at 15 W. We designate the smaller ACR-MS as ACR-MS(S) and the larger one as ACR-MS(L) in this study.

***In Vitro* Release** Figure 1 indicates the *in vitro* release profiles in PBS of ACR from the two kinds of ACR-MS having different sizes. In this release experiment, no degradation product of ACR was detected in the medium. Following the start of incubation, relatively constant release of ACR was observed during the first 10 d, followed by a much slower leakage during the remainder of the incubation period up to 20 d. No "burst" effect in the first few days was detected. Although the released amount of ACR from the two preparations did not differ much up to 7 d, the release rate of ACR from ACR-MS(S) was faster than that from ACR-MS(L). At 20 d, the percentage of the released amount of ACR against loaded ACR in MS was about 75% for (S) size and 45% for (L) size. This is probably because the specific surface area of ACR-MS(S) is greater than that of ACR-MS(L). The SEM observation during *in vitro* incubation, described in our previous report, indicated that MS of LA-oligomer has small cracks over their surface after 1 week, and after 30 d, MS had disintegrated into small fragments.[17] If the MS is regarded as a matrix in which the drug is homogeneously distributed, the Q–$t^{1/2}$ relationship of Higuchi[18] can be expected to hold (Q is the amount of drug released and t is time). However, the ACR release (Fig. 1) did not accord with the Q–$t^{1/2}$ relationship (not shown), probably because of the degradation of MS during the release experiment as aforementioned.

***In Vivo* Experiment** Metabolism of ACR include glycoside- and aglycone-types.[19] After *in vivo* administration to rats in this work, only glycoside-type metabolites (MA144M1 and -N1) were detected in the blood and the lymph of rats, and it has been reported that there is not much difference of antitumor activity between ACR and these two metabolites.[19] Figure 2 indicates total ACR

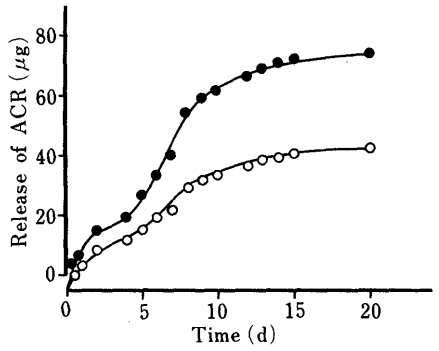

Fig. 1. *In Vitro* Release Profiles of ACR from ACR-MS in PBS
●, ACR-MS (S); ○, ACR-MS (L).

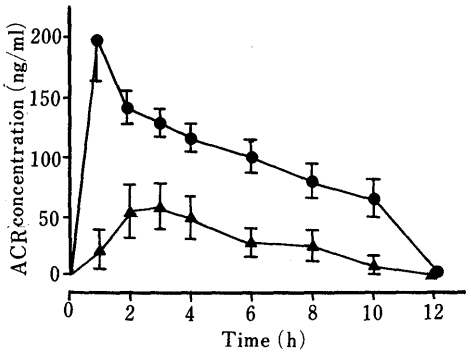

Fig. 2. Lymph and Plasma Concentrations of Total ACR after Intraperitoneal Administration of ACR Dissolved in PBS
●, plasma; ▲, lymph. Results are expressed as the mean±S.E. of at least 3 experiments.

(ACR + two glycoside-type metabolites) levels in the plasma and lymph of the thoracic duct after administration of ACR dissolved in PBS into the peritoneal cavity of rats. The peak level of ACR was detected at 1 h (plasma) and 3 h (lymph), and then the circulating ACR levels in both fluids decreased slowly. Little is known about the lymphatic transfer of ACR, but as can be seen in Fig. 2, the ACR level in the lymph was continuously lower than that in the plasma. The pattern of lymph ACR level was similar to that of plasma level, and ACR in both fluids diminished within 12 h after administration. This fact demonstrates that ACR itself administered in the peritoneal cavity has no lymphotrophy.

Next, we observed ACR levels in both fluids after intraperitoneal administration of ACR-MS suspended in PBS (Fig. 3). In the plasma, ACR levels were extremely low, barely over the detectable limit (10 ng/ml), regardless of the size of ACR-MS, at any sampling time during 2 weeks, and the ACR level in the erythrocytes (not shown) was almost equal to that in the plasma. On the other hand, relatively high levels of ACR were found in the lymph. In particular, during the first 10 d, almost constant lymph levels of ACR were observed (approximate 300—400 ng/ml for ACR-MS(S) and 400—600 ng/ml for ACR-MS(L)). There is some incompatibility between the results of released amoutn of ACR from MS (Fig. 1) and these lymphatic levels of ACR (Fig. 3). We speculate that there may be a difference of lymphotrophy of MS depending on the size. In Table I the ratio of ACR concentration in the lymph to that in the plasma (C_L/C_P) during the sampling period calculated from the data in Figs. 2 and 3 is shown. This C_L/C_P

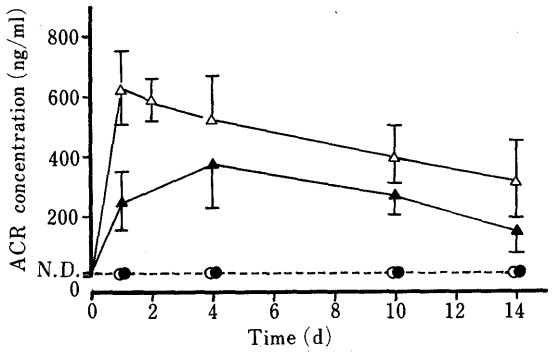

Fig. 3. Lymph and Plasma Concentrations of Total ACR after Intraperitoneal Administration of ACR-MS Suspended in PBS

●, plasma (ACR-MS (S)); ○, plasma (ACR-MS (L)); ▲, lymph (ACR-MS (S)); △, lymph (ACR-MS (L)). Results are expressed as the mean±S.E. of 3—4 experiments for ACR-MS (L) and as the mean±S.E. of 3 experiments for ACR-MS (S). N.D., not detected.

TABLE I. Ratio of ACR Levels in Lymph to Plasma (C_L/C_P)

Formulation	C_L/C_P [a)]
ACR	0.3±0.04
ACR-MS (S)	18.6±3.8
ACR-MS (L)	29.0±6.4

a) Results are expressed as mean±S.E. by calculation from data of C_L and C_P at each sampling time during 12 h as shown in Fig. 2 for ACR, and from those during 14 d (at 1, 4, 10 and 14 d) in Fig. 3 for ACR-MSs.

ratio is considered to indicate the lymphotrophic character of ACR. The results in Table I demonstrate that ACR acquired sustained and selective lymphotrophy through entrapment in MS; that is, we achieved the targeted delivery of ACR into the lymphatics with the aid of MS. The delivery of drugs into the circulation system from the tissue or the cavity is determined by their ability to permeate through the walls of blood capillaries and lymphatic vessels. The permeability of blood capillaries is much less than that of the lymphatic vessel wall, which has large pores and clefts.[20] Therefore, we can establish strategies to deliver drugs selectively into the lymphatics from the interstitum or the cavity with the aid of sieving according to molecular weight or size.

According to this idea, we have reported the improved lymphotrophic delivery of poorly absorbable anticancer drugs such as bleomycin[21] or an analog of 5-fluorouracil[22] by macromolecularization and the use of absorption promoters. There have been some attempts to enhance lymphatic drug delivery by size sieving using emulsions,[23] liposomes[24] or activated carbon particles.[25] However, these drug carriers present problems of stability, poor drug retention or toxicity. The LA-oligomer used in this work is a biocompatible and biodegradable material and the MS composed of this LA-oligomer broke down in the body to smaller fragments[17] and were degraded to bicarbonate and water *via* the monomer of LA. Therefore, LA-oligomer MS is considered to be an excellent and nontoxic drug carrier.

This is the first report in which biodegradable MS, hitherto mostly employed as a material for controlling the release of drugs, is applied as a selective lymphotrophic carrier. The results in this paper offer an interesting and potentially effective method for prevention and treatment of lymphatic tumor metastasis in cancer chemotherapy.

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References

1) R. E. Johnson, *Cancer*, **35**, 1 (1975).
2) S. Wallace, L. Jackson and B. Schaffer, *Radiology*, **76**, 179 (1961).
3) I. M. Ariel and M. I. Resnick, *Surgery*, **55**, 355 (1964).
4) G. Kitchen and M. J. Garrett, *Clin. Radiology*, **22**, 379 (1971).
5) L. V. Leak, *Microvasc. Res.*, **2**, 361 (1970).
6) L. V. Leak, *J. Cell Biol.*, **50**, 300 (1971).
7) J. R. Pappenheimer, *Physiol. Rev.*, **33**, 387 (1953).
8) E. M. Renkin, *The Physiologist*, **7**, 13 (1964).
9) S. Yolles, J. E. Eldridge and J. H. R. Woodland, *Polym. News*, **1**, 9 (1971).
10) N. Mason, C. Thies and T. J. Cicero, *J. Pharm. Sci.*, **65**, 847 (1976).
11) L. M. Sanders, J. S. Kent, G. I. McRae, B. H. Vikery, T. R. Tice and D. H. Lewis, *J. Pharm. Sci.*, **73**, 1294 (1984).
12) Y. Ikada, S.-H. Hyon, K. Jamshidi, S. Higashi, T. Yamamuro, Y. Kakutani and T. Kitsugi, *J. Cont. Release*, **2**, 179 (1985).
13) K. Jamshidi, Doctor Thesis, Kyoto University, (1984).
14) L. R. Beck, D. R. Cowsar, D. H. Lewis, R. J. Cosgrove, C. T. Riddle, S. L. Lowry and T. Epperly, *Fertil. Steril.* **31**, 545 (1979).
15) J. L. Bollman, J. C. Cain and J. H. Grindlay, *J. Lab. Clin. Med.*, **33**, 1349 (1948).
16) T. Ogasawara, Y. Masuda, S. Goto, S. Mori and T. Oki, *Jpn. J. Antibiot.*, **34**, 52 (1981).
17) R. Wada, S.-H. Hyon, Y. Ikada, Y. Nakao, H. Yoshikawa and S. Muranishi, *J. Bioactive Compatible Polym.* **3**, 126 (1988).
18) T. Higuchi, *J. Pharm. Sci.*, **50**, 874 (1961).
19) T. Oki, N. Shibamoto, H. Iguchi and M. Shirai, *Jpn. J. Antibiot.*, **33**, 163 (1980).
20) L. V. Leak, *Fed. Proc.*, **35**, 1863 (1976).
21) H. Yoshikawa, S. Muranishi, C. Kato and H. Sezaki, *Int. J. Pharmaceut.*, **8**, 291 (1981).
22) Y. Kaji, K. Uekama, H. Yoshikawa, K. Takada and S. Muranishi, *Int. J. Pharmaceut.*, **24**, 79 (1985).
23) T. Takahashi, M. Mizuno, Y. Fujita, S. Ueda, B. Nishioka and S. Majima, *Gann*, **64**, 345 (1973).
24) K. Hirano and C. A. Hunt, *J. Pharm. Sci.*, **74**, 915 (1985).
25) A. Hagiwara and T. Takahashi, *Cancer*, **59**, 241 (1987).