MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 29, No. 10, pp. 1149–1153, 2003

RESEARCH PAPER

Continuous Release of Interleukin-2 from Liposomal IL-2 (Mixture of Interleukin-2 and Liposomes) After Subcutaneous Administration to Mice

Eri Kanaoka,* Kouji Takahashi, Takayoshi Yoshikawa, Hiroaki Jizomoto, Yoshitaka Nishihara, and Koichiro Hirano

Drug Metabolism & Pharmacokinetics, Developmental Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka, Japan

ABSTRACT

Recombinant interleukin-2 (IL-2) was strongly and almost completely adsorbed onto small and hydrophobic liposomes by simple mixing under optimal conditions (liposome: DSPC-DSPG; molar ratio, 10:1; 30–50 nm in size, ratio of IL-2 to liposome: 4.0 JRU/nmol lipid). This liposomal IL-2 displayed better distribution after intravenous administration in mice and improved therapeutic effect against experimental M5076 metastases, as reported previously.[1] In this study, the elimination of IL-2 from the dosing area was investigated when the liposomal IL-2 was administered to mice subcutaneously. The results suggest that the release of IL-2 from this liposome was continuous and almost complete. The mean residence time (MRT) of IL-2 in the dosing area was 11.0 ± 1.65 hr. This resulted in the 8-fold times enhancement of MRT in the systemic circulation by the presence of liposomes, and IL-2 was detected in the serum for 2 days. Using this liposomal IL-2 is expected to have the potential to decrease the number of injections and enhance the efficacy of IL-2 in immunotherapies and therapies against tumor.

Key Words: Recombinant interleukin-2 (IL-2); Liposome; Adsorption; Subcutaneous; Mean residence time (MRT).

*Correspondence: Eri Kanaoka, Research Laboratories, Shionogi & Co., Ltd., 12-4, Sagisu 5-chome, Fukushima-ku, Osaka 553-0002, Japan; Fax: +6-6458-0987; E-mail: eri.kanaoka@shionogi.co.jp.

DOI: 10.1081/DDC-120025872
0363-9045 (Print); 1520-5762 (Online)

www.dekker.com

1. INTRODUCTION

Liposomes can incorporate hydrophobic or hydrophilic compounds in lipid bilayers and are often used as drug carriers because they are non-toxic and biodegradable materials.[2–5] By using mice, we showed the efficacy of liposomal IL-2, a simple mixture of recombinant interleukin-2 (IL-2) and liposomes. This preparation could enhance the duration time in the systemic circulation and the delivery of IL-2 to the liver and spleen after intravenous administration, thus resulting in improvement of the therapeutic effect against experimental metastases of M5076.[1,6,7] Interleukin-2 is one of cooperative and not cyto-cidal cytokines, but it has been used in treatments for many kinds of cancer and in immunotherapies.[8–14] Treatment using subcutaneous administration of IL-2 is now performed in clinical studies.[16–18] The efficacy of its direct injection into tumor (melanoma) has also been reported.[15] In this study, liposomal IL-2 was administered subcutaneously to mice, and the release of IL-2 into the systemic circulation and its elimination from the dosing area were investigated. Interleukin-2 was released to the systemic circulation from under the skin, continuously.

2. MATERIALS AND METHODS

Materials

The commercial formulation (Imunace®35, 350,000 JRU/vial) of IL-2 was a product of Shionogi & Co., Ltd. (Osaka, Japan). Distearoyl-L-α-phosphatidylcholine (DSPC) and distearoyl-L-α-phosphatidylglycerol (DSPG) were obtained from Nichiyu Liposome Co., Ltd. (Tokyo, Japan). Tertiary butyl alcohol and maltose were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). NaCl, maltose, NaH_2PO_4, and $Na_2HPO_4 \cdot H_2O$ were purchased from Nacalai Tesque Inc. (Kyoto, Japan). These reagents were all of analytical grade. Dulbecco's phosphate-buffered saline (PBS, Ca^{+2} and Mg^{+2} free) was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Triton-X100 was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The H_2O used was of injectable grade and obtained from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan).

Methods

Liposomes (30–50 nm) were prepared by using a Nanomizer (Sayama Trading, Tokyo, Japan) as reported previously.[19] The liposome size was determined by a quasi-elastic light-scattering method using a submicron particle analyzer (Model N4, Coulter Co., Ltd., FL, USA).

Liposomal IL-2 was prepared as a simple mixture of DSPC-DSPG liposome suspension (molar ratio, 10:1; 30 μmol/mL liposome) and Imunace® (IL-2 lyophilized). The mixture ratio selected was about 6 JRU/nmol lipid, which was the ratio for high association % between DSPC-DSPG liposomes and IL-2, as reported previously.[7]

Five-week-old male mice (BDF1) were obtained from Japan, SLC Inc. IL-2 (1.5×10^6 JRU/kg) or liposomal IL-2 (1.75×10^6 JRU/kg, 300 μmol lipid/kg) was administered subcutaneously into the back of the each mouse anesthetized with diethyl ether. Whole blood was taken from the heart of an anesthetized mouse at each sampling time (1, 3, 6, 12, 24, 48, and 72 hr). The serum was obtained by centrifugation (3000 rev/min, 10 min) and diluted with PBS solution [containing Triton-X100 (2% w/v)] used for IL-2 immunoassay. The tissue of the dosing area (about 2 cm × 2 cm) under the back skin of the mouse was cut off and prepared as 10% homogenates with PBS solution (containing Triton-X100 (2% w/v)] for IL-2 assay. The percent recovery was calculated by using the amount recovered immediately after the subcutaneous administration of the same dose.

The concentration of IL-2 was determined by the immunoassay using the ELISA system (BIOTRAK™, human IL-2) obtained from Amersham Int. Plc. (Buckinghamshire, England). The concentration of phosphatidylcholine was determined by an enzyme method using a PL assay kit obtained from Nippon Shoji Co., Ltd. (Osaka, Japan).

3. RESULTS AND DISCUSSION

Figure 1a shows the time course of percent recovery of IL-2 from the dosing area after subcutaneous administration of liposomal IL-2 to mice. The serum concentration profiles were shown as a reference at the same time comparing with that of free IL-2 (Fig. 1b).[7] Table 1 summarizes the pharmacokinetic parameters after the subcutaneous administration of liposomal IL-2 and free IL-2 to the mice.

Free IL-2 was eliminated from the systemic circulation very quickly after intravenous and subcutaneous administration, and the mean residence time (MRT) was calculated as 0.2 hr and 1.7 hr using

with non-compartmental method, respectively.[7] On the other hand, liposomal IL-2 could control the release of IL-2, and the MRT of IL-2 was enhanced to 12.8 ± 6.33 hr. The MRT of IL-2 remaining in the dosing area was calculated to be 11.0 ± 1.65 hr after administration of liposomal IL-2. The MRT of free IL-2 was not evaluated, but it is clear from the results of the serum concentration that the MRT was enhanced by the presence of liposomes. The MRT of liposomal IL-2 in the dosing area was almost equal to that of the serum concentration. This suggests that the mean absorption time (MAT) of IL-2 from under the skin to the systemic circulation would be very fast. The enhancement of the deposit time of IL-2 in the dosing area is considered to be about 8-fold longer from the results of the serum concentration.

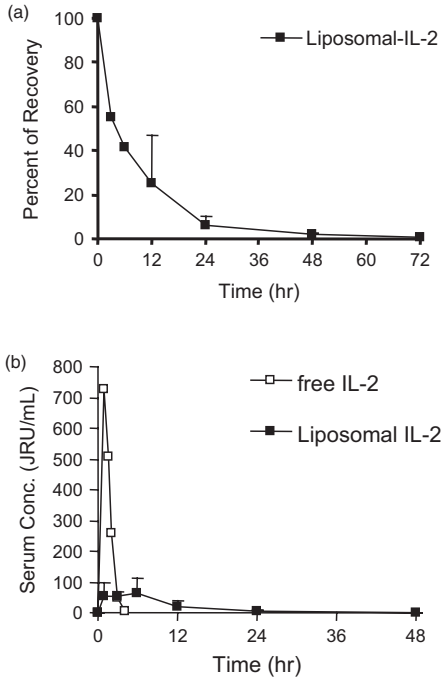

Figure 1. (a) Time course of percentage of IL-2 remaining in the dosing area after subcutaneous administration of liposomal IL-2 to mice. Dose of liposomal IL-2: 1.75×10^6 JRU/kg, 300 μmol lipid/kg, liposome: DSPC-DSPG, 10:1, ($N = 3$). The recovery at time 0 represents 100%. (b) Time courses of serum concentration of IL-2 after subcutaneous administration of free IL-2 and liposomal IL-2 to mice. Dose of free IL-2: 1.5×10^6 JRU/kg, ($N = 2$), dose of liposomal IL-2: 1.75×10^6 JRU/kg, 300 μmol lipid/kg, liposome: DSPC-DSPG, 10:1, ($N = 3$).

The bioavailability of IL-2 after subcutaneous administration of free IL-2 and liposomal IL-2 was 94% and 80%, respectively, which was determined as percent from the AUC (area under the serum concentration) after subcutaneous administration, with reference to the similar data after intravenous administration of free IL-2 reported previously.[7] The bioavailability after subcutaneous administration of liposomal IL-2 was very high.

The AUCs of the two formulations were almost equal. However, the maximum serum concentration of IL-2 was decreased to 1/7 with this liposomal IL-2. The maximum serum concentration of IL-2 after subcutaneous administration of liposomal IL-2 was much lower than that after intravenous administration. The side effects of IL-2 at a high dose and their alleviation by the incorporation in liposomes were reported for the intravenous administration.[2,20] Treatments by low-dose subcutaneous administration and continuous infusion of IL-2 have been done in clinical studies.[18,21] Suitable enhancement of the duration time and a decrease of the maximum serum concentration are often meaningful in the point of decrease of toxicity.

Table 1. Pharmacokinetic parameters of IL-2 in serum and the dosing area after subcutaneous administration to mice.

		Serum				Dosing area
Sample	N	Cmax (%dose/mL)	$AUC_{0-48\,hr}$ (%dose hr/mL)	MRT (hr)	Bioavailability[a] (%)	MRT (hr)
Free IL-2	2	1.88	2.71	1.71	94	NE.[b]
Liposomal IL-2	3	0.27 ± 0.06	2.29 ± 1.32	12.8 ± 6.33	80	11.0 ± 1.65

Pharmacokinetic parameters of IL-2 in serum and remaining in the dosing area were calculated by using with non-compartmental method after subcutaneous administration of free IL-2 and liposomal IL-2. Dose of free IL-2: 1.5×10^6 JRU/kg, ($N = 2$), dose of liposomal IL-2: 1.75×10^6 JRU/kg, 300 μmol lipid/kg, liposome: DSPC-DSPG, 10:1, ($N = 3$).
[a]Bioavailability was calculated by the data of intravenous administration of free IL-2 reported previously.[6] Dose of IL-2: 5×10^5 JRU/kg, $AUC_{(0-\infty)}$ in blood: 2.87% dose hr/mL.
[b]NE: not evaluated.

Usually, liposome is used as a carrier, with drug inside or between its lipid bilayers. However, continuous and complete release of drug from liposomes is thought to be difficult. Sometimes, a part of the incorporated in liposomes is remaining after the end of a continuous release. Our liposomal IL-2 is a simple mixture of lyophilized IL-2 and liposome suspension which were prepared separately. Thus, IL-2 is not incorporated into the liposomes but adsorbed on the surface of them. Our results suggest also that IL-2 is stable on the surface of the liposomes and under the skin. That results in the continuous and almost complete release and a significant enhancement of the duration time of IL-2 in the dosing area. We have preliminary results about the antitumor effect in mice bearing M5076 after the multiple administrations of free IL-2 subcutaneously (data not shown). This effect is supposed to cause by the continuous release of IL-2 to the systemic circulation.

On the other hand, direct injection of IL-2 into a tumor (melanoma) has also been reported.[15,22] It is easy to prospect that the elimination of IL-2 from the tumor would be very fast. So, using this liposomal IL-2 is expected to improve the therapeutic effects or decrease the number of injections, because liposomal IL-2 can enhance the duration time in the dosing area.

4. CONCLUSION

Liposomal IL-2 (mixture of Imunace®, 350,000 JRU, and 2 mL of liposome suspension), prepared separately [DSPC-DSPG (10:1), 30–50 nm in size, 30 μmol/mL] improved the duration time in the systemic circulation of IL-2, when administered to mice subcutaneously. The MRT of this liposomal IL-2 was enhanced to about 8 times that of free IL-2. This enhancement of the duration time of liposomal IL-2 in the dosing area is the potential to improve the therapeutic effect of IL-2 in the local therapies against tumor.

REFERENCES

1. Kanaoka, E.; Takahashi, K.; Yoshikawa, T.; Jizomoto, H.; Nishihara, Y.; Uchida, N.; Maekawa, R.; Hirano, K. A significant enhancement of therapeutic effect against hepatic metastases of M5076 in mice by a liposomal interleukin-2 (mixture). J. Control. Release **2002**, *82*, 183–187.
2. Gause, B.; Longo, D.L.; Janik, J.; Smith, I.I.; Curti, B.; Ochoa, A.; Kopp, W.C.; Anderson, P.; Urb, W.J. A phase I study of liposome-encapsulated IL-2. Proc. Am. Soc. Clin. Oncol. **1993**, *12*, 293.
3. Khanna, C.; Hasz, D.E.; Klausner, J.S.; Anderson, P.M. Aerosol delivery of interleukin-2 liposomes is nontoxic and biologically effective: canine studies. Clin. Cancer. Res. **1996**, *2* (4), 721–734.
4. Konigsberg, P.J.; Godtel, R.; Kissel, T.; Richer, L.L. The development of IL-2 conjugated liposomes for therapeutic purposes. Biochim. Biophys. Acta **1998**, *1370*, 243–251.
5. Cabanes, A.; Even-Chen, S.; Zimberoff, J.; Barenholz, Y.; Kedar, E.; Gabison, A. Enhancement of antitumor activity of polyethylene glycol-coated liposomal doxorubicin with soluble and liposomal IL-2. Clin. Cancer Res. **1999**, *5* (3), 687–693.
6. Hirano, K.; Kanaoka, E.; Takahashi, K.; Yoshikawa, T.; Nishihara, Y.; Jizomoto, H. Biopharmaceutical studies on interleukin-2 simply mixed with liposomes. Prog. Drug Delivery System **1996**, *V*, 113–116.
7. Kanaoka, E.; Takahashi, K.; Yoshikawa, T.; Jizomoto, H.; Nishihara, Y.; Hirano, K. A novel and simple type of liposome carrier for recombinant interleukin-2. J. Pharm. Pharmacol. **2001**, *53* (3), 295–302.
8. Heys, S.D.; Gough, D.B.; Eremin, O. Immunotherapy with interleukin-2: recent developments. Exp. Opin. Invest. Drugs **1996**, *5* (3), 269–288.
9. Semino, C.; Martini, L.; Queirolo, P.; Cangemi, G.; Costa, R.; Alloisio, A.: Ferlazzo, G.; Sertoli, M.R.; Real, U.M.; Ratto, G.B.; Melioli, G. Adoptive immunotherapy of advanced solid tumor: an eight year clinical experience. Anticancer Res. **1999**, *19*, 5645–5649.
10. Savas, B.; Arsla, G.; Gelen, T.; Karpuzoglu, G.; Ozkaynak, C. Multidrug resistant malignant melanoma with intracranial metastasis responding to immunotherapy. Anticancer Res. **1999**, *19*, 4413–4420.
11. Schiphorst, P.P.; Chang, P.C.; Clar, N.; Schoemaker, R.C.; Osanto, S. Pharmacokinetics of interleukin-2 in two anephric patients with metastatic renal cell cancer. Ann. Oncol. **1999**, *10* (11), 1381–1383.
12. Romanelli, F. Interleukin-2 for the management of HIV infection. J. Am. Pharm. Assoc. **1999**, *39* (6), 867–868.

13. Bruton, J.K.; Koeller, J.M. Evaluation new drugs: recombinant interleukin-2. Pharmacotherapy **1994**, *14* (6), 635–656.
14. Maxwell, A.C.A.; Durrant, L.G.; Scholefield, J.H. Immunotherapy for colorectal cancer. Am. J. Surg. **1999**, *64*, 344–348.
15. Den Otter, W.; Cadee, J.; Gavhumende, R.; De Groot, C.J.; Hennink, W.E.; Stewart, R. Effective cancer therapy with a single injection of interleukin-2 at the site of the tumor. Cancer Immunol. Immunother. **1999**, *48* (7), 419–420.
16. Toh, H.C.; McAfee, S.L.; Sackstein, R.; Multani, P.; Cox, B.F.; Colby, C.; Spitzer, T.R. A phase II study of high dose (HD) cyclophsphamide + carboplatin and interleukin-2 (IL2) activated autologus peripheral blood stem cell transplantation (PBSCT) followed by subcutaneous (SC) IL2 therapy in metastatic breast carcinoma (MBC). Proc. Am. Soc. Clin. Oncol. **1999**, *18* (35 Meet.), 47a.
17. Gonzalez-Barca, E.; Granena, A.; Fernandez-Sevilla, A.; Moreno, V.; Salar, A.; Rueda, F.; Garcia, J. Low-dose subcutaneous interleukin-2 in patients with minimal residual lymphoid neoplasm disease. Eur. J. Haematol. **1999**, *62* (4), 231–238.
18. Queirolo, P.; Ponte, M.; Gipponi, M.; Cafiero, F.; Peressini, A.; Semino, C.; Pietra, G.; Lionetto, R.; Vecchio, S.; Ribizzi, I.; Melioli, G.; Sertoli, M.R. Adoptive immunotherapy with tumor-infiltrating lymphocytes and subcutaneous recombinant interleukin-2 plus interferon-alfa-2a for melanoma patients with nonresectable distant disease: a phase I/II pilot trial. Melanoma Istituto Scientifico Tumori Group. Ann. Surg. Oncol. **1999**, *6* (3), 272–278.
19. Kanaoka, E.; Nagata, S.; Hirano, K. The stabilization of aerosolized IFN-γ by liposomes. Int. J. Pharm. **1999**, *188* (2), 165–172.
20. Terry, H. The therapeutic potential and problems of interleukin-2. Chem. Ind. **1993**, *6*, 663–665.
21. Lissoni, P.; Bolis, S.; Mandal'a, M.; Viviani, S.; Pogliani, E.; Barni, S. Blood concentrations of tumor necrosis factor-alpha in malignant lymphomas and their decrease as a predictor of disease control in response to low-dose subcutaneous immunotherapy with interleukin-2. Int. J. Biol. Markers **1999**, *14* (3), 167–171.
22. Dreau, D.; Bosserhoff, A.K.; White, R.L.; Buettner, R.; Holder, W.D. Melanoma-inhibitory activity protein concentrations in blood of melanoma patients treated with immunotherapy. Oncol. Res. **1999**, *11* (1), 55–61.