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A nanometer lipid emulsion, lipid nano-sphere (LNS®), as a parenteral drug carrier for passive drug targeting

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Abstract

We attempted to develop an artificial lipoprotein-like particle, lipid nano-sphere (LNS®), incorporating dexamethasone palmitate (DMP). LNS is 25–50 nm in diameter and is composed of soybean oil and egg lecithin. Potential drug carriers were compared with a conventional fat emulsion for intravenous nutrition, lipid microsphere (LM, $d = 200$–300 nm), which is already used clinically. LM easily entered reticuloendothelial systems, such as the liver, and was rapidly cleared from the circulation. However, LNS showed much higher plasma levels of DMP after intravenous administration to rats and recovered more than 80% of the injected dose in the perfusate in single-pass rat liver perfusion. The calculated volume for the distribution of the lipid emulsion within the liver showed that LNS underwent fenestration and was distributed into the Disse space in the liver. Because of the lower uptake of LNS particles by the liver, LNS showed good recovery from the liver and prolonged the plasma half-life of DMP after intravenous injection. In addition, higher efficiency in the targeting of DMP into inflammation sites and higher anti-inflammatory efficacy were observed in LNS. Thus, LNS easily and selectively passed through the leaky capillary wall by passive diffusion depending on the plasma concentration. Nanometer-sized lipid emulsion particles, LNS, seem to be a promising carrier system for passive drug targeting of lipophilic drugs.

Keywords: Lipid emulsion; Nanoemulsion; Passive drug targeting; Liver perfusion; Dexamethasone palmitate

1. Introduction

Most biopharmacy and pharmacology texts note the importance of plasma proteins, such as albumin and alpha-1-acid glycoprotein, in the ultimate distribution of drug molecules.

Lipoproteins are present in naturally occurring particles in the blood circulation and interstitial fluids, and have the capacity to interact with pharmacological levels of drug molecules. For example, low density lipoprotein (LDL) is a major carrier of cholesterol in the circulation and enters cells by an LDL receptor-mediated endocytotic mechanism. Lipophilic drugs are expected to combine with LDL and are possible carriers for site-specific drug delivery (Rensen et al., 2001; Wasan and Cassidy, 1998). The uptake of lipoproteins by cell types found in numerous tis-

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sues and organs occurs by a transport process known as receptor-mediated endocytosis. Lipoprotein particles, when combined with pharmacological agents, represent unique carrier particles for characterizing drug distribution including extravascular transit, cellular uptake processes, and finally pharmacological responses.

We also showed previously the in vivo efficacy of lipoproteins as carriers of the pharmacological active agent, beta-sitosteryl-beta-D-glucoside (SG). The pharmacological effects were closely related to lipoprotein metabolism and were observed only after intravenous injection of the complexes of SG with lower density lipoproteins (Seki et al., 1985; Sugiyama and Seki, 1991).

On the other hand, numerous artificial drug carriers for drug delivery, such as liposomes and emulsions, have been proposed to improve the distribution of pharmacological active agents. Most exogenous colloidal drug carriers have some difficulty in successful in vivo drug targeting. The critical problem seems to be the apparent non-specific uptake of the carrier particles as a foreign substance by tissues that have developed a reticuloendothelial system, such as the liver and spleen. Many techniques are known to avoid this phenomenon using polyethyleneglycol derivatives (Greenwald et al., 2003; Otsuka et al., 2003).

We developed an artificial lipoprotein-like particle, lipid nano-sphere (LNS®), which is composed of phospholipids and simple lipids and has a similar diameter (25–50 nm) and lipid compositions to endogenous plasma lipoproteins (Seki et al., 1988, 1994; Sugiyama and Seki, 1991). LNS is considered to be a protein-free analogue of plasma lipoproteins. A conventional fat emulsion for intravenous nutrition, lipid microsphere (LM, $d = 200$–300 nm) is already used clinically as a drug carrier (Mizushima, 1996; Yokoyama and Watanabe, 1996). LNS and LM can be manufactured with the same lipids, such as soybean oil and egg lecithin. The major physical difference between LNS and LM is in their particle diameters. By using dexamethasone palmitate (DMP) as an active compound, we studied not only the behavior in the body but also the carrier potential of LNS for site-specific drug delivery to inflamed tissues compared with that of the conventional LM formulation.

2. Materials and methods

2.1. Materials

Purified egg lecithin and soybean oil for parenteral use were purchased from Q.P. Corporation, Tokyo, Japan and Ajinomoto Co., Inc., Tokyo, Japan, respectively.

DMP and ^{3}H-labeled DMP (^{3}H-DMP) were synthesized with dexamethasone (or [6,7-^{3}H]-dexamethasone, 35–50 Ci/mmol, purchased from NEN Life Sci. Product Inc., Boston, MA, USA) and palmitoyl chloride by means of conventional coupling, and were purified in our laboratory (Mizushima et al., 1982a).

All other chemicals were of reagent or analytical grade.

2.2. Preparation of lipid emulsions

The composition and representative diameter of LNS and LM are shown in Table 1. LNS was composed of equal amounts of purified egg lecithin and soybean oil. LM was composed of the same ingredients as LNS and the ratio of egg lecithin to soybean oil was 0.12–1 by weight. The DMP concentration of each lipid emulsion was set at 4 mg/ml (6.3 μmol/ml), and the aqueous phase of both lipid emulsions was 0.24 M glycerin.

DMP (or ^{3}H-DMP) was dissolved in soybean oil and mixed with purified egg yolk lecithin. All ingredients for LNS and LM were homogenized and emulsified in 0.24 M glycerin with a probe type sonicator (Sonifier Model 250D, Branson Ultrasonic Corporation, Danbury, CT, USA), at 80–100 W at 4 °C for 60 and 20 min, respectively. In some cases, the mixtures were emulsified sufficiently with a Microfluidizer™ 110-Y or 110-E/H (Mocrofluidics International Corporation, Boston, MA, USA) at 100 MPa for LNS and at 50 MPa for LM with a Y-type Interaction Chamber by applying 0.2–5% of backpressure (Saheki

Table 1
Composition of LNS and LM

	DMP (mg/ml)	Soybean oil[a] (mg/ml)	Egg lecithin[a] (mg/ml)	Diameter[b] (nm)
LNS	4	50	50	25–50
LM	4	100	12	200–300

[a] Soybean oil and egg lecithin were of parenteral grade.
[b] Diameter was determined by dynamic light scattering.

and Seki, 1998). These two formulations were used without further purification.

The particle size of the lipid emulsions was determined by a laser dynamic light-scattering particle sizer, DLS-700 (Otsuka Electonics, Inc., Osaka, Japan), after diluting the emulsion with distilled water.

For transmission electron microscopy, the samples were placed on a specimen mesh coated with collodion film, negatively stained with 3% sodium phosphotungustate (pH 7.0), and observed with a Hitachi H-7100 transmission electron microscope.

2.3. Plasma and tissue concentrations

Male Sprague–Dawley rats were purchased from Japan S.L.C. (Hamamatsu, Japan) and used for all experiments without fasting at 6–8 weeks of age. LNS or LM containing ^{3}H-DMP was administered intravenously to rats at a dose of 0.5 mg of DMP per kilogram. Venous blood (0.2 ml) was collected into heparin tubes, and plasma was obtained by centrifugation. After rats were sacrificed by collecting blood from the aorta under ether anesthesia, tissues were removed, weighed, and homogenized with methanol (10%, w/v). The supernatant was obtained by centrifugation of the homogenate. The plasma and the supernatant were used to determine total radioactivity by liquid scintillation counting.

2.4. In situ single-pass liver perfusion study

The operative procedure for in situ rat liver perfusion has previously been described in detail (Tyrrell et al., 1977; Nishida et al., 1990; Takino et al., 1995), The perfusate, containing 50% heparinized rat whole blood and 50% Dixon's Krebs bicarbonate buffer (pH 7.4), was circulated using a peristaltic pump at a flow rate of 8 ml/min. LNS or LM containing DMP (16 μg/0.1 ml) was introduced into the portal vein using a six-position rotary valve injector. Every drop of the venous flow samples was collected into tubes and used for HPLC analysis as described elsewhere (Tauchi et al., 1999).

2.5. Delivery into an inflamed site

To determine the efficiency of DMP delivery by LNS or LM into an inflamed site, two different experiments were performed as follows.

An experimental model of pleuritis in rats was induced by an intrapleural injection of 0.1 ml of 2% lambda-carageenin (Sigma-Aldrich, St. Louis, MO, USA) to rats. LNS or LM containing DMP was administered intravenously to the rats at a dose of 2.5 mg/kg of DMP 2 h after the intrapleural injection of carrageenin. After the rats were sacrificed at various intervals after intravenous administration by collecting blood from the aorta under ether anesthesia, the pleural cavity was opened and the pleural exudates collected. An aliquot of the exudate was centrifuged (3000 rpm for 10 min) and the pleural cells were removed. The pleural exudate and the supernatant were used to determine DMP by HPLC analysis as described above.

Another experimental model in rats, carrageenin-induced paw edema was induced by a subcutaneous injection of 0.1 ml of 1% lambda-carrageenin into the right paw. LNS or LM containing ^{3}H-DMP was administered intravenously to the rats at a dose of 0.5 mg/kg of DMP 2 h after carrageenin injection. At 15 min after the intravenous administration of LNS or LM, rats were sacrificed by collecting blood from the aorta under ether anesthesia. Both paws, the inflamed and normal sides, were amputated at ankle level and weighed. The radioactivity in the whole paw was recovered and determined as $^{3}H_2O$ by burning under oxygen with a sample oxidizer.

2.6. Anti-inflammatory activity

To determine the pharmacological potential of the LNS and LM formulations, the most popular and classical assay model, carrageenin-induced paw edema, was used. 0.1 ml of 1% lambda-carrageenin was administered subcutaneously into the right paw. The volume of the paw was recorded over time during the experiment. LNS or LM containing DMP was administered intravenously to the rats 30 min after carrageenin injection. Paw volumes were compared with the control rats to calculate the inhibition percentage.

3. Results and discussion

3.1. Physicochemical properties of LNS

LNS preparations containing DMP in this study showed a similar average particle size with a very

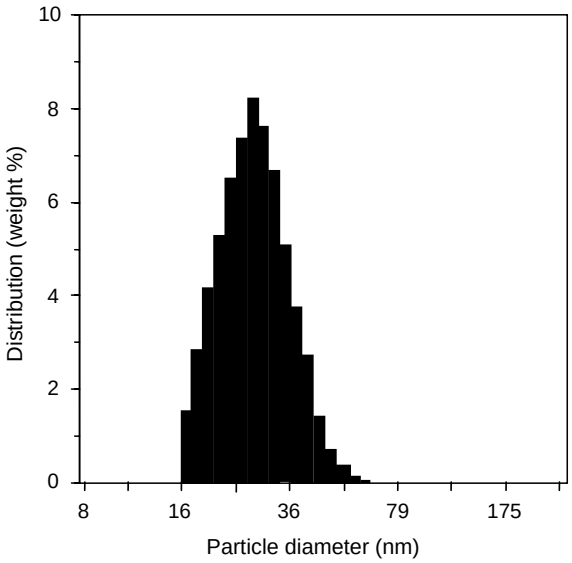

Fig. 1. Distribution of LNS particle diameters measured by dynamic laser light-scattering spectrophotometry.

narrow distribution range to the plasma lipoproteins, especially to low density lipoproteins and to very low density lipoproteins (Fig. 1). LNS and LM ranged from 25–50 nm to 200–300 nm in average diameter, respectively. A representation of the conventional negative stain method for LNS and LM is shown in Fig. 2. There were no differences in diameter by laser particle-size analysis or in morphological observation with transmittance electron microscopy. There was also no difference in diameter by two emulsification methods, i.e. by sonicator and Microfluidizer. The apparent surface potentials of the lipid emulsion particles were estimated with Zetasizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) after diluting the emulsion with distilled water (Zhang and Kirsch, 2003). They were -52.8 ± 3.0 mV for LNS and -49.7 ± 4.2 mV for LM. Both lipid emulsions were stable throughout this study even at room temperature, i.e. there was no change in their diameter during storage for more than a year when they were contained in ampoules with nitrogen gas. No free DMP was found in either lipid emulsion formulation during storage.

As shown in Table 1, LNS consists of a larger amount of purified egg lecithin, to stabilize a large surface area based on the smaller particle size than LM. The relationship between their diameters and the composition ratio of soybean oil to egg lecithin were estimated physicochemically (Handa et al., 1990). The diameter of LNS in this study corresponded well with

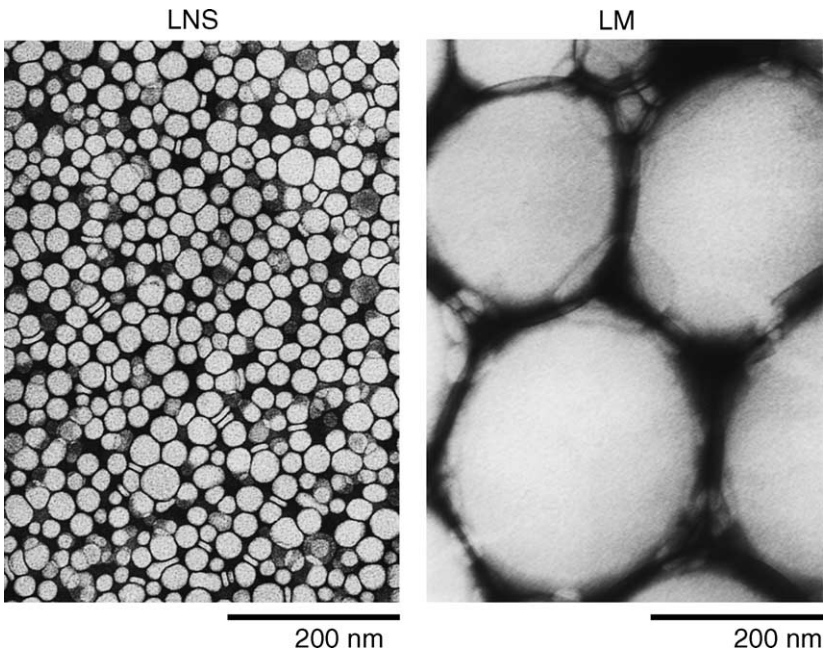

Fig. 2. Electron micrographs of negatively stained LNS and LM.

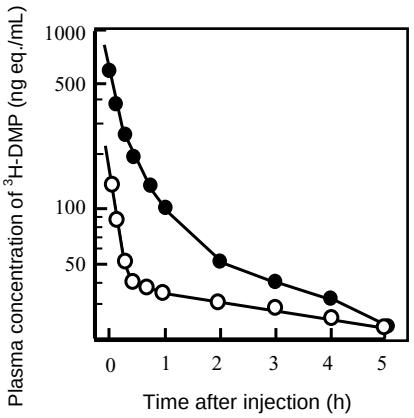

Fig. 3. Plasma radioactivity after the intravenous administration of lipid emulsions incorporating ^{3}H-DMP to rats (dose, 0.5 mg/kg). Each point represents the mean ± S.D. of three rats. Closed circles, LNS; open circles, LM.

the theoretical composition ratio of soybean oil as a core material and lecithin as the surface material of lipid particles. As LNS and LM were prepared using the same soybean oil and egg lecithin, but with different ratios, the major difference in the physicochemical characteristics of LNS and LM seems to be their diameters.

3.2. Plasma and tissue concentrations

Fig. 3 shows the plasma radioactivity levels after an intravenous injection of LNS or LM containing ^{3}H-DMP. The plasma levels after administration of LM decreased rapidly. Even at an equivalent dose (0.5 mg/kg), LNS gave a plasma concentration around three to five times higher than LM up to an hour after intravenous injection. The $AUC_{0-1\,h}$ was 228.4 ng eq./ml h for LNS and 52.0 ng eq./ml h for LM. This difference in plasma concentration disappeared 5 h after injection.

In the tissue distribution study shown in Fig. 4, injected ^{3}H-DMP with LM was found in a relatively high concentration in the liver and spleen. In contrast, tissue distributions of ^{3}H-DMP were very low when administered with LNS. These data suggest that the tissue distribution clearances of LNS were lower, but that the liver and spleen took up LM very rapidly.

A prolonged circulating emulsion carrier system has been reported elsewhere (Takino et al., 1994). We have also reported that LNS showed a higher plasma concentration and a lower uptake of drugs by organs with a reticuloendothelial system after intravenous administration to mice, rats, dogs, and monkeys in comparison with the conventional formulations (Seki et al.,

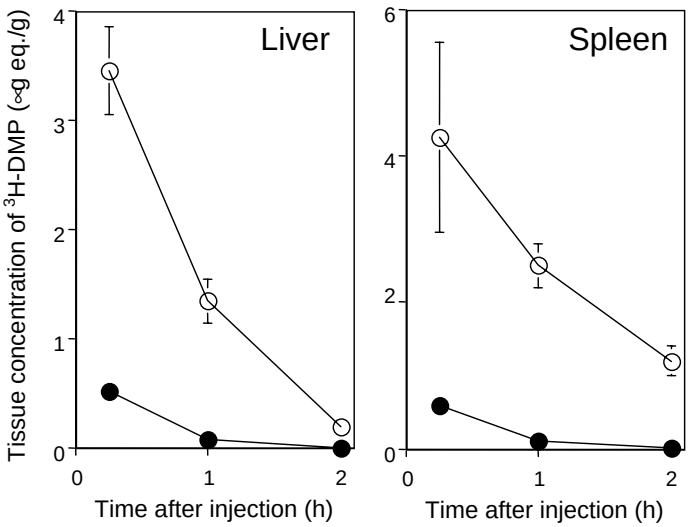

Fig. 4. Tissue distribution of radioactivity after the intravenous administration of lipid emulsions incorporating ^{3}H-DMP to rats (dose, 0.5 mg/kg). Each point represents the mean ± S.D. of three rats. Closed circles, LNS; open circles, LM.

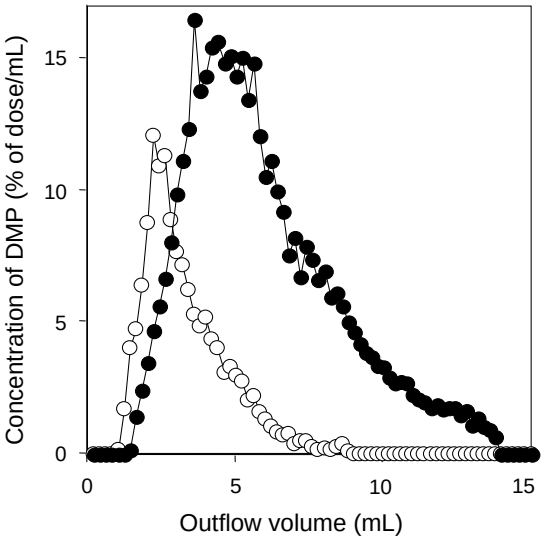

Fig. 5. Typical outflow patterns of DMP obtained for LNS and LM via in situ single-pass liver perfusions in rats. Closed circles, LNS; open circles, LM.

1988, 1994; Fukui et al., 2003a). Isolated rat peritoneal macrophages took up LM but hardly took up any LNS in vitro (data not shown). Our group demonstrated recently that LM delivered larger amounts of radioactivity to non-parenchymal cells than to parenchymal cells in the liver, but almost equal amounts were distributed to both cell types by LNS (Fukui et al., 2003a). These results, including this study, indicate that LNS is hardly recognized as a foreign particle by cells with aggressive endocytotic activity.

3.3. Single-pass liver perfusion study

Fig. 5 shows typical outflow patterns obtained for LNS and LM containing DMP via in situ single pass liver perfusion. The drug recovery and AUC of the DMP administered with LNS from the liver were larger than that with LM, and this was due to the lower uptake of LNS by the liver cells. The active metabolite, dexamethasone, was not found in the outflows during this experiment.

The differences in the disposition parameters of DMP between LNS and LM administration in the liver are clearly shown in Table 2 by a moment analysis of the elution profiles shown in Fig. 5. The recovery for LM was only 30% or less in a single pass from the portal vein to hepatic venous flow but that for LNS was more than 80%. An additional phenomenon found in the results shown in Fig. 5 was similar to size-exclusion chromatography using the liver, i.e. a large molecule/particle eluted faster than a small one. When only the drug recovered in the outflows was used for the calculation ($F = 1$), the small particle (LNS) had a larger volume of distribution within the liver, $V = 6$ ml/liver, than the larger particle (LM, $V = 3$ ml/liver). These values apparently correspond with the volumes of the Disse space and the vascular space in the liver (Weisiger et al., 1986). The calculated volume of the distribution of lipid emulsions within the liver showed that LNS underwent fenestration and was distributed into the Disse space. It is therefore possible that LNS could be distributed not only into the vascular space, but also into the Disse space within the liver. Because of lower uptake by the liver cells, LNS can return to the perfusate and is found in the outflow. We conclude that the higher plasma concentration of the drug administered with LNS was caused by lower uptake of the LNS particles by the liver (Fig. 6).

Table 2
Parameters for in situ single-pass liver perfusion of LNS and LM

Parameters	LNS	LM
AUC (% of dose s/ml)	643.6 ± 35.7	210.5 ± 24.1
t (s)	45.1 ± 4.3	22.7 ± 1.2
Recovery (%)	82.9 ± 4.6	27.9 ± 3.2
T_{max} (s)	30.8 ± 4.3	14.3 ± 3.2
C_{max} (% of dose/ml)	15.6 ± 2.1	12.1 ± 5.6
F (%)	85.6 ± 4.7	28.1 ± 3.2
E (%)	14.4 ± 0.9	71.9 ± 8.2
V (ml)	7.0 ± 0.4	10.8 ± 1.1
V (ml) in $F = 1$	6.0 ± 0.3	3.0 ± 0.3

Each value represents the mean ± S.D. in three experiments.

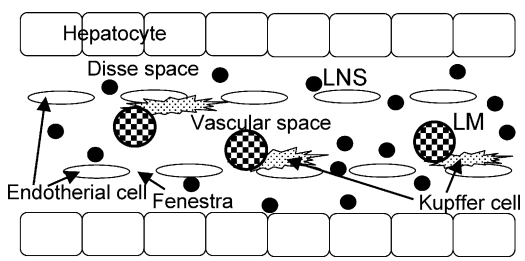

Fig. 6. Schematic illustration of distribution of LNS and LM in the liver in relation to the vascular structure.

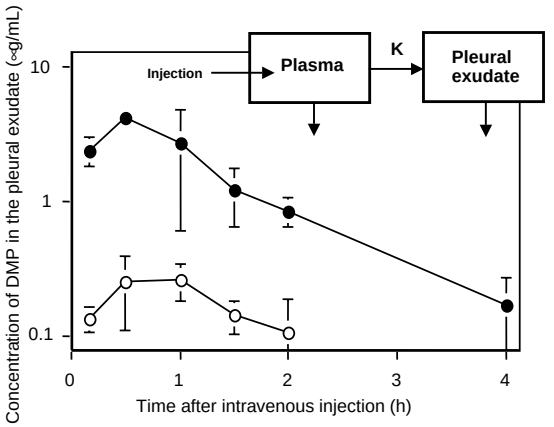

Fig. 7. Concentration of DMP in the pleural exudate after the intravenous administration of lipid emulsions, incorporating DMP, to rats with experimental pleuritis (dose, 2.5 mg/kg). Each point represents the mean ± S.D. of five rats. Closed circles, LNS; open circles, LM.

3.4. Delivery into an inflamed site

LNS showed a higher concentration of DMP not only in plasma but also in pleural exudate at the inflamed site after intravenous administration to an experimental model of carrageenin-induced pleuritis in rats (Fig. 7). The AUC's for both lipid emulsions were 5.29 and 0.53 μg h/ml, respectively. The rate constant (K) for DMP from the circulation to the pleural exudate at the inflamed site obtained with LNS was 0.0025 h^{-1}, greater than that with LM (0.00018 h^{-1}).

In another evaluation in rats with carrageenin-induced paw edema, the concentration of radioactivity in the inflamed right paw after LNS administration was compared with that of LM. As seen in Table 3, LNS showed a more than three times higher concentration of radioactivity than LM in the inflamed paw after intravenous administration to rats with an experimental model of carrageenin-induced paw edema.

Table 3
Distribution of radioactivity in the paws 15 min after the intravenous administration of lipid emulsions incorporating ^{3}H-DMP to rats with experimental paw edema (dose, 0.5 mg/kg)

	LNS	LM	LNS/LM
Inflamed paw (ng/paw)	475 ± 175	154 ± 17	3.08
Right side (ng/g)	204 ± 53	64 ± 8	3.19
Control paw (ng/paw)	164 ± 19	89 ± 24	1.84
Left side (ng/g)	94 ± 8	52 ± 14	1.80
Inflamed/control	2.17	1.23	1.76
Plasma (ng/ml)	448 ± 38	122 ± 12	3.67

Each value represents the mean ± S.D. of three rats.

The mechanism of LNS transportation from the blood stream to the inflamed site is considered a passive diffusion of the particles through the leaky capillary wall. It is well known that the capillary wall leaks due to an inflammatory reaction. As LNS could pass through the fenestra in the liver in the single-pass liver perfusion experiment, it is possible that LNS can pass through the leaky capillary wall at the inflamed site. The LNS has no protein or sugar moiety for site-specific recognition, but it has ultra-fine nano-particles that can pass easily and selectively through a leaky capillary wall accompanying an inflammatory reaction. The potential of LNS for delivering DMP to the inflamed site can therefore be classified into the category of passive drug targeting.

For the same reason, LNS is an effective carrier of lipophilic antimicrobial and antifungal agents, such as amphotericin B, as reported recently (Fukui et al., 2003a). We have also reported that the superior delivery of amphotericin B into inflamed and infectious sites was accomplished by using LNS as a drug carrier (Fukui et al., 2003b).

3.5. Anti-inflammatory activity of LNS-DMP

Fig. 8 shows the dose–response curves of lipid emulsions in the inhibition of carrageenin-induced paw edema in rats. The ED_{50} of LNS-DMP and LM-DMP was around 0.01 and 0.03 mg/kg of DMP, respectively. DMP administered with LNS showed pharmacologically a three times higher efficacy than that with LM in the inhibition of paw edema induced by carrageenin. It apparently corresponded with the drug concentration ratio, 3.19, in the inflamed site, as shown in Table 3 with the same experimental model.

Mizushima et al. reported that LM containing DMP had significantly higher anti-inflammatory efficacy than a conventional water-soluble steroid, dexamethasone phosphate (Mizushima et al., 1982b; Mizushima, 1996). His group showed that a possible mechanism for LM was targeting macrophages accumulated in the inflamed sites. In this report, LNS showed that higher drug levels in the pleural exudate and superior pharmacological activity were found in LNS, sug-

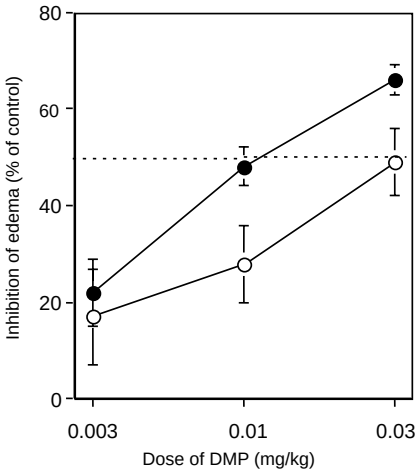

Fig. 8. Dose–response curves of lipid emulsions in the inhibition of carrageenin-induced paw edema in rats. Pharmacological potentials of lipid emulsions evaluated as inhibiting carrageenin-induced paw edema in rats. Each point represents the mean ± S.D. of six rats. Closed circles, LNS; open circles, LM.

gesting that LNS is an excellent carrier for DMP with a different targeting mechanism.

4. Conclusions

From a pharmaceutical standpoint, synthetic lipoprotein-like lipid particles, such as LNS and LM, have advantages in mass production and quality control. This study showed that LNS had superior efficacy to LM as an exogenous parenteral carrier for site-specific drug delivery.

The major features of LNS were: (1) a highly uniform size with stability in various conditions, (2) a higher plasma concentration of drugs and lower hepatic uptake with a larger distribution volume within the liver, (3) improved distribution of drugs into inflammation sites by passive delivery, and (4) easily application to production on an industrial scale with conventional ingredients.

LNS has potential as an effective carrier of drugs for the treatment of diseases such as inflammation, infection, and cancer. LNS shows higher biocompatibility because it is similar to an endogenous lipoprotein and can be composed of the same lipids established as safe with LM, and is digested by the physiological metabolic pathways of lipids. LNS can be sterilized by filtration with a conventional 0.2 μm membrane, freeze-dried using a certain cryoprotectant and rehydrated completely with no changes (Seki et al., 1997). For example, our group reported recently that LNS incorporating amphotericin B, a lyophilized product, is stable at room temperature for at least 2 years with no change in visual appearance, reconstitution time, pH after reconstitution, particle size, zeta potentials, or drug concentration (Fukui et al., 2003b). We are studying several promising new formulations to improve the efficacy of pharmacological active agents with conventional dosage forms. Our studies started with plasma lipoproteins and we are now interested in LNS as a novel and bio-compatible drug carrier for parenteral use in site-specific drug targeting (Sugiyama et al., 1997).

Acknowledgements

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References

Fukui, H., Koike, T., Saheki, A., Sonoke, S., Seki, J., 2003a. A novel delivery system for amphotericin B with Lipid Nano-Sphere (LNS®). Int. J. Pharm. 265, 37–45.

Fukui, H., Koike, T., Nakagawa, T., Saheki, A., Sonoke, S., Tomii, Y., Seki, J., 2003b. Comparison of LNS-AmB, a novel low-dose formulation of amphotericin B with lipid nano-sphere(LNS®), with commercial lipid-based formulations. Int. J. Pharm. 267, 101–112.

Greenwald, R.B., Choe, Y.H., McGuire, J., Conover, C.D., 2003. Effective drug delivery by PEGylated drug conjugates. Adv. Drug Deliv. Rev. 55, 217–250.

Handa, T., Saito, H., Miyajima, K., 1990. Phospholipid monolayers at the triolein–saline interface: production of microemulsion particles and conversion of monolayers to bilayers. Biochemistry 29, 2884–2890.

Mizushima, Y., Hamano, T., Yokoyama, K., 1982a. Use of a lipid emulsion as a novel carrier for corticosteroids. J. Pharm. Pharmacol. 34, 49–50.

Mizushima, Y., Hamano, Y., Yokoyama, K., 1982b. Tissue distribution and anti-inflammatory activity of corticosteroids incorporated in lipid emulsion. Ann. Rehum. Dis. 41, 263–267.

Mizushima, Y., 1996. Lipid microsphere (lipid emulsions) as a drug carrier—an overview. Adv. Drug Deliv. Rev. 20, 113–115.

Nishida, K., Tonenaga, C., Nakane, S., Takakura, Y., Hashida, M., Sezaki, H., 1990. Effect of electric charges on the hepatic uptake of macromolecules in the rat liver. Int. J. Pharm. 65, 7–17.

Otsuka, H., Nagasaki, Y., Kataoka, K., 2003. PEGylated nanoparticles for biological and pharmaceutical applications. Adv. Drug Deliv. Rev. 55, 403–419.

Rensen, P.C.N., de Vrueh, R.L.A., Kuiper, J., Bijsterbosch, M.K., Biessen, E.A.L., van Berkel, T.J.C., 2001. Recombinant lipoproteins: lipoprotein-like lipid particles for drug targeting. Adv. Drug Deliv. Rev. 47, 251–276.

Saheki, A., Seki, J., 1998. Method of producing emulsions and an emulsification apparatus. United States Patent #5,843, 334.

Seki, J., Azukizawa, M., Sugiyama, M., 1988. In vivo evaluation of plasma lipoproteins (LDL) and its protein-free analogue as carriers of pharmacological agents. In: Abstracts of 2nd International ISSX Meeting, Kobe, Japan, p. 53.

Seki, J., Okita, T., Watanabe, M., Nakagawa, T., Honda, K., Tatewaki, N., Sugiyama, M., 1985. Plasma lipoproteins as drug carriers: pharmacological activity and disposition of the complex of beta-sitosteryl-beta-D-glucoside with plasma lipoproteins. J. Pharm. Sci. 74, 1259–1264.

Seki, J., Sasaki, H., Doi, M., Yoshikawa, H., Takahashi, Y., Yamane, S., Fukui, H., Sonoke, S., Yamamoto, H., Hirose, M., Ezure, Y., Ando, T., Ushimaru, K., Sugiyama, M., 1994. Lipid nano-sphere (LNS), a protein-free analogue of lipoproteins, as a novel drug carrier for parenteral administration. IV. J. Control. Release 28, 352–353.

Seki, J., Yamamoto, H., Yamane, S., Takahashi, Y., Ushimaru, K., 1997. Lyophilized fatty emulsions and a process for the production thereof. United States Patent #5,635,491.

Sugiyama, M., Okita, A., Seki, J., 1997. Drug carriers. United States Patent #5,651,991.

Sugiyama, M., Seki, J., 1991. In vivo application of lipoproteins as drug carriers: pharmacological evaluation of sterylglucoside–lipoprotein complexes. In: Shaw, J.M. (Ed.), Lipoproteins as Carriers of Pharmacological Agents. Marcel Dekker, Inc., New York, pp. 315–350.

Takino, T., Konishi, K., Takakura, Y., Hashida, M., 1994. Long circulating emulsion carrier systems for highly lipophilic drugs. Biol. Pharm. Bull. 17, 121–125.

Takino, T., Nagahama, E., Sakaeda, T., Yamashita, F., Takakura, Y., Hashida, M., 1995. Pharmacokinetic disposition analysis of lipophilic drugs injected with various lipid carriers in the single-pass rat liver perfusion system. Int. J. Pharm. 114, 43–54.

Tauchi, Y., Takase, M., Zushida, I., Chono, S., Sato, J., Ito, K., Morimoto, K., 1999. Preparation of a complex of dexamethasone palmitate-low density lipoprotein and its effect on form cell formation of murine peritoneal macrophages. J. Pharm. Sci. 88, 709–714.

Tyrrell, D.A., Richardson, V.J., Ryman, B.E., 1977. The effect of serum protein fractions on liposome–cell interactions in cultured cells and perfused rat liver. Biochim. Biophys. Acta 497, 469–480.

Wasan, K.M., Cassidy, S.M., 1998. Role of plasma lipoproteins in modifying the biological activity of hydrophobic drugs. J. Pharm. Sci. 87, 411–424.

Weisiger, R.A., Mendel, C.M., Cavalieri, R.R., 1986. The hepatic sinusoid is not well-stirred: estimation of the degree of axial mixing by analysis of lobular concentration gradients formed during uptake of thyroxine by the perfused rat liver. J. Pharma. Sci. 75, 233–237.

Yokoyama, K., Watanabe, M., 1996. Limethason as a lipid microsphere preparation: an overview. Adv. Drug Deliv. Rev. 20, 195–201.

Zhang, X., Kirsch, L.E., 2003. An Assessment of techniques for evaluating the physical stability of parenteral emulsions. PDA J. Pharm. Sci. Tech. 57, 300–315.