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Research paper

Edaravone-loaded liposomes for retinal protection against oxidative stress-induced retinal damage

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ABSTRACT

To optimize the retinal protective effects of submicron-sized liposomes (ssLips) containing edaravone for intravitreal administration, we investigated the effects of liposomal formulation on the pharmacological effects. Loading of edaravone into ssLips of around 50% entrapment efficiency was achieved by a calcium acetate gradient method. The *in vitro* radical-scavenging capacity of edaravone-loaded ssLip based on egg phosphatidylcholine (EPC-ssLip) and L-α-distearoyl phosphatidylcholine (DSPC-ssLip) was determined in RGC-5, a neuronal precursor cell line that can be differentiated to resemble retinal ganglion cells. Edaravone-loaded EPC-ssLip scavenged intracellular H_2O_2 radical more strongly than DSPC-ssLip, although there was only a small difference in cellular uptake of edaravone into RGC-5. An *in vivo* *N*-methyl-D-aspartate (NMDA)-induced disease model was used to investigate the retinal protective effects in mice. The edaravone-loaded EPC-ssLip significantly reduced NMDA-induced ganglion cell layer (GCL) cell death compared with free edaravone. Such protective effect was small in the case of DSPC-ssLip. These results may be related to the release profile of the edaravone from ssLips across the inner layers of the retina including GCL, indicating effective retinal protection of EPC-ssLip compared to that of DSPC-ssLip. EPC-ssLip is a promising carrier for edaravone in treating oxidative stress-induced retinal diseases.

1. Introduction

Age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa, and optic nerve damage associated with glaucoma are some of the vision impairments afflicting the posterior segment of the eye [1]. These diseases occur via a variety of mechanisms involving, for example, oxidative stress [2], excitatory amino acid (glutamate) [3], nitric oxide [4], or reduced retinal perfusion [5]. Of these mechanisms, oxidative stress, leading to the formation of free radicals, has been implicated as part of the final common pathway for neurotoxicity in a variety of acute and chronic neurodegenerative diseases [6].

Edaravone, a potent free radical scavenger, has been shown to have protective effects against cerebral ischemia–reperfusion injuries in a variety of experimental animal models [7]. The clinical efficacy of edaravone against ischemic brain attack was demonstrated in a randomized, placebo-controlled, double-blind study, and indeed it has been prescribed clinically in Japan for the treatment of acute brain infarction since 2001 [8]. Edaravone was also reported to be a candidate for the treatment of retinal diseases, since edaravone protected against oxidative stress-induced apoptotic retinal cell death through its reactive oxygen species (ROS)-scavenging capacity [9].

Intravitreal injection is a common way to deliver drugs to the retina. Although direct intravitreal injection offers high concentrations of drugs in the retina, the short half-life of this method necessitates frequent administration [10], which is accompanied by the risks of vitreous hemorrhage, retinal detachment, and/or endophthalmitis [11]. In order to enhance bioavailability and avoid repeated intraocular surgical procedures, nano-sized drug delivery systems have been studied extensively [12,13]. Many features of liposomes such as low toxicity, biodegradation, and lack of immunogenicity make them useful in drug delivery [14]. Encapsulation of biologically active molecules in liposomes may increase their bioavailability and may induce prolonged residence in the vitreous

Abbreviations: ssLip, submicron-sized liposomes; DSPC-ssLip, ssLip based on L-α-distearoyl phosphatidylcholine; EPC-ssLip, ssLip based on egg phosphatidylcholine; ROS, reactive oxygen species; RGC-5, neuronal precursor cell line; H_2DCF, dichlorodihydrofluorescein; CM-H_2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate; NMDA, *N*-methyl-D-aspartate; GCL, ganglion cell layer; IPL, inner plexiform layer.

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body, eliminating the need for repeated intraocular injections. The use of liposomes as carriers is also expected to decrease the dose size of administered drugs, thereby reducing their side effects [15].

The use of liposomes for intravitreal administration has been reported by numerous studies [16,17]. However, most of these have only evaluated the drug concentration in the vitreous body. There are only a few reports evaluating the pharmacological effect of liposome-entrapped drugs in the retina [12]. Several reports have found that the cationic nanoparticles aggregated in the vitreous and could not diffuse into the retina [18,19]. The purpose of this study was to optimize the pharmacological effects of liposomes containing edaravone for intravitreal administration. Effect of liposomal formulation on retinal protection was evaluated using NMDA-induced disease model.

2. Materials and methods

2.1. Materials

L-α-distearoyl phosphatidylcholine (DSPC) and egg phosphatidylcholine (EPC) were purchased from Nippon Oil and Fats Co., Ltd. (Tokyo, Japan). 3-Methyl-1-phenyl-2-pyrazolin-5-one (edaravone, MW; 174.2) and H_2O_2 were purchased from Wako Pure Chemicals (Osaka, Japan). Cholesterol and *N*-methyl-D-aspartate (NMDA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). 2-Morpholinoethanesulfonic acid monohydrate (Mes) was purchased from Nakalai Tesque (Kyoto, Japan). Hank's balanced salt solution (HBSS) was purchased from GIBCO BRL (Grand Island, NY, USA). 5-(and-6)-Chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) was purchased from Invitrogen (Carlsbad, CA, USA). Isoflurane was purchased from Nissan Kagaku (Tokyo, Japan). All other chemicals were commercial products of reagent grade.

2.2. Preparation of edaravone-loaded liposomes

Edaravone-loaded liposomes were prepared using the calcium acetate gradient method [20]. The calcium acetate gradient method takes advantage of the large difference in permeability coefficients across lipid bilayers of the cation and acetic acid molecules. A plausible mechanism of drug uptake in response to the transmembrane calcium acetate gradient method is shown in Fig. 1. When transmembrane gradients of calcium acetate are created, membrane-impermeable calcium ions remain trapped in the liposome internal phase while membrane-permeable protonated acetic acid molecules leak out and behave as proton shuttles. The leakage of protonated acetic acid from the liposomes increases the liposome internal pH (Fig. 1B). This pH imbalance (inside base, outside acid) serves as an efficient driving force to load and accumulate weak acids inside lipid vesicles. Weakly acidic molecules, such as edaravone in its protonated (uncharged) form, are pumped into the slightly alkaline liposome internal phase by diffusing through the lipid bilayers. Then, edaravone loses its proton and assumes its charged, membrane-impermeable form (Fig. 1C).

Phospholipid (DSPC or EPC) and cholesterol (8:1 M ratio) were dissolved in a small amount of chloroform in a round-bottom flask and dried in a rotary evaporator under reduced pressure at 40 °C to form a thin lipid film. The film was dried in a vacuum oven overnight to ensure complete removal of the solvent. Subsequently, the lipid film was hydrated at 70 °C with calcium acetate solution (120 mM) by vortexing. The obtained multilamellar vesicles were frozen and thawed in a water bath maintained at 40 °C; this freeze–thaw cycle was repeated 4 times. The submicron-sized liposomes (ssLips) were prepared using an extruder (LipoFast™-Pneumatic; Avestin, Inc., Ottawa, Canada) with a size-controlled

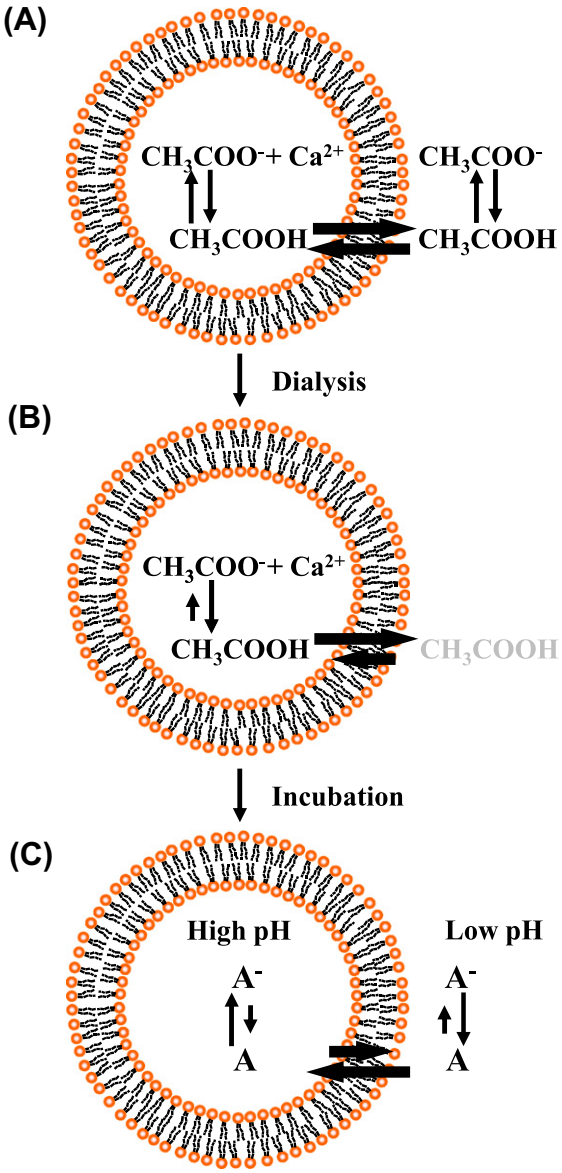

Fig. 1. A plausible mechanism of drug uptake in response to the transmembrane calcium acetate gradient method. (A) At equilibrium the concentration of the protonated (uncharged) acetic acid is the same in both liposome internal and external phases. (B) When transmembrane gradients of calcium acetate are created by dialysis, calcium ions remain trapped in the liposome internal phase while membrane-permeable protonated acetic acid molecules leak out and lead to an increase in the liposome internal pH. (C) Uncharged weakly acidic molecules are pumped into the liposome internal phase and assume their charged, membrane-impermeable forms.

polycarbonate membrane (0.1 μm membrane filter pore size; Whatman Japan KK, Tokyo, Japan). Extrusion was performed 41 times under nitrogen pressure (200 psi). To create a calcium acetate concentration gradient across the liposomal membrane, the calcium acetate of the external liposome medium was replaced with HBSS-Mes buffer (pH 6.0) at 4 °C in two dialysis steps. Edaravone was dissolved in HBSS–Mes buffer and was mixed with ssLips. Remote loading was achieved by incubation of the ssLips at 37 or 60 °C. The final phospholipid concentration in the resultant liposomal suspension was 20.4 μmol/ml.

Conventional liposomes as a reference were prepared by a hydration method. The lipid film was hydrated with edaravone dissolved in HBSS–Mes buffer. The obtained multilamellar vesicles were freeze–thawed and then downsized by the extruder.

2.3. Characterization of liposomes

The particle size of ssLips was measured with an aliquot of the liposomal suspension diluted with a large amount of distilled water by the dynamic light scattering method (Zetasizer Nano ZS, Malvern, Worcestershire, UK). The zeta potential of ssLips was measured using the laser Doppler method (Zetasizer Nano ZS). Each batch was analyzed in triplicate. To determine the efficiency of edaravone into ssLips, edaravone-loaded ssLips were separated from free edaravone by ultracentrifugation (231,000*g*, 45 min) at 4 °C. The edaravone concentration in the supernatant was determined by HPLC carried out under the following conditions: column, ODS-3; mobile phase, methanol/0.1% TFA solution = 1/1; wavelength for spectrophotometry, 240 nm. The entrapment efficiency of ssLips was calculated by the following equation: % entrapment efficiency = $(A_{total} - A_{free}/A_{total}) \times 100$ (1), where A_{total} was the total drug amount in ssLip when methanol was added to the ssLip and A_{free} was the free drug amount in ssLip.

2.4. RGC-5 culture

The neuronal precursor cell line (RGC-5) that can be differentiated to resemble retinal ganglion cells was a gift from Dr. R. Agarwal (Department of Pathology and Anatomy, UNT Health Science Center Fort Worth, TX, USA). RGC-5 were cultured under standard conditions (5% CO_2, 95% humidified air, 37 °C) in DMEM (Sigma–Aldrich) containing 10% FBS (GIBCO), 100 IU/ml streptomycin, and 100 IU/ml penicillin (GIBCO).

2.5. Uptake study

RGC-5 cells were seeded at 6×10^4 cells/well onto 12-well plates. Twenty-four hours later, the culture medium was replaced. The samples containing edaravone were added to the cells and incubated at 37 °C for 1 h. Following incubation, cells were washed twice with ice-cold HBSS–Mes buffer to terminate the uptake experiment. Then, the cells were lysed with Triton X-100 in acetate buffer for 2 h. After the addition of methanol, aliquots from each well were shaken for 1 h. The samples were centrifuged at 36,670*g*, and the edaravone concentration in the supernatant was measured using HPLC. The amount of uptake was normalized to the protein content of each well. The amount of protein in the cell lysate was measured by a BCA protein assay kit (Bio-Rad, CA, USA).

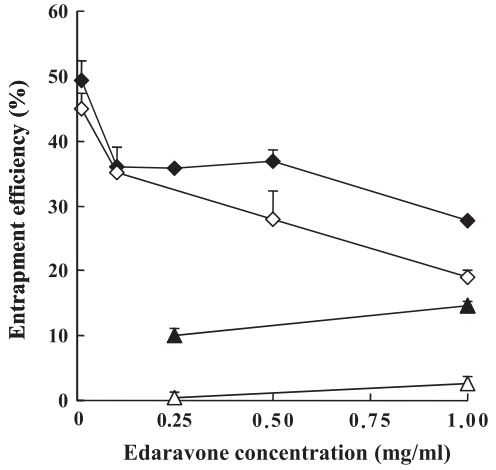

Fig. 2. Changes in the entrapment efficiency of edaravone into liposomes under different loading methods and drug concentrations. ♦, EPC-ssLip and ◇, DSPC-ssLip prepared by the calcium acetate gradient method. ▲, EPC-ssLip and Δ, DSPC-ssLip prepared by the hydration method. Data are shown as mean ± SEM ($n = 3$).

2.6. Radical-scavenging capacity assay

RGC-5 cells were seeded at 1×10^4 cells/well onto 96-well plates. Twenty-four hours later, the culture medium was replaced. The samples containing edaravone (20 μg/ml) or the vehicle (HBSS–Mes buffer) were added to the cells, and incubated at 37 °C for 1 h. The samples were removed and a radical probe CM-H_2DCFDA (10 μM, DMEM containing 1% FBS), which can permeate cell membranes, was loaded into the cells by incubation for 20 min at 37 °C. Once inside the cell, CM-H_2DCFDA is cleaved by intracellular esterases, resulting in a charged form (H_2DCF) that is much better retained by cells than the parent compound (CM-H_2DCFDA) [21]. Then, DMEM was removed and PBS was added to remove the extra probe. H_2O_2 (1 mM) was added to generate the radical species. The radical species (H_2O_2) oxidizes the nonfluorescent H_2DCF to fluorescent dichlorofluorescein. Fluorescence was measured for various intervals using Skanlt RE for Varioskan Flash 2.4 (Thermo Fisher Scientific, Waltham, MA, USA) at excitation/emission wavelengths of 485/535 nm.

2.7. Animals

Six-week-old male albino ddY mice (Japan SLC, Hamamatsu, Japan) were used. They were kept under controlled lighting conditions (12 h/12 h light/dark). All experiments were approved and monitored by the Institutional Animal Care and Use Committee

Table 1
Effect of incubation condition on entrapment efficiency of edaravone into ssLips.

Incubation condition		Phospholipid	Particle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
Time (min)	Temperature (°C)				
10	37	DSPC	124.7	−0.1	23.4
10	60	DSPC	121.6	−1.5	4.1
60	37	DSPC	121.4	1.2	24.7
10	37	EPC	93.9	8.4	29.3
60	37	EPC	90.3	8.1	25.2

Concentration of edaravone was 1 mg/ml.

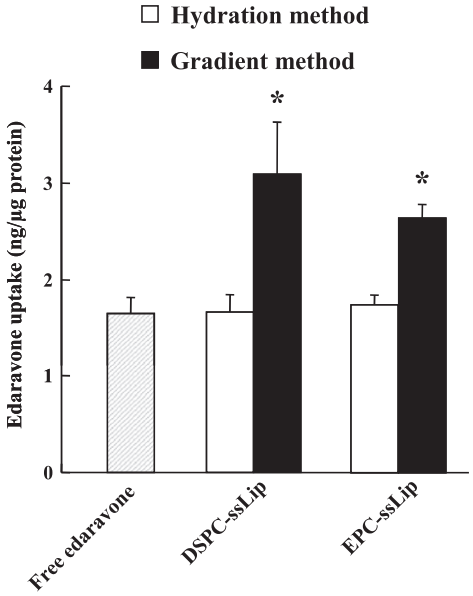

Fig. 3. Comparison of cellular uptake of free edaravone and edaravone-loaded liposomes into RGC-5. Data are shown as mean ± SEM ($n = 3$). $^*P < 0.05$ versus free edaravone treatment.

of Gifu Pharmaceutical University. Mice were anesthetized with 3.0% isoflurane and maintained with 1.5% isoflurane in 70% N_2O and 30% O_2 via an animal general anesthesia machine (Soft Lander; Sin-ei Industry Co. Ltd., Saitama, Japan).

2.8. NMDA-induced retinal damage

To evaluate the protective effects of edaravone, retinal damage was induced by the intravitreal injection of *N*-methyl-D-aspartate (NMDA) into mouse eyes. The cells in the ganglion cell layer (GCL) are exquisitely sensitive to the effects of both glutamate and its analog NMDA. NMDA-induced calcium entry and reactive oxygen species (ROS)-production are well recognized to be mediators of ischemic neuronal damage, and activation of NMDA receptors reportedly generated free radicals and reduced antioxidant ability in the rat hippocampus [22].

Retinal damage was induced by the injection (2 μl/eye) of 2.5-mM NMDA in HBSS–Mes buffer. NMDA solution was injected into the vitreous body of the left eye under the above-mentioned anesthesia. One drop of 0.01% levofloxacin ophthalmic solution (Santen Pharmaceuticals Co. Ltd., Osaka, Japan) was dropped into the treated eye immediately after the intravitreal injection. Seven days after the injection, eyeballs were enucleated for histological analysis. For comparative purposes, untreated retinas were also investigated. Edaravone (1 or 5 nmol), edaravone-loaded liposomes, void liposomes, or vehicle (HBSS–Mes buffer) were co-administered with NMDA at 5 nmol/eye.

The enucleated eyes were fixed overnight in 4% paraformaldehyde at 4 °C. Six paraffin-embedded sections (thickness, 4 μm) were cut parallel with the maximal circumference of the eyeball through the optic disk. These sections were then stained with hematoxylin and eosin. The damage induced by NMDA was evaluated using three sections from each eye for the morphometric analysis. Light microscope images were photographed using a digital camera (COOLPIX 4500; Nikon, Tokyo, Japan), and the cell counts in the GCL at a distance between 375 and 675 μm from the optic disk (nasal and temporal portions) was determined (three sites per section) and then averaged to give a single value. Data from three sections (selected randomly from the six sections) were averaged for each eye and used to evaluate the cell count in the GCL.

2.9. Statistical analysis

Data are presented as mean ± SEM. Statistical comparisons were made using one-way ANOVA followed by Tukey's test. Values of $P < 0.05$ were considered to indicate statistical significance.

3. Results and discussion

3.1. Entrapment efficiencies and loading conditions of edaravone into liposomes

Fig. 2 shows changes in the entrapment efficiency of edaravone into ssLip under different loading methods and drug concentrations. ssLip based on EPC (EPC-ssLip) showed higher entrapment efficiency at all edaravone concentrations in comparison with ssLip based on DSPC (DSPC-ssLip). The extremely low entrapment efficiency of edaravone into DSPC-ssLip (less than 2.6%) implies difficulty of edaravone entrapment into DSPC-ssLip by the hydration method.

The effects of incubation temperature and time on entrapment efficiencies of ssLip prepared by the calcium acetate gradient method were examined as listed in Table 1. DSPC-ssLips incubated for 10 min at 37 or 60 °C exhibited entrapment efficiencies of 23.4% and 4.1%, respectively. The entrapment efficiency of DSPC-ssLip incubated at 37 °C for 10 min was equivalent to that incubated for 60 min. In the case of EPC-ssLip incubated at 37 °C, on the other hand, the entrapment efficiency slightly decreased from 29.3% to 25.2% as the duration increased from 10 to 60 min, respectively. From these results, we identified loading at 37 °C for 10 min as the optimal incubation condition. Calcium ions act as a reservoir inside liposomes to sustain the salt gradient across the membrane. The appropriate incubation condition was selected not only to contribute to the remote loading of edaravone but also to avoid leakage of calcium ions. We confirmed that the entrapment efficiency of edaravone was almost unchanged when edaravone-loaded ssLips were stored at 4 °C for 12 h (data not shown).

As shown in Fig. 2, the drug concentration affected the entrapment efficiency. The entrapment efficiency of edaravone decreased with the increase in edaravone concentration. Maximal entrapment efficiencies of around 50% could be obtained at a low concen-

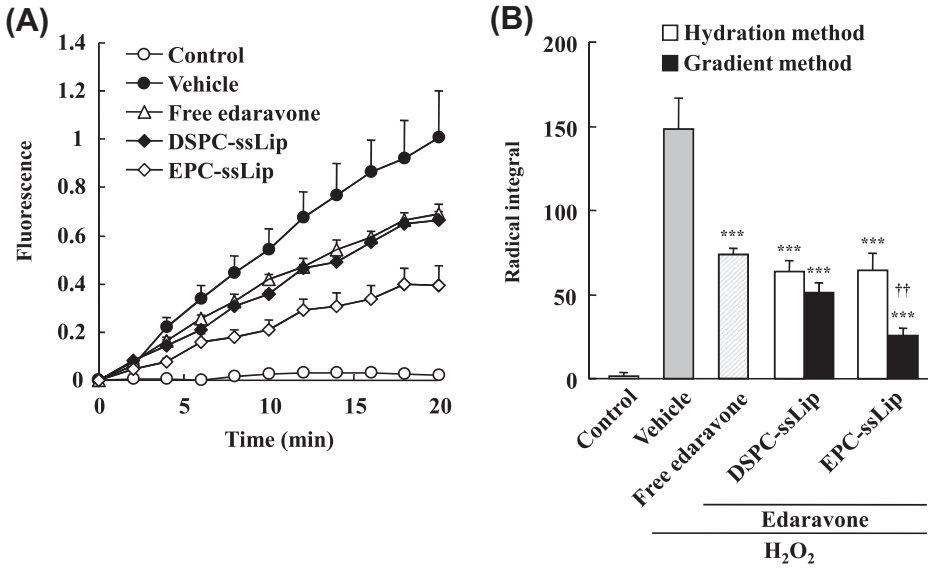

Fig. 4. Time-kinetic data for antioxidant activities of free edaravone and edaravone-loaded liposomes against the production of radical species (H_2O_2) in RGC-5. (A) Fluorescence of DCFH oxidation induced by H_2O_2 was measured for various times after treatment of edaravone (20 μg/ml). (B) Integrals of reactive oxygen species production from time-kinetics graphs. Data are shown as mean ± SEM ($n = 6$). $^{***}P < 0.001$ versus vehicle treatment. $^{††}P < 0.01$ versus free edaravone treatment.

tration of edaravone (0.01 mg/ml) by the calcium acetate gradient method. The dissociation of edaravone in the liposome internal phase may be induced by the decrease in internal pH. Qiu et al. reported that the pH gradient and the internal buffering capacity are very important for remote loading [23]. A high level of accumulated edaravone may deplete the interior buffering capacity and collapse the pH gradient, inhibiting further drug uptake.

3.2. Cellular uptake and radical-scavenging activity of edaravone-loaded liposomes

The cellular uptake of free edaravone and edaravone-loaded ssLips in RGC-5 is shown in Fig. 3. Liposome entrapment was found to significantly enhance the uptake of edaravone. Liposomal phospholipid types did not affect the incorporation of edaravone into RGC-5. Uptake of edaravone-loaded ssLips by the hydration method was not different from than that in free edaravone treatment. The incorporation of edaravone into the liposome internal phase may be necessary to enhance the cellular uptake of edaravone into RGC-5.

A radical-scavenging capacity assay was used with the ROS-sensitive probes of CM-H_2DCFDA to investigate the effect of edaravone-loaded ssLips on intracellular radical production. The kinetics of ROS reactivity after H_2O_2 treatment monitored as fluorescence generation is illustrated in Fig. 4A. As shown in Fig. 4B, treatment with edaravone significantly reduced intracellular radical generation in comparison with treatment with the vehicle. In addition, edaravone-loaded EPC-ssLip reduced the intracellular radical generation more strongly than free edaravone treatment ($P < 0.01$). Enhancement of edaravone uptake into RGC-5 cells contributed to this effective activity of edaravone-loaded ssLips. The antioxidant activity of void liposomes was negligible, because edarav-

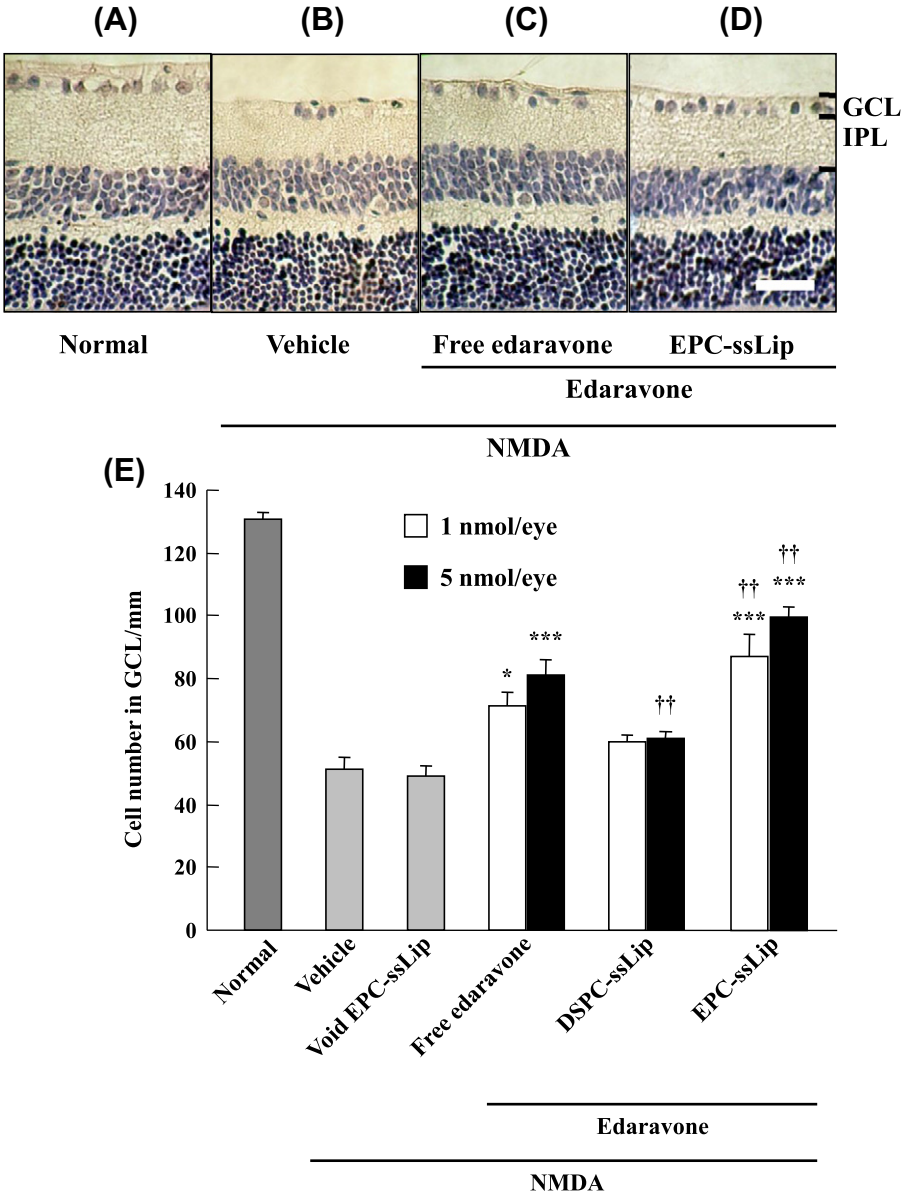

Fig. 5. Effects of free edaravone and edaravone-loaded liposomes on retinal damage induced by intravitreal injection of NMDA in mice. (A) Untreated, (B) NMDA-treated, (C) NMDA plus free edaravone (5 nmol/eye) treated, and (D) retinal cross-sections treated by NMDA plus edaravone (5 nmol/eye)-loaded EPC-ssLip at 7 days after intravitreal injection of NMDA (5 nmol/eye). (E) Retinal damage was evaluated by counting cell numbers in GCL at 7 days after intravitreal injection of NMDA (5 nmol/eye). Data are shown as mean ± SEM ($n = 7$ or 8). Scale bar represents 25 μm. $^{*}P < 0.05$, $^{***}P < 0.001$ versus vehicle treatment. $^{\dagger\dagger}P < 0.01$ versus free edaravone treatment.

one-loaded ssLips by the hydration method had no effect on the intracellular radical generation compared with free edaravone treatment.

Edaravone-loaded DSPC-ssLip was slightly more effective, but there was no significant difference between the effects of free edaravone. This result may be explained by the difference in the rate of edaravone leakage from DSPC-ssLips and EPC-ssLips in RGC-5 cells. We previously reported that DSPC-ssLip was more rigid than EPC-ssLip [24]. It is well known that the rigidity of liposomes determines the rate of drug leakage and the rate of natural degradation after uptake by cells [25,26]. Rigid liposomes are less susceptible to lysosomal enzyme activities than fluid liposomes, causing a delay in liposomal degradation after uptake of the vesicles by cells. Bakker-Woudenberg et al. reported a correlation between the relatively slow degradation of the rigid liposomes and a delayed intracellular release of the encapsulated ampicillin, as reflected in delayed intracellular drug availability [26]. The slow degradation of DSPC-ssLip causing a release of edaravone from liposomes may affect the weak radical-scavenging activity.

3.3. NMDA-induced retinal damage

To evaluate the protective effects of edaravone against retinal damage, NMDA was intravitreally injected into mouse eyes. Intravitreal injection of NMDA decreased the cell number in GCL in the mouse retina (versus the untreated normal retina) (Fig. 5A, B, and E). Edaravone co-administered with NMDA significantly reduced the cell loss in GCL (Fig. 5C and E). When edaravone-loaded EPC-ssLip was co-administered with NMDA, the cell loss in GCL was protected against compared with the vehicle and free edaravone treatment (Fig. 5D and E). It is noteworthy that the protective effect against GCL cell death for low concentrations of edaravone-loaded EPC-ssLip (1 nmol/eye) was higher than that for high concentrations of free edaravone (5 nmol/eye). Therefore, entrapment of edaravone into EPC-ssLip was quite effective in protecting against cell death if a low concentration of edaravone was administrated. We confirmed that the effect of the liposome itself against NMDA-induced retinal damage was negligible, since there was no statistical difference between NMDA alone and NMDA with void EPC-ssLip.

As shown in Fig. 3, liposomes achieved the efficient intracellular delivery of edaravone into RGC-5. The uptake of edaravone into GCL may also be enhanced by liposome entrapment. Several authors indicated that the duration of therapeutic levels in the vitreous body can be increased by liposome encapsulation of a drug. Gupta et al. reported that the terminal elimination constant of fluconazole-loaded liposomes from the vitreous was seven times less than that of the plain drug [27]. Barza et al. reported that the half-life ^{51}Cr-EDTA in the vitreous humor of normal eyes was prolonged by 3.5 to 11-fold by liposome encapsulation [28]. The longer residence time in the retina of edaravone-loaded liposomes compared with free edaravone also reflected a significant enhancement of the retinal protection of edaravone-loaded EPC-ssLip.

On the other hand, the remarkable protection of edaravone loading was not observed for the administration of edaravone-loaded DSPC-ssLip (Fig. 5E). Therefore, DSPC-ssLip was estimated as a less effective carrier of edaravone than EPC-ssLip, in terms of protective effects on NMDA-induced retinal damage. The intraocular behavior of drug-loaded ssLips, i.e., leakage of drug from ssLips, degradation of ssLips and trans-retinal movement, may have contributed to the protective effects of edaravone-loaded ssLips. Some layers of the retina show formidable barriers against the trans-retinal penetration of macromolecules [29]. However, the trans-retinal movement of the nanoparticles with diameters around 100 nm has been demonstrated [30]. In the present study, ssLips having particle size around 100 nm may have been able to penetrate from the inner to the outer side of the retina. The difference in NMDA-induced GCL cell death may relate to the rigidity of liposomes. The DSPC-liposome having higher rigidity may behave an inefficient release of edaravone to EPC-ssLip during the distribution throughout the retinal inner layers of GCL, resulting in an inferior effect on retinal protection compared to the EPC-ssLip system.

4. Conclusions

The calcium acetate gradient method used for the preparation of liposomes yielded high drug entrapment efficiency of water-soluble drugs. Edaravone-loaded liposomes significantly scavenged radical generation in RGC-5 and reduced oxidative stress-induced retinal damage more strongly than free drug via intravitreal injection. Enhancement of cellular uptake and prolongation of vitreous residence time of edaravone contributed to these remarkable effects. The phospholipid type of liposomes remarkably affected the pharmacological effect after intravitreal administration. Liposomes with a fluid rather than rigid structure appear to be good candidates for the delivery of therapeutic molecules such as edaravone in the treatment of oxidative stress-related retinal diseases.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2011.01.019.

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