Review

Innovations in Liposomal DDS Technology and Its Application for the Treatment of Various Diseases

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Liposomes have been widely used as drug carriers in the field of drug delivery systems (DDS), and they are thought to be ideal nano-capsules for targeting DDS after being injected into the bloodstream. In general, DDS drugs meet the needs of aged and super-aged societies, since the administration route of drugs can be changed, the medication frequency reduced, the adverse effects of drugs suppressed, and so on. In fact, a number of liposomal drugs have been launched and used worldwide including liposomal anticancer drugs, and these drugs have appeared on the market owing to various innovations in liposomal DDS technologies. The accumulation of long-circulating liposomes in cancer tissue is driven by the enhanced permeability and retention (EPR) effect. In this review, liposome-based targeting DDS for cancer therapy is briefly discussed. Since cancer angiogenic vessels are the ideal target of drug carriers after their injection and are critical for cancer growth, damaging of these neovessels has been an approach for eradicating cancer cells. Also, the usage of liposomal DDS for the treatment of ischemic stroke is possible, since we observed that PEGylated liposomes accumulate in the site of cerebral ischemia in transient middle cerebral artery occlusion (t-MCAO) model rats. Interestingly, liposomes carrying neuroprotectants partly suppress ischemia/reperfusion injury of these model rats, suggesting that the EPR effect also works in ischemic diseases by causing an increase in the permeability of the blood vessel endothelium. The potential of liposomal DDS against life-threatening diseases might thus be attractive for supporting long-lived societies.

Key words liposome; drug delivery system (DDS); ischemic stroke; cancer; positron emission tomography (PET) imaging

1. INTRODUCTION

The average lifetime in the world, especially that in Japan and other developed countries, has continuously increased since the middle of the 20th century. According to elongation of the lifespan in aged and super-aged societies, the population of elderly often requires various medications. Therefore, the development of oral drugs has proceeded, since oral drugs can be taken non-invasively and are suitable for self-medication. In contrast, many kinds of injection drugs are increasing in usage of liposomal DDS and current status of liposomal drugs have been presented in several review articles.6–8 In liposomal DDS, the circulation time of liposomes is an important factor affecting the outcome of the therapy. Some commercialized liposomal drugs such as AmBisome® and Doxil® are characterized by long circulation. In the case of Doxil®, PEGylated liposomes encapsulating doxorubicin, this liposomal drug is expected to accumulate passively in tumor tissues because of its enhanced permeability and retention (EPR) effect.9,10 In the case of AmBisome, the rigid bilayer of these liposomes is composed of distearoylphosphatidylcholine and cholesterol, enabling a long-circulation time. The EPR effect was originally reported by Matsumura and Maeda,11 and it is now believed to be the main reason for the accumulation of long-circulating liposomes in cancer tissues or inflammatory sites.12 This long-circulation time characteristic of DDS carriers is also important for the development of active targeting DDS. Liposomes modified with active targeting probes can interact with the target molecules or accumulate in the target tissues during circulation in the bloodstream.

2. CANCER NEOVASCULAR-TARGETED DDS

Firstly we developed several liposomal drugs for the treatment of cancer.13–15 After that, to achieve active targeting...
liposomal drugs for the purpose of cancer treatment, we selected tumor angiogenic vessels as a target for the delivery of anticancer drugs. Liposomes were decorated with PEG; and a 5-mer short peptide, APRPG, having affinity for vascular endothelial growth factor receptor-1 (VEGFR-1), was conjugated to the free end of the PEG chain. Membrane type-1 matrix metalloproteinase and binding immunoglobulin protein/glucose-regulated protein 78 (BiP/GRP78) were also expressed several specific molecules in comparison with normal or tumor angiogenic vessels. Angiogenic endothelial cells are non-malignant, normal cells, but they undergo growth during the angiogenic process. Therefore, those cells are specifically damaged by anticancer drugs. The eradication of neovessels by delivering anticancer drugs to the vessels would induce the cut off of the oxygen and nutrient supply to tumor cells, thus causing tumor regression; and so we designated this therapy as anti-neovascular therapy (ANET). In this therapy, anticancer drugs are expected to be efficiently delivered to angiogenic endothelial cells, since such drugs contained in liposomes firstly meet blood vessels after injection.

Angiogenesis is critical for the growth of solid tumors: Tumor cells obtain oxygen and nutrients from the neovessels whose formation they stimulate. Angiogenic endothelial cells are non-malignant, normal cells, but they undergo growth during the angiogenic process. Therefore, those cells are specifically damaged by anticancer drugs. The eradication of neovessels by delivering anticancer drugs to the vessels would induce the cut off of the oxygen and nutrient supply to tumor cells, thus causing tumor regression; and so we designated this therapy as anti-neovascular therapy (ANET). In this type of therapy, anticancer drugs are expected to be efficiently delivered to angiogenic endothelial cells, since such drugs contained in liposomes firstly meet blood vessels after injection.

### Table 1. Lipid Composition and Characteristics of Several Kinds of Liposomes Described in This Review

<table>
<thead>
<tr>
<th>Liposomal composition</th>
<th>Particle size (nm)</th>
<th>ζ-Potential (mV)</th>
<th>Entrap. eff. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Anti-neovascular therapy] (ref. 27)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont-PEG-Lip (DSPC : Chol : DSPE-PEG = 10 : 5 : 1)</td>
<td>134.9 ± 5.6</td>
<td>+2.2 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>APRPG-PEG-Lip (DSPC : Chol : DSPE-PEG-APRPG = 10 : 5 : 1)</td>
<td>128.0 ± 4.1</td>
<td>−0.1 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Cont-PEG-Lip (DOX)</td>
<td>163.0 ± 2.5</td>
<td>−10.6 ± 6.4</td>
<td>&gt;90</td>
</tr>
<tr>
<td>APRPG-PEG-Lip (DOX)</td>
<td>144.9 ± 2.8</td>
<td>−13.0 ± 2.1</td>
<td>&gt;90</td>
</tr>
<tr>
<td>[Anti-neovascular PDT] (ref. 42)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont-Lip (BPD-MA)</td>
<td>131.2 ± 2.3</td>
<td>−7.4 ± 5.1</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Cont-PEG-Lip (BPD-MA)</td>
<td>151.4 ± 3.5</td>
<td>−7.7 ± 6.8</td>
<td>&gt;90</td>
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<tr>
<td>Cont-Lip (DSPC-PEG = 20 : 1)</td>
<td>135.7 ± 1.9</td>
<td>−5.8 ± 2.9</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Cont-Lip (DSPC-PEG-APRPG = 20 : 1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cont-Lip (DSPC-PEG-DiI-labeled)</td>
<td>99.4 ± 0.5</td>
<td>−0.4 ± 0.0</td>
<td></td>
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<tr>
<td>AEPO-Lip (ref. 76)</td>
<td>129.0 ± 1.0</td>
<td>+0.3 ± 1.3</td>
<td>34.8</td>
</tr>
<tr>
<td>DSPC : Chol : DSPE-PEG : AEPO = 20 : 10 : 1 : 1.0−3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusudil-Lip (ref. 77)</td>
<td>125.6 ± 3.6</td>
<td>−1.6 ± 0.6</td>
<td>75.7</td>
</tr>
<tr>
<td>Cont-Lip (DSPC-PEG = 10 : 5 : 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FK506-Lip (ref. 86)</td>
<td>109.5 ± 4.3</td>
<td>−7.2 ± 0.7</td>
<td>39.8</td>
</tr>
<tr>
<td>DSPC : DSPC-PEG : FK506 = 20 : 10 : 0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) DOX and fasudil were encapsulated into liposomes by remote-loading method. BPD-MA and FK506 were entrapped in the liposomal bilayer because of their hydrophobic characteristics. b) AEPO was conjugated on the top of PEG chain in DSPE-PEG. Therefore, entrap efficiency indicate the modification percent of AEPO. Abbreviations: Cont, control; DSPC, distearoyl phosphatidylcholine; DSPE, distearoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; entrap. eff., entrapping efficiency.

Biography
Dr. Naoto Oku was born in Setagaya, Tokyo, Japan in 1952. He received his Ph.D. degree from the University of Tokyo in 1980 under the supervision of Prof. Shoshichi Nojima. After having received his Ph.D. degree, he became a postdoctoral fellow research associate at Northwestern University, Evanston, Illinois, U.S.A. Then, he became assistant professor of the Faculty of Pharmaceutical Sciences, Setsunan University in 1983, and was thereafter promoted to associate professor in 1987. In 1991, he moved to the School of Pharmaceutical Sciences, the University of Shizuoka, and was promoted to full professor in 1998. He was honored by receiving the Pharmaceutical Society of Japan Award for Encouragement of Research in 1995, Innovation of the 12th Nagai Award of the Japan Society of Drug Delivery System in 2012, The Takeru & Aya Higuchi Memorial Prize of the Academy of Pharmaceutical Science and Technology, Japan in 2015, and the Pharmaceutical Society of Japan Award in 2016. He has spent about 40 years doing research on liposomal DDS as his major research field.
tion of them into bloodstream and, thus, easily interact with them. Lipid composition and characteristics of liposomes used for ANET and other studies described in the review are shown in Table 1. Recently, Hansen et al. determined the distribution of copper-64 encapsulated in PEGylated liposomes and evaluated the EPR effect in 11 canine cancer patients with spontaneous solid tumors. As a result, the EPR effect was strongly dependent on the tumor type: Six out of 7 carcinomas displayed high uptake of liposomes, whereas only 1 of 4 sarcomas did so. This finding also supports the idea that active targeting of blood vessels is a promising system for drug delivery besides the EPR effect. To enhance the targeting ability of the liposomal carrier, we also developed dual targeting liposomes having 2 different probes recognizing different targets on the liposomal surface.

In active targeting DDS technology, the anti-neovascular strategy of chemotherapy has another attractive feature. Anti-cancer drugs encapsulated in liposomes are rather slowly and sustainably released from the liposomes depending on the liposomal composition, type of encapsulated drug, and the encapsulation technique. Low-dose anticancer drugs working for a long period of time are known to damage neovessels and to induce cytostasis of cancerous cells. Anti-cancer drugs would be the most effective at the maximum tolerated dose (MTD), because this schedule has a potent effect in cancer patients. Kerbel and colleagues reported that repeated low-dose administration, a schedule referred to as metronomic-dosing chemotherapy, suppresses the growth of cancer cells without causing severe side effects and that this therapy was effective for the treatment of anticancer drug-resistant tumors.

In our study using drug-resistant tumor-bearing mice, anti-neovascular therapy with APRPG-modified liposomal doxorubicin effectively suppressed tumor growth in these animals. These results suggest that the metronomic chemotherapy has the potential to eradicate angiogenic endothelial cells in the tumor bed with reduced side effects and that ANET is similarly effective as metronomic chemotherapy for eradicating cancer without causing severe side effects.

According to the success of ANET in animal experiments, we applied cancer photodynamic therapy (PDT) for targeting cancer neovessels. Benzoporphyrin derivative monocoid ring A (BPD-MA or verteporfin) had been developed as the first liposomal drug in Japan. This compound is suitable for liposomalization because of its hydrophobicity, and it releases singlet oxygen upon laser irradiation. We thus sought to deliver BPD-MA to cancer angiogenic endothelial cells and damaged these cells by laser irradiation. For this purpose, laser irradiation was performed early after the injection of liposomal BPD-MA into tumor-bearing mice, laser irradiation (689 nm 150 J/cm^2) was performed. The tumor volume was monitored every day after PDT of PEG-liposomal BPD-MA-treated mice (gray circles) or APRPG-PEG-liposomal BPD-MA-treated mice (black circles). C. Schema of PDT with liposomal BPD-MA. Liposomal BPD-MA passively accumulates in the interstitial space of the tumor, and singlet oxygen generated by laser irradiation does not affect either endothelial cells or tumor cells very much. In contrast, APRPG-PEG-modified liposomes are taken up by angiogenic endothelial cells; and the cells are damaged (shown as dark gray) after laser irradiation, leading to eradication of the tumor cells. (A and B are modifications of figures presented in ref. 42.)

Interestingly, BPD-MA in PEGylated liposomes and that in APRPG-PEG-modified liposomes similarly accumulated in tumors of tumor-bearing mice 3 h after injection (Fig. 1A). In contrast, BPD-MA in APRPG-PEG-modified liposomes induced drastic tumor regression compared with that obtained with PEGylated liposomes after irradiation of laser given 3 h after injection of the liposomes (Fig. 1B). This result indicates that accumulation of liposomal drugs in tumor tissues is less important than the topological intratumoral distribution of liposomal drugs especially for PDT. In the case of PEG-
(siRNA) as well as plasmid DNA. Non-viral gene transfection carriers are classified as nanoparticles including cationic lipid complexes53) and polyethylenimine-based cationic polymers.54) For obtaining advantages of both cationic liposomes and polycations, we developed polycation liposomes (PCLs) for the purpose of gene transfection55–57) and for RNA interference (RNAi) therapy.58–60) To improve the gene-silencing effect of PCLs, we synthesized various polycationic lipids61) or modified PCL-siRNA complexes with APRPG-peptides or other specific antibodies for active targeting of the complex.55–57) Those PCL carriers were also used for the delivery of anti-angiogenic microRNA (miRNA), which is expected to normalize angiogenic vessels and to improve the delivery of anticancer drugs.61)

3. LIPOSOMAL TRAFFIC IN THE BRAIN

For the purpose of evaluating liposomal traffic in a living body, we firstly encapsulated [18F]-2-deoxy-2-fluoro glucose ([18F]FDG) into liposomes and then examined their time-dependent distribution by use of positron emission tomography (PET).62–64) Liposomal distribution was affected by charge, size, modification with PEG, and so on.65,66) By the way, the microdosing trial, a phase 0 study, has become popular as a useful strategy for developing new drugs. In this type of study, a very low dose of drug without pharmacological or toxicological effects is used for detecting metabolites analyzed by accelerator mass spectrometry (AMS) or by use of some other analytical apparatus, and for evaluating pharmacokinetics by PET or some other methods.67,68) Since DDS drugs using liposomes or lipid nanoparticles as carriers will be continuously developed, to label pre-formulated liposomes with a positron emitter, an optimized [18F]-compound, 1-[18F]fluoro-3,6-dioxatetracosane, and a direct liposome modification method called the “solid-phase transition method” were developed. By this method, dried 1-[18F]fluoro-3,6-dioxatetracosane molecules are directly incorporated into the lipid bilayer of preformed liposomes and then used for determining liposomal traffic in a living animal by PET.69) Firstly, rats bearing a brain glioma were imaged by use of [18F]labeled PEGylated liposomes with a size of 100 nm in diameter. Liposomes did not enter into the normal brain tissue, probably due to the presence of the blood–brain barrier (BBB). In contrast, the liposomes easily entered into the site of the glioma, because of the lack of a functional BBB. In addition, the smallest tumor imaged by the liposomes was only 1 mm in width70) (Fig. 2).

By chance, Kawaguchi et al. found that the penumbra region in a brain ischemic rat model was protected from damage by the injection of liposome-encapsulated hemoglobin (LEH).71) Therefore, LEH was labeled with 1-[18F]fluoro-3,6-dioxatetracosane, and the distribution of LEH in the rat brain was imaged by using a small animal PET apparatus. As a result, [18F]-labeled LEH gradually penetrated into the penumbra region where the brain was protected from damage, thus suggesting that LEH delivered oxygen into the ischemic brain.72) In the case of LEH having a diameter of 230 nm, it was possible that the liposomes flowed to the vessels beyond the occlusion site, since LEH was small enough to pass through the occlusion. On the other hand, since it is known

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**Fig. 2. Imaging of Rat Brain Glioma by [18F]-Labeled Liposomes**

APRPG-PEGylated liposomes (DSPC: cholesterol:DSPE-PEG-APRPG=10:5:1 as molar ratio, 107 nm sized) were positron emitter-labeled with 1-[18F]fluoro-3,6-dioxatetracosane and injected into a tail vein of brain glioma-bearing rat. Horizontal (left) and vertical (upper right) PET images are shown. The ovals show the brain area; and the arrowheads, liposomes accumulated at the site of the glioma. The lower right image show a brain slice with a glioma 1 mm in width. (Slight modification of figures from ref. 70.)

**Fig. 3. Localization of PEGylated Liposomes in Brain Ischemic Site of t-MCAO Model Rats**

A. Schema of liposomal penetration into brain parenchyma under conditions of ischemia or ischemia/reperfusion. B. Damaged region of brain after 1-h occlusion followed by 0 or 3-h reperfusion. Brain slices were stained with 2,3,5-triphenyltetrazolium chloride (TTC). White areas (indicated by white arrow) show the damaged regions; and red areas, the surviving regions (ref. 75 with modification). C. The t-MCAO rats treated 1 h in infract were injected with DiIC18 fluorescence-labeled PEGylated liposomes after 0 or 3 h of reperfusion. Then the rats were sacrificed at 1 h after the injection, their brains were dissected and DiI fluorescence localization was observed with an in vivo imaging system (IVIS). The left hemispheres of the brain slices are the non-ischemic side; and the right hemispheres, the ischemic side. Gradation bar shows the relative levels of fluorescence intensity (ref. 76). D. Size-dependent accumulation of PEG-liposomes in the ischemic region of t-MCAO rats. The particle size of DiI-labeled PEG-liposomes was adjusted by extrusion and determined as 109, 213 or 848 nm. t-MCAO rats were intravenously injected with PEG-liposomes 1 h after reperfusion following a 1-h occlusion. The brains of the rats were dissected 1 h after the injection, and sliced into 2-mm coronal sections. Then, the fluorescence of DiI-labeled PEG-liposomes was imaged with the IVIS (ref. 77).
that the BBB is disrupted during occlusion, it is possible that the small-sized liposomes entered into the brain tissue. Therefore, fluorescence-labeled liposomes were injected into transient middle cerebral artery occlusion (t-MCAO) model rats just after reperfusion (Fig. 3). Liposomes accumulated in the ischemic side of the brain. Moreover, liposomes approximately 100 nm in size accumulated to a greater extent than those about 200 nm in size; and those about 800 nm in size did not accumulate in the ischemic brain. Figure 3 also shows that once accumulated the liposomes resided there for a long period of time.

4. TREATMENT FOR ISCHEMIC STROKE WITH LIPOSOMAL NEUROPROTENCTANTS

As described above, liposomes might accumulate in the interstitial space of the brain parenchyma after ischemia/reperfusion; and so we next examined the neuroprotective effect of liposomal drugs on ischemia/reperfusion (I/R) injury. Cerebrovascular disorder is the 4th leading cause of death in Japan, and the disease in 1st position regarding the necessity of long-term care. About 60% of patients with this disorder have a cerebral infarction; and the rest, cerebral and subarachnoid hemorrhage. In clinical settings, thrombolytic therapy with tissue plasminogen activator (t-PA) is the only acceptable therapeutic agent used worldwide. However, since there are several limitations regarding the use of t-PA, such as a narrow therapeutic time window (TTW) and safety concerns about the risk of cerebral hemorrhage, patients given t-PA treatment are very few. Moreover, the production of reactive oxygen species and inflammatory cytokines after reperfusion causes I/R injury, which sometimes leads to a poor prognosis for patients such as those with hemiplegia. Therefore, the development of more widely applicable and effective therapies has been awaited.

Fig. 4. Therapeutic Effect of AEPO-Liposomes on Brain Injury in the t-MCAO Rats

A. Schema of AEPO-decorated liposomes. AEPO was conjugated to the free end of the PEG chain. B. Biodistribution of AEPO-liposomes in the t-MCAO model rats. AEPO was labeled with [3H]. The t-MCAO model rats were injected with [3H]-labeled AEPO or [3H]-AEPO liposomes via a tail vein. Biodistribution of each sample was determined by measuring the radioactivity in each organ at 3 h after the injection. Data are presented as the mean±S.D. (n=5). Significant differences are indicated as follows: **p<0.01, ***p<0.001 vs. AEPO value, ##p<0.01 vs. non-ischemic hemisphere. C. Transient-MCAO model rats were injected via a tail vein with PBS, AEPO or AEPO-liposomes immediately after the start of reperfusion. Then accumulation of [125I] in the brain was imaged during the long period of time.

Fig. 5. Neuroprotection by Liposomal Fasudil against Brain I/R Injury

A. Schema of liposomal fasudil. B. Accumulation of fasudil liposomes in I/R region of t-MCAO rats was determined as follows: t-MCAO rats were intravenously injected with DiIC18-labeled fasudil liposomes just after the start of reperfusion, and brain sections were prepared at 24 h after reperfusion. Then, DiI fluorescence was imaged by the IVIS. C. TTC staining of brain section was performed after 24 h of reperfusion, as described in “B.” At 24 h after the start of reperfusion, brain sections were immunostained for MPO to visualize neutrophils and counterstained with hematoxylin. MPO-positive cells (brown) are indicated by black arrows (ref. 77 with modification).

Fig. 6. Neuroprotectant Delivery with Liposomes during Occlusion

A. PEGylated liposomes were labeled with 1-[18F]fluoro-3,6-dioxatetracosane by solid-phase transition method and injected intravenously into t-MCAO rats 1 h after start of occlusion. Then accumulation of [18F] in the brain was imaged during 0–10 and 110–120 min after injection. Red circle shows the brain region. B. MCAO rats were intravenously injected with [3H]-labeled PEG-liposomes after 1 h of occlusion. The liposomal accumulation was determined by radioactivity at 1 and 3 h after injection. Significant difference; **p<0.01. C. Transient-MCAO model rats were intravenously injected with PBS, or FK506-liposomes after 1 h of occlusion. At 1 h after injection, the brains of the rats were reperfused. At 24 h after the onset of occlusion, brain sections were prepared and stained with TTC for evaluating damaged-brain volume (“B” and “C” from ref. 92 with modification).
For the treatment of cerebral I/R injury, we selected asialo-erythropoietin (AEPO) as a neuroprotectant, since this compound is known to act in a neuroprotective manner after cerebral ischemia without any hematopoietic effects.\(^5\) AEPO-modified PEGylated liposomes (AEPO-liposomes; Fig. 4A) were injected at various time points after the start of reperfusion in t-MCAO model rats. \(^{21}\)AEPO showed rapid clearance from the bloodstream and less accumulation in the ischemic brain region in comparison with \(^{21}\)AEPO-modified liposomes\(^6\) (Fig. 4B). Treatment with AEPO-liposomes significantly reduced the area of 2,3,5-triphenyltetrazolium chloride (TTC) staining-defined cerebral lesions following cerebral I/R injury\(^7\) (Fig. 4C). Cerebral I/R injury is accompanied by a neuropathological disorder, resulting in motor function deficits. A single administration of AEPO-liposomes significantly suppressed the motor function deficits evaluated by a 21-point scoring system. At day 7 after the start of reperfusion, AEPO-liposome treated group had strongly recovered from function deficits and from paralysis of the right forepaw\(^8\) (Fig. 4D).

Next, we examined low-molecular-weight drugs instead of AEPO for the treatment of I/R injury, since such drugs, in general, are less expensive to prepare and easier to handle. FK506 (tacrolimus) has the potential to suppress cerebral I/R injury.\(^9\) We found that FK506 entrapped in liposomal membranes accumulated in the ischemic brain parenchyma of t-MCAO rat model after reperfusion.\(^10\) Histological analysis indicated that treatment with the liposomes strongly suppressed neutrophil invasion and apoptosis of brain cells.

Similarly, we entrapped fasudil hydrochloride, a Rho-associated kinase (ROCK) inhibitor, into liposomes\(^7\) (Fig. 5A). This drug is used for the treatment of cerebral vasospasm, and it is known to be safe and effective for the treatment of ischemic stroke.\(^7\) Fasudil can be encapsulated into liposomes efficiently by using the remote loading method.\(^9\) Fluorescence-labeled fasudil-liposomes accumulated in the ischemic site of the brain and remained there for at least 24 h (Fig. 5B). Fasudil-liposomes suppressed neutrophil invasion into the ischemic site (Fig. 5D), suggesting that fasudil suppressed the inflammation, which suppression relates to the neuroprotective effect. Moreover, the therapeutic effect of liposomal fasudil against I/R injury was higher than that of free fasudil (Fig. 5C), implying that liposomialization facilitated the delivery of fasudil to the ischemic site or maintained the appropriate drug concentration at the site.

Next, we examined the effect of a liposomal neuroprotectant during occlusion; because even during occlusion, it is possible that liposomes in the residual flow in the blood vessels are able to penetrate into the brain tissue through an incomplete BBB. The usefulness of liposomal DDS for the treatment of ischemic stroke, especially I/R injury after reperfusion, is discussed above. However, the most desirable innovation would be a drug that can expand the TTW of t-PA or reduce the risk of hemorrhage by t-PA treatment. If an increase in the permeability of the BBB would start at the time of occlusion, fluorescence-labeled liposomes injected during occlusion would accumulate in the ischemic brain hemisphere. In fact, the liposomes actually accumulated in the brain parenchyma during occlusion, indicating that water flow in a blood vessel is not completely stopped by an occlusion.\(^9\)

For evaluation of the liposomal trafficking in the ischemic brain, liposomes were labeled with 1-\(^{18}\)F]fluoro-3,6-dioxatetracosane by use of the solid-phase transition method. The blood flow in the ischemic hemisphere was reduced just after occlusion, although the accumulation of \(^{18}\)F radioactivity in this hemisphere gradually increased compared with that in the non-ischemic hemisphere.\(^9\) This increase in \(^{18}\)F in the ischemic hemisphere suggests that at least a part of the liposomes that had accumulated in the ischemic hemisphere were localized in the brain tissues (Fig. 6A). These data are consistent with the distribution of \(^1\)H-labeled liposomes injected into t-MCAO rats during occlusion\(^7\) (Fig. 6B). We next investigated the effect of a liposomal neuroprotectant on the occluded site prior to the recovery of blood flow. For this purpose, FK506-entrapped PEGylated liposomes were intravenously injected into MCAO rats after a 1-h occlusion. FK506 is known to inhibit activation of calcineurin by ischemia and to suppress inflammation.\(^9\) In fact, treatment of MCAO rats with FK506-liposomes significantly suppressed the oxidative stress determined by the production of superoxide anion in brain slices, as well as brain cell damage\(^9\) (Fig. 6C). Moreover, treatment with FK506-liposomes before reperfusion significantly ameliorated motor function deficits of the rats examined 1 week after occlusion,\(^9\) suggesting that liposomes could carry a neuroprotectant to both core and penumbra regions of the brain during the occlusion and thus prevent brain damage.

5. CONCLUSION AND PROSPECTS

In this review, I discussed our research on liposomal DDS, such as anti-neovascular cancer chemotherapy, antiangiogenic photodynamic therapy, carrier development for gene therapy, therapy with nuclear medicine therapy, and others. Especially, I focused on the liposomal DDS for the treatment of ischemic stroke, since this field was just started by the discovery of liposomal accumulation in ischemic site of the brain parenchyma. I hope that effective DDS drugs will be developed for future therapy of ischemic diseases as well as for cancer therapy.

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Conflict of Interest The author declares no conflict of interest.

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