Department of Pharmaceutics[1], Faculty of Pharmaceutical Sciences, Hokkaido University of Science, Creation Research Institute of Life Science in KITA-no-DAICHI[2], Division of Pharmaceutics[3], Hokkaido Pharmaceutical University School of Pharmacy, Sapporo, Japan

Development of LOX-1 antibody modified immuno-liposomes as drug carriers to macrophages in atherosclerotic lesions

K. Togami[1,2,3,*], X. Zhan[3], K. Ishizawa[1,3], K. Miyakoshi[3], A. Miyao[3], P. Quan[3,#], S. Chono[1,2,3,*]

Received January 26, 2023, accepted June 26, 2023

**Corresponding authors: Kohei Togami, PhD, and Sumio Chono, PhD, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University of Science, 7-Jo 15-4-1 Maeda, Teine, Sapporo, Hokkaido 006-8585, Japan*
k-togami@hus.ac.jp; s-chono@hus.ac.jp
[#]On leave from Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang, Liaoning 110016, China

Pharmazie 78: 113-116 (2023) *doi: 10.1691/ph.2023.3004*

We developed a drug delivery system for atherosclerotic lesions using immuno-liposomes. We focused on enhancing the delivery efficiency of the liposomes to macrophages in atherosclerotic lesions by antibody modification of lectin-like oxidized low-density lipoproteins (LDL) receptor 1 (LOX-1). The cellular accumulation of the liposomes in foam cells induced by oxidized LDL (oxLDL) in Raw264 mouse macrophages was evaluated. The cellular accumulation of LOX-1 antibody modified liposomes in oxLDL-induced foam cells and untreated Raw264 cells was significantly higher compared with that of unmodified liposomes. The liposomes were also administered intravenously to *Apoe*^{shl} mice as an atherosclerosis model. Frozen sections were prepared from the mouse aortas and observed by confocal laser microscopy. The distribution of LOX-1 antibody modified liposomes in the atherosclerotic regions of *Apoe*^{shl} mice was significantly greater compared with that of unmodified liposomes. The results suggest that LOX-1 antibody modified liposomes can target foam cells in atherosclerotic lesions, providing a potential route for delivering various drugs with pharmacological effects or detecting atherosclerotic foci for the diagnosis of atherosclerosis.

1. Introduction

Atherosclerosis is a chronic inflammatory disease characterized by the formation of plaques in the intima of the aorta, lipid deposition, production of extracellular matrix, and infiltration of macrophages (Weber and Noels 2011). This pathological lesion results from a combination of risk factors, including dyslipidemia, hypertension, and diabetes mellitus (Goyal et al. 2012). The growth of plaques causes a narrowing of blood vessels and if the plaque ruptures, a blood clot forms, leading to a potential fatal outcome, such as myocardial infarction or stroke (Braganza 2001; Libby et al. 2011). The progression of atherosclerosis is closely associated with the presence of low-density lipoproteins (LDL) in the blood. Oxidized LDL (oxLDL) binds to the lectin-like oxidized LDL receptor 1 (LOX-1) on vascular endothelial cells, which results in pathological changes, such as the expression of adhesion factors, chemokines, and the release of nitric oxide (Kattoor et al. 2019).
The LOX-1 receptor is not only expressed on vascular endothelial cells, but also on macrophages and it is involved in the phagocytosis of oxLDL in conjunction with CD36 and scavenger receptor type I (Kattoor et al. 2019). This results in the transformation of foam cells from macrophages, which are rich in cholesterol esters. Foam cells maintain a persistent chronic inflammatory state by acquiring the properties of inflammatory macrophages, such as the production of inflammatory cytokines, growth factors, and reactive oxygen species (Goyal et al. 2012). This process stimulates vascular smooth muscle cells to secrete extracellular matrix proteins, such as collagen and elastin, thereby promoting the formation of atherosclerotic plaques (Bobryshev et al. 2016). In this advanced plaque, inflammatory mediators produced by macrophages induce apoptosis and secrete extracellular matrix-degrading enzymes by smooth muscle cells, thereby increasing the susceptibility of the plaque to rupture. Unstable plaques dominated by macrophages are considered to be a potentially fatal condition, particularly with respect to the formation of thrombi.
To develop therapeutic and diagnostic strategies for atherosclerosis that target the regulation of macrophage function, an efficient drug delivery system is required. In this study, we developed immuno-liposomes modified with a LOX-1 antibody and evaluated their capacity to target atherosclerotic lesions.

2. Investigations and results

2.1. Physical characteristics of LOX-1 antibody modified liposomes

The particle size and the zeta potential of the LOX-1 antibody modified liposomes are shown in Table. The particle size and zeta potential of LOX-1 antibody modified liposomes and unmodified liposomes as a control were approximately 130 nm and -16 mV, respectively. The conjugation efficiency of the LOX-1 antibody to the liposomes was 36.3%.

Table: Antibody conjugation and physical characteristics of liposomes

	Particle size (nm)	Polydispersity index	Zeta potential (mV)
Unmodified liposomes	128 ± 4	0.062 ± 0.020	-16.1 ± 0.6
LOX-1 antibody modified liposomes	132 ± 2	0.095 ± 0.011	-16.4 ± 2.2

Each point represents the mean ± SD (n = 3).

2.2. OxLDL-induced cholesterol-rich foam cells

The amount of cholesterol in oxLDL-treated mouse macrophages (Raw264 cells) is shown in Fig. 1A. OxLDL treatment significantly increased cellular cholesterol in Raw264 cells. The images of Oil red O staining of oxLDL-treated Raw264 cells are shown in Fig. 1B. Red-stained oil droplets were observed in OxLDL-treated Raw264 cells. These results indicate that foam cells are induced by oxLDL treatment of Raw264 cells.

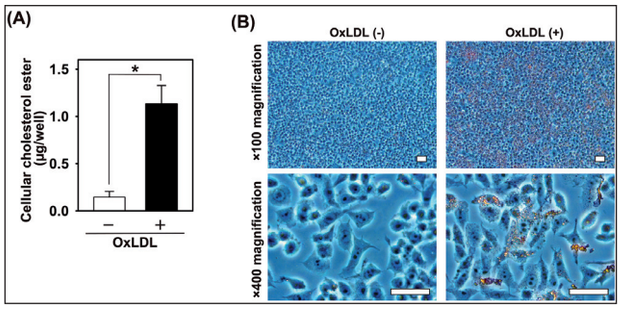

Fig. 1: Foam cell formation following treatment of Raw264 cells with oxidized low-density lipoprotein (oxLDL). Raw264 cells were incubated with oxLDL (20 μg/mL) for 24 h at 37°C. (A) Accumulation of cholesterol ester in the cell extracts. Each data represents the mean ± S.D. (n = 5). *$p < 0.01$. (B) Foam cell formation from Raw264 cells as determined by Oil red O staining. The cells were observed by phase contrast microscopy (×100 and ×400). The measurements were performed three times with similar results. The scale bar is 100 μm.

2.3. LOX-1 receptor expression on the foam cells

The expression profiles of LOX-1 in cells are shown in Fig. 2. LOX-1 receptor was detected in Raw264 cells with and without oxLDL treatment. LOX-1 expression was not altered by oxLDL treatment.

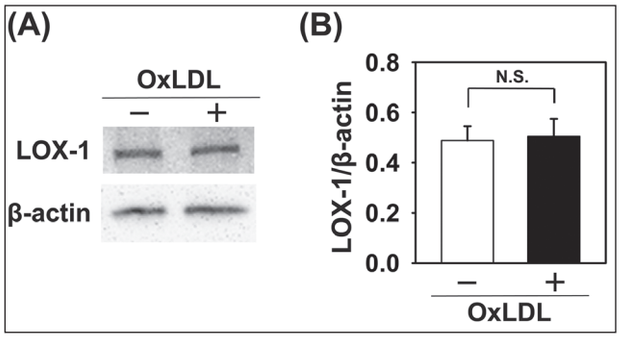

Fig. 2: Immunoblot images showing the expression of the LOX-1 receptor in Raw264 cells after treatment with oxLDL (20 μg/mL) for 24 h at 37°C. The densities of the LOX-1 bands was normalized to that of β-actin and shown as a ratio. Each point represents the mean ± S.D. (n = 3). N.S.: not significant.

2.4. Cellular accumulation of LOX-1 antibody modified liposomes

The cellular accumulation of LOX-1 antibody modified liposomes in oxLDL-treated foam cells and untreated Raw264 cells is shown in Fig. 3A. The cellular accumulation of LOX-1 antibody modified liposomes was significantly higher than that of unmodified liposomes in oxLDL-treated foam cells and untreated Raw264 cells. Fluorescent images detailing the intracellular distribution of immuno-liposomes in these cells are shown in Fig. 3B. The fluorescence of both immuno-liposomes and unmodified liposomes was widely observed in each cell type. The fluorescence of immuno-liposomes was higher compared with that of unmodified liposomes in both foam and Raw264 cells.

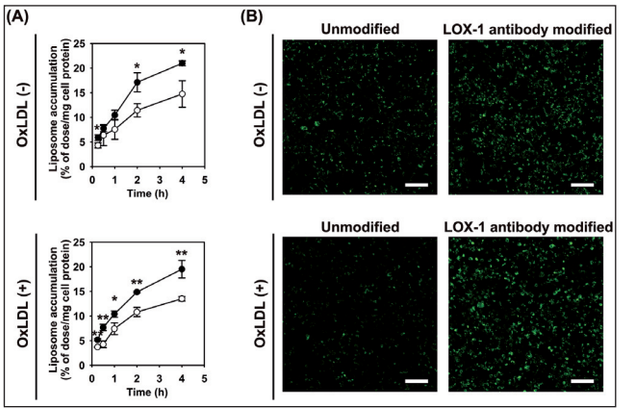

Fig. 3: Cellular accumulation of unmodified and LOX-1 antibody modified liposomes in Raw264 cells after the application of oxLDL. Raw264 cells were incubated with oxLDL (20 μg/mL) for 24 h at 37°C. (A) Cellular accumulation of the liposomes after incubation at 37°C for 0.25, 0.5, 1, 2, and 4 h. (○) Unmodified liposomes, (●) LOX-1 antibody modified liposomes. *$p < 0.05$, **$p < 0.01$; significantly different from the unmodified liposomes. Each point represents the mean ± SD (n = 3). (B) Fluorescent images showing the cellular accumulation of liposomes after incubation at 37°C for 4 h. The scale bar is 100 μm.

2.5. Aortic pathology in KOR-Apoe^{shl} mice

The pathological features of the aortas of KOR-*Apoe*^{shl} mice (Matsushima et al. 1999) are shown in Fig. 4. The arteries of KOR-*Apoe*^{shl} mice were stained red (Fig. 4A) by Oil red O staining. In frozen sections, the aortic region of KOR-*Apoe*^{shl} mice was enlarged (Fig. 4B). The aortic region of KOR-*Apoe*^{shl} mice showed fat accumulation by Oil red O staining and localized macrophages by immunostaining.

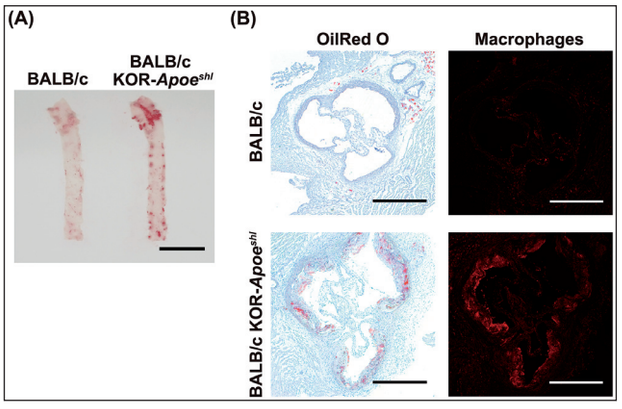

Fig. 4: Pathologic features and macrophage localization in the aorta of KOR-*Apoe*^{shl} mice. (A) Oil Red O-stained slitting aorta from KOR-*Apoe*^{shl} mice. Scale bar is 5 mm. (B) The images of OilRed O-stained and macrophages immunostained frozen sections of the aorta from KOR-*Apoe*^{shl} mice. The scale bar is 500 μm.

2.6. Distribution of LOX-1 antibody modified liposomes in the aortic region

Fluorescence images illustrating the distribution of LOX-1 antibody modified liposomes in aorta sections of KOR-*Apoe*^{shl} mice are shown in Fig. 5. The fluorescence of LOX-1 antibody modified liposomes was observed in the aortic region, whereas the fluorescence of unmodified liposomes was not observed (Fig. 5A). The fluorescence intensity of LOX-1 antibody modified liposomes in the aortic region was significantly higher compared with that of unmodified liposomes (Fig. 5B).

3. Discussion

In this study, we developed LOX-1 antibody modified liposomes as drug carriers to target atherosclerotic lesions. Liposomes are

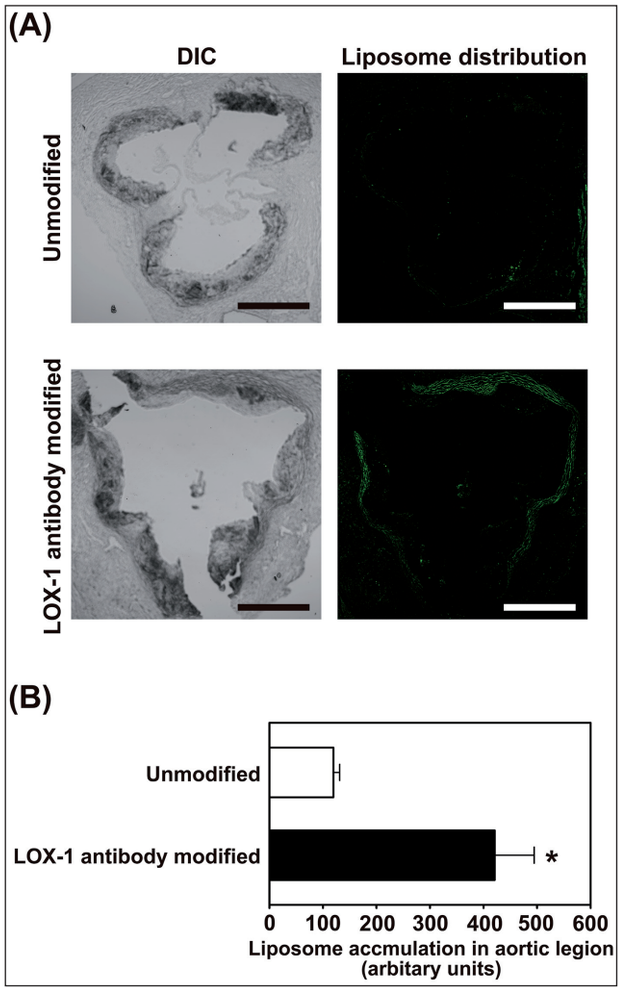

Fig. 5: Liposome distribution in the aorta of KOR-*Apoe*^{shl} mice after intravenous administration of LOX-1 antibody modified liposomes. After treatment for 3 h, the aortas were excised and frozen sections were prepared. (A) Images of the liposome distribution in frozen aorta sections. Scale bar is 500 µm. (B) The fluorescence intensity of the liposomes in the aortic lesion was calculated using ImageJ software. Each value represents the mean ± S.D. (n = 3). *$p < 0.01$; significantly different from the unmodified liposomes.

effective for a range of cardiovascular diseases and conditions and have potential as a treatment for atherosclerosis (Levchenko et al. 2012). Furthermore, the integration of liposomes with conventional diagnostic tools, such as contrast agents, may enhance plaque detection. The primary approach to targeting atherosclerotic plaques is by enhancing the permeability and retention effect of the endothelium that is damaged by atherosclerosis. This facilitates the extravasation of liposomes from the bloodstream to the plaque site (Mulder et al. 2004). In addition, surface modification of liposomes with polyethylene glycol (PEG) increases circulation time, providing increased opportunities for targeting plaque (Sawant and Torchilin 2012). The distribution of atherosclerotic lesions may also be enhanced by optimizing the particle size of the liposomes (Chono et al. 2006). Although these strategies for targeting atherosclerotic plaque produced promising experimental outcomes, clinical implementation has been challenging. One of the obstacles encountered is the effect of blood flow into the vessels, which exerts shear stress on the endothelial wall. This process leads to the displacement of particles from the target site and diminishes the duration of interaction between particles and their targets within the plaque. To address this challenge, an alternative approach for the treatment of atherosclerosis using liposomes involves the incorporation of an active targeting function that enables liposomes to specifically target macrophages within the plaque.

We examined cholesterol-accumulating foam cells and measured their expression of LOX-1. Using the Raw264 cell line, the results indicated that the expression of LOX-1 did not change with or without oxLDL treatment. However, other studies using other macrophage cell lines have demonstrated that oxLDL treatment increases LOX-1 expression (Lu et al. 2019; Wang et al. 2020). Therefore, while an increase in liposome uptake by LOX-1 antibody modification was similar with and without oxLDL treatment in the present study, the actual accumulation of LOX-1 antibody modified liposomes in foam cells may be higher. To enhance recognition by foam cells, the incorporation of phosphatidylserine into the lipid membranes of liposomes was shown to be beneficial (Harel-Adar et al. 2011). By combining this strategy with an active targeting approach utilizing LOX-1 antibody modified liposomes in future studies, it may be possible to further enhance the accumulation of liposomes onto macrophages within the plaque.

In this study, we utilized KOR-*Apoe*^{shl} mice as a model for atherosclerosis. These mice are stable and spontaneously develop atherosclerosis. Their aorta and foam cells express LOX-1, which is similar to that observed in human aortic plaques (Liu et al. 2017; Matsushima et al. 1999; Matsushima et al. 2001). The LOX-1 antibody modified liposomes prepared in this study accumulated efficiently in the atherosclerotic plaques. The upregulation of LOX-1 on the vascular endothelium in response to inflammatory cytokines and oxidative stress suggests that liposomes targeting this receptor may effectively target the endothelium of atherosclerotic lesions (Di Pietro et al. 2016). This concept has been supported by previous studies in which the LOX-1 receptor in the vascular endothelium was targeted by intravenous injection of liposomes modified with a LOX-1 antibody in mice (Li et al. 2010). The present study suggests that these liposomes may potentially target both macrophages and vascular endothelium in atherosclerotic lesions, providing an efficient route for the delivery of various drugs with pharmacological effects or to detect atherosclerotic foci for the diagnosis of atherosclerosis. There has been a growing interest in using liver X receptor agonists to control atherosclerosis by suppressing inflammation, promoting cholesterol efflux, and regulating apoptosis (Barrett 2020). Future studies will need to test the applicability of such drug-encapsulated LOX-1 antibody modified liposomes for the treatment of atherosclerosis in vivo to confirm their therapeutic potential. In addition, the use of these liposomes in combination with existing plaque imaging techniques, such as ultrasound, magnetic resonance imaging, computed tomography, as well as nuclear imaging methods, such as positron emission tomography and SPECT (Groenendyk and Mehta 2018; Sanz and Fayad 2008), may improve the precision and sensitivity of diagnosing atherosclerosis.

4. Experimental

4.1. Materials

Oxidized low-density lipoprotein (OxLDL) was purchased from Alfa Aesar (Heysham, Lancashire, UK). Rat monoclonal anti-LOX-1 antibody was purchased from R&D Systems Inc. (Catalog number: MAB1564, Minneapolis, MN, USA). Egg-yolk phosphatidylcholine (EPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*n*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2000}), DSPE-PEG_{2000}-maleimide, and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-DPPE) were purchased from NOF Co. (Tokyo, Japan). Cholesterol (Chol) was purchased from Wako Pure Chemicals Co. Ltd. (Osaka, Japan). 2-iminothiolane was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other reagents were commercially available and were of analytical grade.

4.2. Cell culture

Raw264 (Riken Cell Bank, Tsukuba, Japan) cells were maintained in Eagle's medium containing 1% non-essential amino acid solution, 10% heat-inactivated fetal bovine serum, and 40 µg/mL gentamicin. The cells were maintained in a humidified atmosphere with 5% CO_2 at 37°C. Cells with passage numbers 11–16 were used.

4.3. Foam cell induction

Raw264 cells were seeded into 48-well culture plates (5.0×10^5 cells/well) or 2-well chamber slides (2.0×10^5 cells/well). After incubating for 2 h, OxLDL (125 µg/mL) was added to the cells and they were incubated in 5% CO_2 at 37°C for 24 h. The intracellular cholesterol content was determined using the Total cholesterol and cholesteryl

ester colorimetric/fluorometric assay kit (Bio Vision Inc., Milpitas, CA, USA). Oil Red O staining of Raw264 cells was done using chamber slides and the cells were observed under an inverted phase microscope (Axio Vert. A1; Zeiss, Oberkochen, Germany).

4.4. Immunoblotting for LOX-1 receptor on the cells

Raw264 cells were treated with OxLDL (125 µg/mL) and incubated for 24 h at 37 °C in 5% CO_2. Cell lysates were prepared using RIPA buffer (ThermoFischer Scientific Inc, Waltham, MA, USA). Protein was separated by sulfate-polyacrylamide gel electrophoresis using 12% gels (10 µg protein/lane), transferred to polyvinylidene difluoride membranes (10 V, 30 min), and blocked with 0.1% Tween 20 and 5% non-fat milk for 1 h at room temperature. The membranes were incubated with rat monoclonal anti-LOX-1 antibody (1:1000 dilution) or mouse monoclonal anti-β-actin antibody (Sigma-Aldrich Co., St. Louis, MO, USA, Catalog number: A5441 1:2000 dilution) for 1 h at room temperature. The membranes were incubated with horseradish peroxidase-conjugated anti-rat (R&D Systems, Catalog number: HAF005 1:1000 dilution) or anti-mouse IgG secondary antibodies (GE Healthcare, Pollards Wood, UK, Catalog number: NA931 1:2000 dilution). Antibody-protein complexes were detected using an enhanced chemiluminescene assay (GE Healthcare). Relative protein expression levels were quantified based on band densities determined with ImageJ software version 1.53c (National Institutes of Health, Bethesda, MD, USA).

4.5. Preparation of immuno-liposomes

Immuno-liposomes were prepared as previously described (Lopes de Menezes et al. 1999). Briefly, EPC, Chol, DSPE-PEG_{2000}, DSPE-PEG_{2000}-MAL, and NBD-DPPE at a lipid molar ratio of 25/10/0.8/0.2/5 were dissolved in chloroform, followed by evaporation to obtain a thin film. To obtain liposomes, the film was completely hydrated by vortexing with 25 mM HEPES buffer (pH 7.4 in 140 mM NaCl). The liposomes were then extruded ten times through polycarbonate filters with a pore size of 400, 200, and 100 nm (Nuclepore; Whatman, Piscataway, NJ, USA).

Rat monoclonal anti-LOX-1 antibody (100 µg) and 2-iminothiolane (5.5 µg) were mixed in 25 mM HEPES buffer (pH 8.0) and incubated for 1 h at room temperature. Unreacted 2-iminothiolane was removed by ultrafiltration using an Amicon Ultra-0.5 centrifugal filter (molecular weight cutoff: 3000 Da). To modify the liposome surface with antibody, the recovered thiolated anti-LOX-1 antibody was mixed with the liposomes (2 µmol phospholipids) and incubated for 18 h at room temperature.

To compare the effect of LOX-1-antibody, modified and unmodified liposomes were prepared using the same method as above with EPC, Chol, DSPE-PEG_{2000}, and NBD-DPPE at a lipid molar ratio of 25/10/10/5. The particle size and the zeta potential of the liposomes were determined by photon correlation spectroscopy using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK).

4.6. Cellular accumulation experiment of immuno-liposomes

Cellular accumulation experiments were performed as described previously. Raw264 (5.0×10^5) cells were seeded into 48-well culture plates and incubated for 2 h at 37 °C in a 5% CO_2 humidified incubator. The liposomes (5 nmol phospholipid/well) were added to the cells and incubated at 37 °C. The cells were extracted with 400 µL of 2 M NaCl. The NBD-DPPE concentration in the cell extracts was measured using a microplate reader (Powerscan HT). The protein concentration in cell the extracts was determined using the Coomassie protein assay reagent (Pierce Chemical Company, Rockford, IL, USA) with bovine serum albumin as a standard.

To measure the accumulation and localization of the liposomes in cells, confocal microscopy was performed. Briefly, Raw264 cells (2.0×10^6 cells/well) were seeded onto 2-well glass chamber slides and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. The liposomes (20 nmol phospholipid/well) were added to the cells, which were then incubated for 4 h at 37°C. The medium was removed by aspiration and the cells were washed twice with PBS, followed by fixation with 4% *p*-formaldehyde for 10 min at room temperature. The fluorescence of NBD-DPPE as a marker of liposomes was observed using a laser-scanning confocal microscope (LSM 700; Zeiss).

4.7. KOR-Apoe^{shl} mice as an atherosclerosis model

BALB/c KOR-*Apoe*^{shl} mice aged 7 weeks and weighing 19–24 g were purchased from Japan SLC (Shizuoka, Japan). The mice were fed a high-fat diet (D12108C, Research Diets Inc, New Brunswick, NJ, USA) for 8 weeks, euthanized by cervical dislocation, and the aorta was immediately excised. The aorta was cut open, and Oil red O staining was performed. An aorta taken from another individual was fixed with 4% paraformaldehyde overnight. After immersion in 30% sucrose solution overnight, they were embedded in O.C.T. compound (Sakura Finetek USA, Inc, Torrance, CA, USA) using liquid nitrogen. Frozen sections (10 µm) were prepared using a cryostat and directly mounted onto glass slides (Matsunami Trading Company Ltd., Osaka, Japan). Oil red O staining was performed on frozen aorta sections to evaluate the histological features in the aortic lesion. The animal experiments described here were conducted in accordance with the Guiding Principles for the Care and Use of Experimental Animals (Hokkaido Pharmaceutical University). The Ethics Committee approved all experimental protocols for Animal Experiments at Hokkaido Pharmaceutical University (approval number: H28-003).

4.8. Immunostaining for macrophages in aortic sections

After blocking with Blocking One Histo (Nacalai Tesque, Inc., Kyoto, Japan) at room temperature for 15 min, frozen aorta sections were incubated with rat monoclonal macrophage/monocyte antibody (GeneTex, Inc. Irvine, CA, USA, Catalog number: GTX39773) at a 1:25 dilution at 4°C overnight. The specimens were washed 3 times in PBS for 5 min and then incubated with Alexa Fluor 568-conjugated anti-rat IgG secondary antibodies (Abcam, Cambridge, UK, Catalog number: ab175475) at a 1:200 dilution for 1 h at room temperature. The localization of macrophages was determined using a laser-scanning confocal microscope (LSM 700).

4.9. Intravenous administration of LOX-1 antibody modified liposomes

The LOX-1 antibody modified and unmodified liposomes (100 µmol phospholipids/kg body wt.) were administered intravenously to the BALB/c KOR-*Apoe*^{shl} mice via the tail. After 3 h of liposome administration, the aortas were excised. Frozen sections were prepared in the same manner as described above and analyzed using a laser-scanning confocal microscope (LSM 700).

4.10. Statistical analysis

Statistical analyses were conducted using a Student t-test with SPSS software version 27 (IBM Inc., Armonk, NY, USA). A p-value of <0.05 was considered statistically significant.

Acknowledgement: This work was supported by JSPS KAKENHI Grant Number JP16K13049 provided by the Japan Society for the Promotion of Science.

Conflicts of interest: None declared.

References

Barrett TJ (2020) Macrophages in atherosclerosis regression. Arterioscler Thromb Vasc Biol 40: 20–33.

Bobryshev YV, Ivanova EA, Chistiakov DA, Nikiforov NG, Orekhov AN (2016) Macrophages and their role in atherosclerosis: pathophysiology and transcriptome analysis. BioMed Res Int 2016: 9582430.

Braganza DM, Bennett MR (2001) New insights into atherosclerotic plaque rupture. Postgrad Med J 77: 94–98.

Chono S, Tauchi Y, Morimoto K (2006) Influence of particle size on the distributions of liposomes to atherosclerotic lesions in mice. Drug Dev Ind Pharm 32: 125–135.

Di Pietro N, Formoso G, Pandolfi A (2016) Physiology and pathophysiology of oxLDL uptake by vascular wall cells in atherosclerosis. Vascul Pharmacol 84: 1–7.

Goyal T, Mitra S, Khaidakov M, Wang X, Singla S, Ding Z, Liu S, Mehta JL (2012) Current concepts of the role of oxidized LDL receptors in atherosclerosis. Curr Atheroscler Rep 14: 150–159.

Groenendyk JW, Mehta NN (2018) Applying the ordinal model of atherosclerosis to imaging science: a brief review. Open Heart 5: e000861.

Harel-Adar T, Ben Mordechai TB, Amsalem Y, Feinberg MS, Leor J, Cohen S (2011) Modulation of cardiac macrophages by phosphatidylserine-presenting liposomes improves infarct repair. Proc Natl. Acad Sci U S A 108: 1827–1832.

Kattoor AJ, Goel A, Mehta JL (2019) LOX-1: regulation, signaling and its role in atherosclerosis. Antioxidants (Basel) 8: 218.

Levchenko TS, Hartner WC, Torchilin VP (2012) Liposomes in diagnosis and treatment of cardiovascular disorders. Methodist deBakey Cardiovasc J 8: 36–41.

Li D, Patel AR, Klibanov AL, Kramer CM, Ruiz M, Kang BY, Mehta JL, Beller GA, Glover DK, Meyer CH (2010) Molecular imaging of atherosclerotic plaques targeted to oxidized LDL receptor LOX-1 by SPECT/CT and magnetic resonance. Circ Cardiovasc Imaging 3: 464–472.

Libby P, Ridker PM, Hansson GK (2011) Progress and challenges in translating the biology of atherosclerosis. Nature 473: 317-325.

Liu L, Nagai I, Gao Y, Matsushima Y, Kawai Y, Sayama K (2017) Effects of catechins and caffeine on the development of atherosclerosis in mice. Biosci Biotechnol Biochem 81: 1948–1955.

Lopes de Menezes DE, Kirchmeier MJ, Gagne J-F, Pilarski LM, Allen TM (1999) Cellular trafficking and cytotoxicity of anti-CD19-targeted liposomal doxorubicin in B lymphoma cells. J Liposome Res 9: 199–228.

Lu J, Chen X, Xu X, Liu J, Zhang Z, Wang M, Li X, Chen H, Zhao D, Wang J, Zhao D, Cong D, Li X, Sun L (2019) Active polypeptides from hirudo inhibit endothelial cell inflammation and macrophage foam cell formation by regulating the LOX-1/LXR-α/ABCA1 pathway. Biomed Pharmacother 115: 108840.

Matsushima Y, Hayashi S-I, Tachibana M (1999) Spontaneously hyperlipidemic (SHL) mice: Japanese wild mice with apolipoprotein E deficiency. Mamm Genome 10: 352–357.

Y, Sakurai T, Ohoka A, Ohnuki T, Tada N, Asoh Y, Tachibana M (2001) Four strains of spontaneously hyperlipidemic (SHL) mice : phenotypic distinctions determined by genetic backgrounds. J Atheroscler Thromb 8: 71–79.

Mulder WJ, Strijkers GJ, Griffioen AW, van Bloois L, Molema G, Storm G, Koning GA, Nicolay K (2004) A liposomal system for contrast-enhanced magnetic resonance imaging of molecular targets. Bioconjug Chem 15: 799–806.

Sanz J, Fayad ZA (2008) Imaging of atherosclerotic cardiovascular disease. Nature 451: 953–957.

Sawant RR, Torchilin VP (2012) Challenges in development of targeted liposomal therapeutics. A.A.P.S. J 14: 303–315.

Wang H, Li Y, Zhang X, Xu Z, Zhou J, Shang W (2020) DPP-4 inhibitor linagliptin ameliorates oxidized LDL-induced THP-1 macrophage foam cell formation and inflammation. Drug Des Devel Ther 14: 3929–3940.

Weber C, Noels H (2011) Atherosclerosis: current pathogenesis and therapeutic options. Nat Med 17: 1410–1422.