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# Functional involvement of scavenger receptor class B, type I, in the uptake of α-tocopherol using cultured rat retinal capillary endothelial cells

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**Purpose:** α-Tocopherol is an essential micronutrient acting as an antioxidant in the retina. However, the molecular mechanism of its retinal uptake from the circulating blood remains to be determined. The purpose of this study was to elucidate the contribution of scavenger receptor class B, type I (SR-BI), to the uptake of high-density lipoprotein (HDL)-associated α-tocopherol (α-tocopherol-HDL) using a conditionally immortalized rat retinal capillary endothelial cell line (TR-iBRB2 cells), as an in vitro inner blood-retinal barrier model.
**Methods:** An uptake study of α-tocopherol-HDL was performed using TR-iBRB2 cells. The expression of SR-BI protein was determined by immunoblot and immunohistochemical analyses. RNA interference was done to clarify the relationship between SR-BI protein expression and the uptake of α-tocopherol-HDL by TR-iBRB2 cells.
**Results:** [$^{14}$C]α-tocopherol-HDL uptake by TR-iBRB2 cells exhibited a time-dependent increase and a temperature-dependence with an 88% reduction for 90 min at 4 °C compared with that at 37 °C. The uptake of [$^{14}$C]α-tocopherol-HDL was inhibited by BLT-1, a specific inhibitor of the SR-BI-mediated lipid transfer between HDL and cells, in a concentration-dependent manner with an $IC_{50}$ of 23.2 nM. SR-BI protein expression was detected in TR-iBRB2 cells and SR-BI immunostaining was observed along the rat retinal capillaries. Inhibition of SR-BI protein expression by SR-BI siRNA resulted in a 24.4% reduction in [$^{14}$C]α-tocopherol-HDL uptake.
**Conclusions:** Our findings strongly suggest that SR-BI at the inner blood-retinal barrier is responsible for α-tocopherol uptake from the circulating blood and plays a key role in maintaining α-tocopherol in the neural retina.

Vitamin E is a family of essential micronutrients composed of lipid-soluble tocopherols and tocotrienols that have potent antioxidant activity [1,2]. Systemic vitamin E administration has been proposed to have preventive and therapeutic effects in human retinopathies [3,4]. In contrast, prolonged vitamin E deficiency may lead to retinal degeneration [5]. The retina is the only tissue in which light is directly focused on cells and causes free radical oxidation. Therefore, the retina is necessary to be protected against oxidative stress by the constant supply of antioxidants. Among the vitamin E family, α-tocopherol is the major constituent found in mammalian tissues and has the highest biological activity [1]. Weaned rats fed a vitamin E deficient diet for 15 weeks manifested an approximate eightfold reduction in the concentration of vitamin E in the neural retina, compared with those fed a regular diet (about 10 μg versus 78 μg of vitamin E/g dry weight retina) [6]. Such evidence strongly suggests that the retina possesses an efficient uptake system for α-tocopherol from the circulating blood to protect itself against oxidative stress. It is important to identify the transport mechanisms for α-tocopherol as far as the supply of antioxidants to the neural retina is concerned, in order to increase our knowledge of α-tocopherol transport to the retina.

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The nutrient supply to the retina from the circulating blood is regulated by the blood-retinal barrier (BRB), which is composed of retinal capillary endothelial cells (inner BRB) and retinal pigment epithelial cells (RPE, outer BRB) [7,8]. We have found that vitamin C, which is also a well-known antioxidant, is supplied from the circulating blood to the retina via a facilitative glucose transporter 1 (GLUT1) at the inner BRB [9]. Therefore, it is conceivable that the inner BRB has an active system to supply α-tocopherol to the retina. Hydrophobic α-tocopherol is exclusively associated with lipoproteins, such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL) in the circulating blood. Quantitatively, 80-95% of the total plasma α-tocopherol is associated with HDL among mouse plasma lipoproteins [10], although Voegele et al. found human serum albumin exhibited no binding to D-α-tocopherol based on surface plasmon resonance experiments [11]. The cellular uptake of HDL-associated lipids, particularly cholesteryl esters, is reported to be mediated by scavenger receptor class B, type I (SR-BI) [12]. Moreover, a previous study using SR-BI-deficient mice has shown the defective uptake of α-tocopherol from plasma lipoproteins to specific tissues, such as ovary, testis, lung, and brain [13]. These lines of evidence prompted us to hypothesize that SR-BI plays a role in the uptake of HDL-associated α-tocopherol at the inner BRB.

The purpose of the present study was to investigate whether SR-BI was functionally involved in the uptake of

HDL-associated α-tocopherol at the inner BRB. The characteristics of HDL-associated α-tocopherol transport were examined using a conditionally immortalized rat retinal capillary endothelial cell line (TR-iBRB2 cells), as an in vitro inner BRB model [14]. The expression of SR-BI protein at the inner BRB was determined by immunoblot and immunohistochemical analyses. We used RNA interference to further clarify the relationship between SR-BI protein expression and the uptake of HDL-associated α-tocopherol by TR-iBRB2 cells.

## METHODS

*Animals:* Six-week-old male Wistar rats and male ddY mice were purchased from SLC (Shizuoka, Japan). The investigations using rats and mice described in this report conformed to the provisions of the Animal Care Committee, University of Toyama (number 2006-4) and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

*Uptake study of high-density lipoprotein-associated [$^{14}$C]α-tocopherol ([$^{14}$C]α-tocopherol-high-density lipoprotein) in TR-iBRB2 cells:* TR-iBRB2, the conditionally immortalized rat retinal capillary endothelial cell line that we established [14], was used as an in vitro inner BRB model to characterize [$^{14}$C]α-tocopherol-HDL transport. [$^{14}$C]α-tocopherol-HDL was generated in all experiments by incubation of ethanolic [$^{14}$C]α-tocopherol solution (12.5 μCi; 50 mCi/mmol, GE Healthcare, Piscataway, NJ) with HDL from human plasma (1.5 mg protein; Merck, Darmstadt, Germany) in 2 ml extracellular fluid (ECF) buffer (122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.4 mM $K_2HPO_4$, 10 mM D-glucose, and 10 mM HEPES, pH 7.4) at 37 °C for 3 h. Non HDL-associated [$^{14}$C]α-tocopherol was removed by size-exclusion chromatography on a PD-10 column (GE Healthcare). TR-iBRB2 cells from passages 27-38 were seeded onto rat tail collagen-type I-coated tissue culture dishes (BD Biosciences, San Jose, CA). The cells were cultured at 33 °C in Dulbecco's modified Eagle's medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal bovine serum (Moregate, Bulimba, Australia) in 5% $CO_2$-air. For the uptake study, cells ($5x10^4$ cells/cm$^2$) were cultured at 33 °C for two days on rat tail collagen-type I-coated 24-well plates (BD Biosciences, San Jose, CA) and washed with 1 mL ECF buffer at 37 °C. Uptake was initiated by applying 200 μl ECF buffer containing HDL-associated [$^{14}$C]α-tocopherol (0.5 μCi/ml, 10 μM) at 37 °C or on ice (4 °C). For the uptake study, except for the inhibition study, we used the ECF buffer that was free of serum and other proteins, such as albumin. For the inhibition study, TR-iBRB2 cells were preincubated with ECF buffer containing 0.5% dimethyl sulphoxide (DMSO) and 0.5% bovine serum albumin (BSA) in the presence or absence of block lipid transport-1 (BLT-1; Chembridge, San Diego, CA) at 37 °C for 60 min. Uptake was subsequently performed using ECF buffer containing HDL-associated [$^{14}$C]α-tocopherol (0.5 μCi/ml, 10 μM), 0.5% DMSO, and 0.5% BSA in the presence or absence of BLT-1 at 37 °C for 60 min. DMSO and BSA were supplemented in the ECF buffer at each concentration of 0.5% to maintain BLT-1 solubility [15]. We confirmed that there was no difference in [$^{14}$C]α-tocopherol-HDL uptake (cell:medium ratio) for 90 min in the presence or absence of 0.5% DMSO and 0.5% BSA in the uptake buffer. This suggests that the uptake of [$^{14}$C]α-tocopherol-HDL by TR-iBRB2 cells is not influenced by DMSO and BSA supplemented in the uptake buffer. After a predetermined time period, uptake was terminated by removing the solution, and cells were immersed in ice-cold ECF buffer. The cells were then solubilized in 1N NaOH and subsequently neutralized with 1N HCl. Radioactivity was measured by liquid scintillation counting, and the protein content was determined using a kit (DC; Bio-Rad. Hercules, CA) with BSA as a standard.

*Immunoblot analysis:* To prepare protein samples of whole lysate, TR-iBRB2 cells were lysed with lysis buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), and a protease-inhibitor cocktail (Sigma, St Louis, MO). The lysate was centrifuged at 12,000 g for 5 min, and the supernatants were collected. Protein samples of the crude membrane fraction were prepared by homogenizing mouse liver and TR-iBRB2 cells using the nitrogen cavitation technique (800 psi, 20 min, 4 °C) in a buffer containing 10 mM HEPES-NaOH (pH 7.5), 1 mM EDTA, 1 mM EGTA, 320 mM sucrose, and a protease-inhibitor cocktail (Sigma). Homogenized samples were centrifuged at 2,000 g for 15 min. The supernatants were centrifuged at 100,000 g for 1 h, and a crude membrane fraction was obtained from the pellets. The pellets were suspended in the buffer and the protein concentration of samples was measured using a kit (DC; Bio-Rad). Protein samples (25 or 50 μg) were resolved by 7.5% SDS polyacrylamide gel electrophoresis (SDS-PAGE, Bio-Rad) and subsequently electrotransferred to nitrocellulose membranes. Membranes were incubated with a blocking agent solution (Block Ace; Dainippon Sumitomo Pharma Co, Osaka, Japan), prior to incubation with rabbit polyclonal anti-SR-BI antibody (1:2000, Novus Biologicals, Littleton, CO) or mouse monoclonal anti-β-actin antibody (1:5000, Sigma) as the primary antibody at 4 °C for 16 h. The membranes were subsequently incubated with horseradish peroxidase conjugated anti-rabbit or mouse IgG. Bands were visualized using an enhanced chemiluminescence kit (GE healthcare).

*Immunohistochemical analysis:* Under deep pentobarbital anesthesia (50 mg/kg body weight, intraperitoneally), rats were perfused transcardially with 4% formaldehyde in 0.1 M phosphate buffer. Both eyeballs were isolated and immersed in 30% sucrose in 0.1 M phosphate buffer. Sections (20 μm in thickness) were cut from the frozen eye with a cryostat (CM1900; Leica, Heidelberg, Germany) and mounted on silanated glass slides (Dako, Carpinteria, CA). After incubation with 10% goat serum (Nichirei, Tokyo, Japan) for 30 min at room temperature, sections were then incubated with rabbit polyclonal anti-SR-BI antibody (1:200, Novus Biologicals) and guinea pig polyclonal anti-GLUT1 antibody (0.5 μg/ml), [16] for 16 h at room temperature. Sections were subsequently incubated with FITC-conjugated anti-rabbit and Cy3-conjugated anti-guinea pig IgG antibodies for 2 h at room tempera-

ture. Sections were then mounted on coverslips using mounting medium (Vectashield; Vector Laboratories, Burlingame, CA) and viewed using a confocal laser microscope (LSM510; Carl Zeiss Meditec, Oberkochen, Germany).

*SR-BI siRNA preparation and transfection into TR-iBRB2 cells:* The siRNA targeted to rat SR-BI mRNA (GenBank accession number, NM_031541) was designed based on the corresponding region of human SR-BI siRNA reported by Voisset et al. [17]. The siRNA sequence containing 3'-dTdT extensions (5'-GCA GCA GGU GCU CAA GAA UTT-3') was chemically synthesized by Japan Bio Services Co. (Saitama, Japan). Double-stranded siRNA was generated according to the manufacturer's instructions. According to the manufacturer, non-specific control siRNA Duplex VIII (Dharmacon, Lafayette, CO) exhibits no RNAi effect. Its target sequence is 5'-ACU CUA UCU GCA CGC UGA C-3'. No rat gene sequences with homology to non-specific control siRNA were found by a Blast search. TR-iBRB2 cells were plated on a rat tail collagen type I-coated six well plate (Asahi Techno Glass Co., Tokyo, Japan) at 4 x $10^5$ cells/well, and grown for 24 h at 33 °C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in 5% $CO_2$-air. SR-BI and non-specific control siRNAs were then transfected using Lipofectamine 2000 and Opti-MEMI reduced serum medium (Invitrogen, Carlsbad, CA). Protein expression and uptake activity were examined 32 h after transfection. For the uptake study, cells were washed with 2.5 ml ECF buffer at 37 °C. Uptake was initiated by applying 1 ml ECF buffer containing HDL-associated [$^{14}$C]α-tocopherol (0.5 μCi/ml, 10 μM) at 37 °C. The sequential procedures were performed as described in this section.

*Data analysis:* All data represent the mean±SEM. Statistical significance of differences among means of several groups was determined by one-way analysis of variance followed by the modified Fischer's least-squares difference method.

## RESULTS

*Characteristics of [$^{14}$C]α-tocopherol-high-density lipoprotein uptake by TR-iBRB2 cells:* [$^{14}$C]α-tocopherol-HDL uptake was investigated using TR-iBRB2 cells as an in vitro model of the inner BRB. As shown in Figure 1A, [$^{14}$C]α-tocopherol-HDL uptake by TR-iBRB2 cells exhibited a time-dependent increase for 90 min at 37 °C, and was reduced by 88% for 90 min at 4 °C compared with that at 37 °C, suggesting that the uptake by TR-iBRB2 cells is mediated by an energy-dependent process. An inhibition study using BLT-1, a specific inhibitor of SR-BI-mediated lipid transfer [15], was also performed to clarify the involvement of SR-BI in α-tocopherol-HDL uptake by TR-iBRB2 cells (Figure 1B). [$^{14}$C]α-tocopherol-HDL uptake was inhibited by BLT-1 in a concentration-dependent manner with a 50% inhibition concentration ($IC_{50}$) value of 23.2 nM. The uptake exhibited a maximum inhibition of 57% by BLT-1 at concentrations over 1 μM.

*Expression of SR-BI protein in TR-iBRB2 and retinal capillary endothelial cells:* The expression of SR-BI protein was determined in TR-iBRB2 cells by immunoblot analysis (Figure 1C). Mouse liver was used as a positive control because anti-SR-BI antibody was raised against the mouse sequence of SR-BI as an antigen. Single bands of SR-BI were detected in all samples at about 75 kDa, which is comparable with previous reports (81-84 kDa, [18,19]). The molecular size of SR-BI protein in TR-iBRB2 cells was slightly lower than that in mouse liver. This may be due to the difference in glycosylation between the mouse and rat tissues. The localization of SR-BI in the retinal capillaries was determined by immunohistochemical analysis (Figure 1D). Around the outer plexiform layer, low to moderate immunoreactivities of SR-BI (red, Figure 1D,F) partially overlapped that of GLUT1 (green, Figure 1E,F), which is known to be expressed in retinal capillaries [20], and was also detected beyond areas of GLUT1 expression.

*Effect of SR-BI siRNA on SR-BI protein expression and [$^{14}$C]α-tocopherol-high-density lipoprotein uptake by TR-iBRB2 cells:* The effect of siRNA concentrations on SR-BI protein expression in TR-iBRB2 cells, was determined using immunoblot analysis (Figure 2A). After treatment with SR-BI siRNA for 36 h, SR-BI siRNA significantly reduced the levels of SR-BI protein expression in TR-iBRB2 cells in a concentration-dependent manner. In contrast, the SR-BI protein levels were not affected by any concentration of non-specific control siRNA. The level of β-actin protein was unchanged by any concentration of SR-BI and non-specific control siRNA. As shown in Figure 2B, [$^{14}$C]α-tocopherol-HDL uptake by TR-iBRB2 cells treated with 200 nM SR-BI siRNA for 36 h was reduced by 24.4% in the absence of BLT-1 and was reduced by 53.3% in the presence of 100 nM BLT-1, compared with that of a non-specific control in the absence of BLT-1, respectively. In contrast, [$^{14}$C]α-tocopherol-HDL uptake by TR-iBRB2 cells treated with 200 nM non-specific siRNA was reduced by 40.8% in the presence of 100 nM BLT-1, compared with that in the absence of BLT-1 (Figure 2B).

## DISCUSSION

The present study demonstrated the functional involvement of SR-BI in the uptake of HDL-associated α-tocopherol by retinal capillary endothelial cells using an in vitro inner BRB model, TR-iBRB2 cells. Although the uptake process of fat-soluble micronutrients like vitamin E was assumed to be passive diffusion across the plasma membrane, recent reports have proposed SR-BI-mediated transport of α-tocopherol in the brain capillary endothelial cells and enterocytes [18,19,21]. In this study, [$^{14}$C]α-tocopherol-HDL uptake by TR-iBRB2 cells exhibited a time-dependent increase and did not reach "steady-state" at least for 90 min, suggesting that, for the most part [$^{14}$C]α-tocopherol-HDL uptake by TR-iBRB2 cells depends on influx mechanism(s). [$^{14}$C]α-tocopherol-HDL uptake by TR-iBRB2 cells was reduced by 88% for 90 min at 4 °C compared with that at 37 °C (Figure 1A). This suggests the involvement of an energy-dependent carrier-mediated process, rather than passive diffusion. In turn, this raises the question as to how [$^{14}$C]α-tocopherol-HDL is taken up into TR-iBRB2 cells. The uptake of [$^{14}$C]α-tocopherol-HDL by TR-iBRB2 cells was inhibited by BLT-1, a specific inhibitor of the SR-BI-mediated lipid transfer between HDL and cells [15], in a

concentration-dependent manner with an $IC_{50}$ value of 23.2 nM (Figure 1B). This corresponding $IC_{50}$ value is close to the reported values for the uptake of [$^{3}$H]cholesterol ester-HDL and [$^{125}$I]-HDL binding using LDL-receptor deficient Chinese hamster ovary cells (IdlA) with high expression of murine SR-BI (110 and 88 nM, respectively) [15]. These pieces of evidence strongly suggest that BLT-1 exhibits a specific inhibitory effect on SR-BI-mediated [$^{14}$C]α-tocopherol-HDL uptake

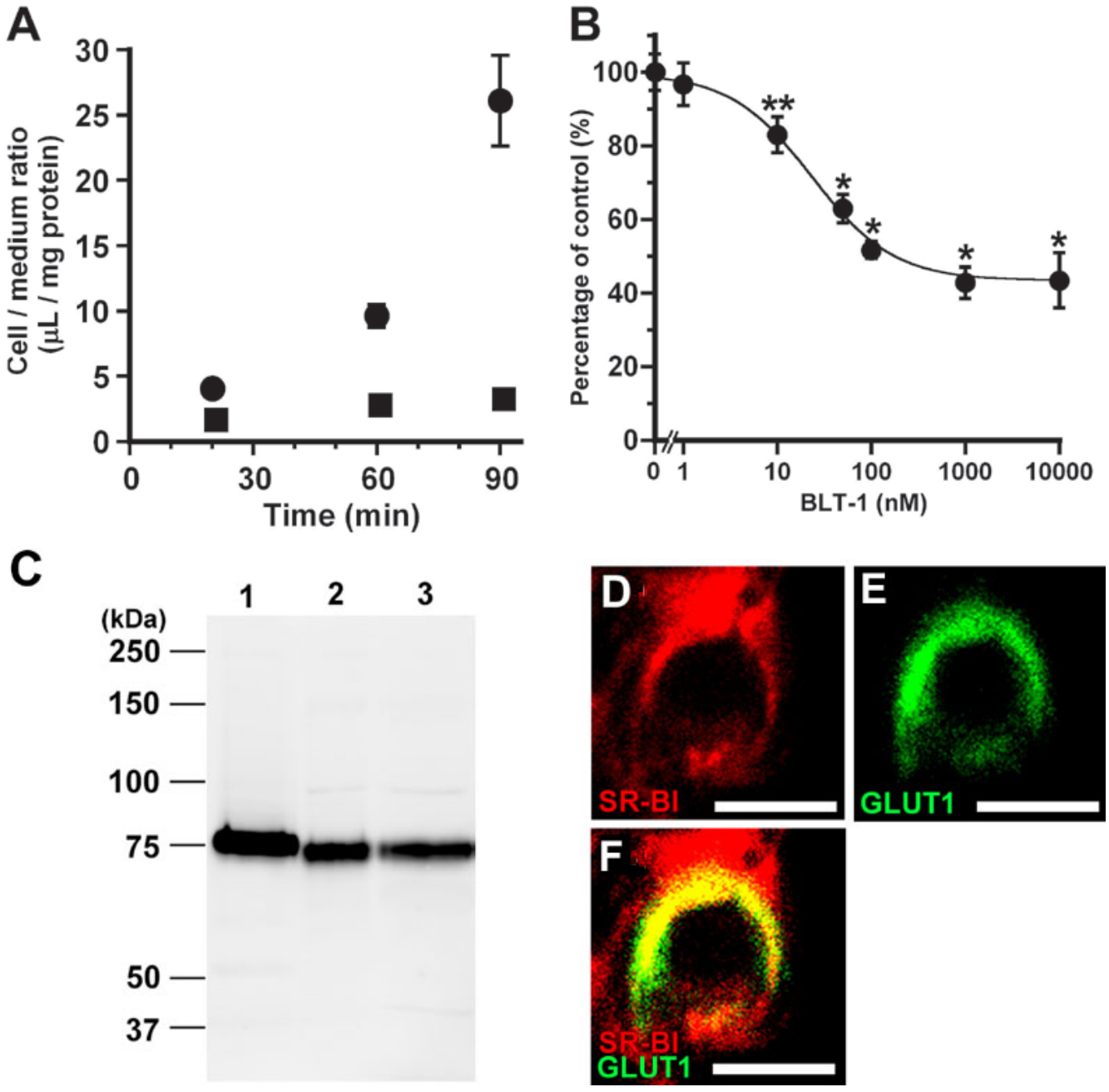


Figure 1. Characteristics of [$^{14}$C]α-tocopherol-high-density lipoprotein uptake and scavenger receptor class B, type I protein expression in TR-iBRB2 cells. **A**: [$^{14}$C]α-tocopherol-high density lipoprotein (HDL) uptake by TR-iBRB2 cells exhibited a time-dependent increase and a temperature-dependence. The [$^{14}$C]α-tocopherol-HDL (10 μM of [$^{14}$C]α-tocopherol) uptake was performed at 37 °C (circle) or 4 °C (square). Each point represents the mean±SEM (n=4). **B**: The [$^{14}$C]α-tocopherol-HDL uptake was inhibited by block lipid transport-1 (BLT-1), a potent inhibitor of SR-BI, in a concentration dependent manner with an $IC_{50}$ of 23.2 nM. Each point represents the mean±SEM (n=4). Asterisk (*) and double asterisk (**) represent statistical significance of differences from control, *p<0.01 and **p<0.05, respectively. **C**: Immunoblot analysis of SR-BI was performed using mouse liver (lane 1), membrane fraction of TR-iBRB2 cells (lane 2), and whole lysate of TR-iBRB2 cells (lane 3). Mouse liver was used as a positive control. **D**-**F**: SR-BI immunoreactivities were detected in rat retinal capillary endothelial cells by immunofluorescence. Rat retina was stained with anti-SR-BI antibody (red; **D**, **E**) and anti-GLUT1 antibody (green, **E**, **F**). Scale bars represents 5 μm.

by TR-iBRB2 cells. The maximum inhibition by BLT-1 reached 57% at concentrations over 1 μM BLT-1 (Figure 1B). Accordingly, SR-BI appears to make more than a 50% contribution to the uptake of α-tocopherol by retinal capillary endothelial cells. It is also possible that [$^{14}$C]α-tocopherol-HDL does not internalize into the cells and only distributes to the plasma membrane. Indeed, the selective uptake of lipid including cholesterol ester is generally considered to be a non-endocytotic mechanism. In this model, the HDL-apolipoproteins do not enter the cell, while cholesterol ester is first transferred to a reversible plasma membrane pool and then to an irreversible cell surface-remote compartment [22-24]. Gu et al. [25] proposed that the most likely mechanism for SR-BI-mediated lipid uptake is a combination of HDL-binding to SR-BI and SR-BI-facilitated lipid transfer from the HDL particle into the cell. This mechanism is possibly comparable with the SR-BI-mediated α-tocopherol transport at the inner BRB. Further studies that incorporate molecular imaging are needed to clarify the intracellular trafficking of α-tocopherol in retinal capillary endothelial cells and to determine whether the α-tocopherol transfer reaction requires specific interactions of SR-BI with additional cellular components. Moreover, the fact that BLT-1 did not fully inhibit [$^{14}$C]α-tocopherol-HDL uptake raises the possibility that an alternative transport system may also be involved. Indeed, based on their studies with SR-BI-deficient mice, Mardones et al. [13] suggested SR-BI-dependent and SR-BI-independent pathways for the tissue uptake of α-tocopherol. It will be intriguing, in future studies, to investigate the SR-BI-independent pathway for the uptake of α-tocopherol by retinal capillary endothelial cells.

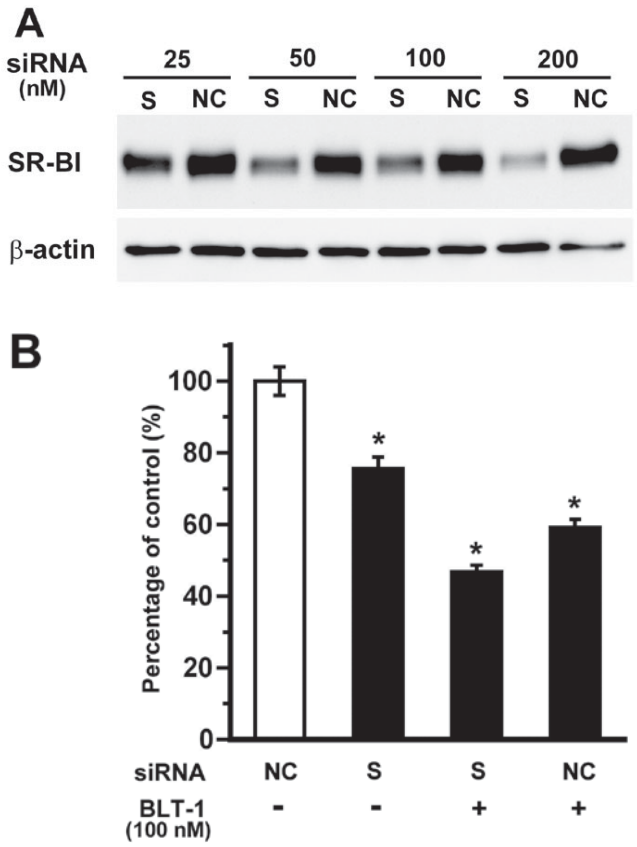


Figure 2. Effect of scavenger receptor class B, type I siRNA on protein expression of scavenger receptor class B, type I and [$^{14}$C]α-tocopherol-high-density lipoprotein uptake in TR-iBRB2 cells. **A**: Immunoblot analysis of scavenger receptor class B, type I (SR-BI) and β-actin in whole lysate of TR-iBRB2 cells. TR-iBRB2 cells were treated with different concentrations of SR-BI siRNA (S) or non-specific siRNA (control, NC). **B**: [$^{14}$C]α-tocopherol-high-density lipoprotein (HDL; 10 μM of [$^{14}$C]α-tocopherol) uptake was performed at 37 °C for 90 min after treatment of 200 nM SR-BI (S) or non-specific control (NC) siRNAs for 36 h in the absence (-) or presence (+) of 100 nM BLT-1. Each column represents the mean±SEM (n=6-7). Asterisk (*) represents statistical significance of differences from control, *p<0.01.

Immunoblot analysis revealed that SR-BI protein was expressed in TR-iBRB2 cells (Figure 1C), suggesting the expression of SR-BI at the inner BRB. Immunohistochemical analysis further confirmed that SR-BI protein is localized in retinal capillary endothelial cells (Figure 1D-F). We detected low to moderate immunoreactivities, including those in capillaries around the outer plexiform layer, although it has been reported that no reactivity was detected around that region in the monkey retina [26]. This discrepancy in capillary expression may be due to the differences in species, antibodies produced by different companies, or the gain level of a confocal scanning microscope. Our observations are comparable with those at the blood-brain barrier (BBB), since SR-BI is localized to the brain capillary endothelial cells [18,19]. It has been reported that porcine brain capillary endothelial cells possess an uptake system for HDL-associated α-tocopherol, via SR-BI [18]. Therefore, it appears that the lower level of α-tocopherol in the brain of SR-BI knockout mice depends on an SR-BI deficiency at the BBB. Since α-tocopherol deficiency is associated with oxidative stress, SR-BI at the inner BRB may be also involved in the accumulation of α-tocopherol, as an effective antioxidant in the neural retina. Our present report is the first to demonstrate SR-BI protein expression at the inner BRB. Moreover, previous immunohistochemical studies have shown that SR-BI proteins are found in the choriocapillaris, ganglion cells and Müller glial cells as well as the photoreceptors in the monkey retina [26]. Tserentsoodol et al. [26] have proposed a mechanism of lipid transport including HDL via SR-BI as well as SR-BII in the retina. HDL in the circulating blood enters the retina via SR-BI or SR-BII in the RPE. The RPE takes up lipoprotein particles and transfers the lipids to endogenous apoAI- and apoE-containing HDL-like particles. These HDL-like particles are then transported by the ATP-binding cassette (ABC) transporter A1 (ABCA1) out of the RPE and delivered to the photoreceptors via SR-BI and SR-BII receptors. Future studies may clarify how HDL-α-tocopherol has access to photoreceptor cells by SR-BI. We also found SR-BI immunoreactivity in the GLUT1-negative cells surrounding the capillaries, suggesting SR-BI expression in Müller glial cells as well as neuronal cells.

RNA interference was carried out to examine whether SR-BI directly contributes to the uptake of [$^{14}$C]α-tocopherol-HDL

by TR-iBRB2 cells. To our knowledge, there is no direct evidence that SR-BI is involved in HDL-associated α-tocopherol uptake in cells. In the present study, the inhibition of SR-BI protein expression by SR-BI siRNA resulted in a 24.4% reduction in [$^{14}$C]α-tocopherol-HDL uptake by TR-iBRB2 cells (Figure 2). The uptake by TR-iBRB2 cells treated with SR-BI siRNA was further reduced by 53.3% in the presence of 100 nM BLT-1, compared with that of a non-specific control in the absence of BLT-1. This additional reduction by BLT-1 can be due to the fact that TR-iBRB2 cells expressed SR-BI protein even after siRNA treatment and that BLT-1 inhibited the residual function of SR-BI in TR-iBRB2 cells. Furthermore, the uptake by TR-iBRB2 cells treated with non-specific siRNA was reduced by 40.8% in the presence of 100 nM BLT-1, compared with that in the absence of BLT-1. This inhibitory effect by BLT-1 was nearly comparable with that of TR-iBRB2 cells treated with 200 nM SR-BI siRNA (53.3% inhibition), suggesting the specificity of BLT-1-mediated inhibition of SR-BI. These pieces of evidence support the idea that SR-BI makes almost a 50% contribution to the uptake of α-tocopherol at the inner BRB and provides a pathway facilitating a continuous supply of α-tocopherol as the essential micronutrient to the neural retina.

Although it has been also reported that LDL receptor is involved in LDL-associated α-tocopherol transport [27,28], there were no differences in α-tocopherol concentrations in LDL-receptor deficient mice in tissues such as liver, kidney, testis, lung, and brain [13]. Taking into consideration the fact that most plasma α-tocopherol is associated with HDL among mouse plasma lipoproteins [13], it is likely α-tocopherol was transferred from HDL in the circulating blood to retinal capillary endothelial cells, at least in part, via SR-BI. Selective uptake of HDL-associated α-tocopherol by the retinal capillary endothelial cells via SR-BI is most likely the first step facilitating the supply of α-tocopherol to the neural retina. However, the subsequent steps of intracellular as well as efflux transport of α-tocopherol across the inner BRB remains unclear. Further studies should provide new insights into the molecular mechanisms regulating the transcellular transport of α-tocopherol across the inner BRB. α-Tocopherol transfer protein (α-TTP) and ABCA1 have been identified as the molecules involved in the intracellular trafficking and the efflux transport of α-tocopherol, respectively [29]. When α-TTP-knockout mice were fed a vitamin E-deficient diet, they exhibited vitamin E deficiency, lipid peroxidation in the retina, and degenerative damage to the retina with aging [30]. Future studies are need to examine the physiological contribution of α-TTP as well as ABCA1 to the transcellular and efflux transport of α-tocopherol across the inner BRB.

In conclusion, this is the first report to demonstrate that the transport of HDL-associated α-tocopherol, at least in part, is mediated by SR-BI at the inner BRB. The functional role of SR-BI at the inner BRB appears to involve the constant supply of α-tocopherol from the circulating blood to the retina to protect the neural retina against oxidative stress. Our present data may assist in the design of a suitable vitamin E dosage regimen for pharmacological therapies of a variety of retinopathies.

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