Review

Transporters for the Intestinal Absorption of Cholesterol, Vitamin E, and Vitamin K

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Humans cannot synthesize fat-soluble vitamins such as vitamin E and vitamin K. For this reason, they must be obtained from the diet via intestinal absorption. As the deficiency or excess of these vitamins has been reported to cause several types of diseases and disorders in humans, the intestinal absorption of these nutrients must be properly regulated to ensure good health. However, the mechanism of their intestinal absorption remains poorly understood. Recent studies on cholesterol using genome-edited mice, genome-wide association approaches, gene mutation analyses, and the development of cholesterol absorption inhibitors have revealed that several membrane proteins play crucial roles in the intestinal absorption of cholesterol. Surprisingly, detailed analyses of these cholesterol transporters have revealed that they can also transport vitamin E and vitamin K, providing clues to uncover the molecular mechanisms underlying the intestinal absorption of these fat-soluble vitamins. In this review, we focus on the membrane proteins (Niemann-Pick C1 like 1, scavenger receptor class B type I, cluster of differentiation 36, and ATP-binding cassette transporter A1) that are (potentially) involved in the intestinal absorption of cholesterol, vitamin E, and vitamin K and discuss their physiological and pharmacological importance. We also discuss the related uncertainties that need to be explored in future studies.

Key words: ABCA1, CD36, Ezetimibe, NPC1L1, SR-BI

Introduction

The excess intake of cholesterol (**Fig. 1A**) due to the westernization of eating habits has led to an increased number of patients worldwide with lifestyle-related diseases such as dyslipidemia and obesity. Meanwhile, the moderate intake of antioxidant and/or anticalcification lipids such as vitamin E (**Fig. 1B**) and vitamin K (**Fig. 1C**) is reported to reduce the risk of atherosclerosis and cardiovascular events[1, 2]. The appropriate regulation of intestinal lipid absorption is therefore important for a healthy life and is also considered an attractive strategy to prevent lifestyle-related diseases.

It was long assumed that dietary cholesterol and fat-soluble vitamins, including vitamin E and vitamin K, were absorbed via a passive diffusion process across the plasma membrane of enterocytes. However, recent advances in genomic analyses[3-8], studies with genome-edited mice[9-14], and the development of ezetimibe (**Fig. 1D**), a cholesterol absorption inhibitor clinically used for dyslipidemia[15, 16], have revealed that several membrane transporters play key roles in intestinal cholesterol absorption. Interestingly, the identification of such cholesterol transporters has also provided novel insights into the molecular mechanism underlying the absorption of fat-soluble vitamins because most of these transporters can transport vitamin E and vitamin K in addition to cholesterol.

In this review, we provide an overview of the recent findings on intestinal cholesterol transporters that are also (potentially) involved in the absorption of vitamin E and vitamin K and discuss clinical topics related to the pharmacological effects of ezetimibe on the absorption of these fat-soluble vitamins (**Fig. 2**).

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A sterols

cholesterol

β-sitosterol

B vitamin E isoforms

tocopherol

tocotrienol

	R1	R2	R3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

C vitamin K

vitamin K_1
(phylloquinone)

vitamin K_2
(menaquinone-n)

n (n=1~13)

D ezetimibe

E BLT-1

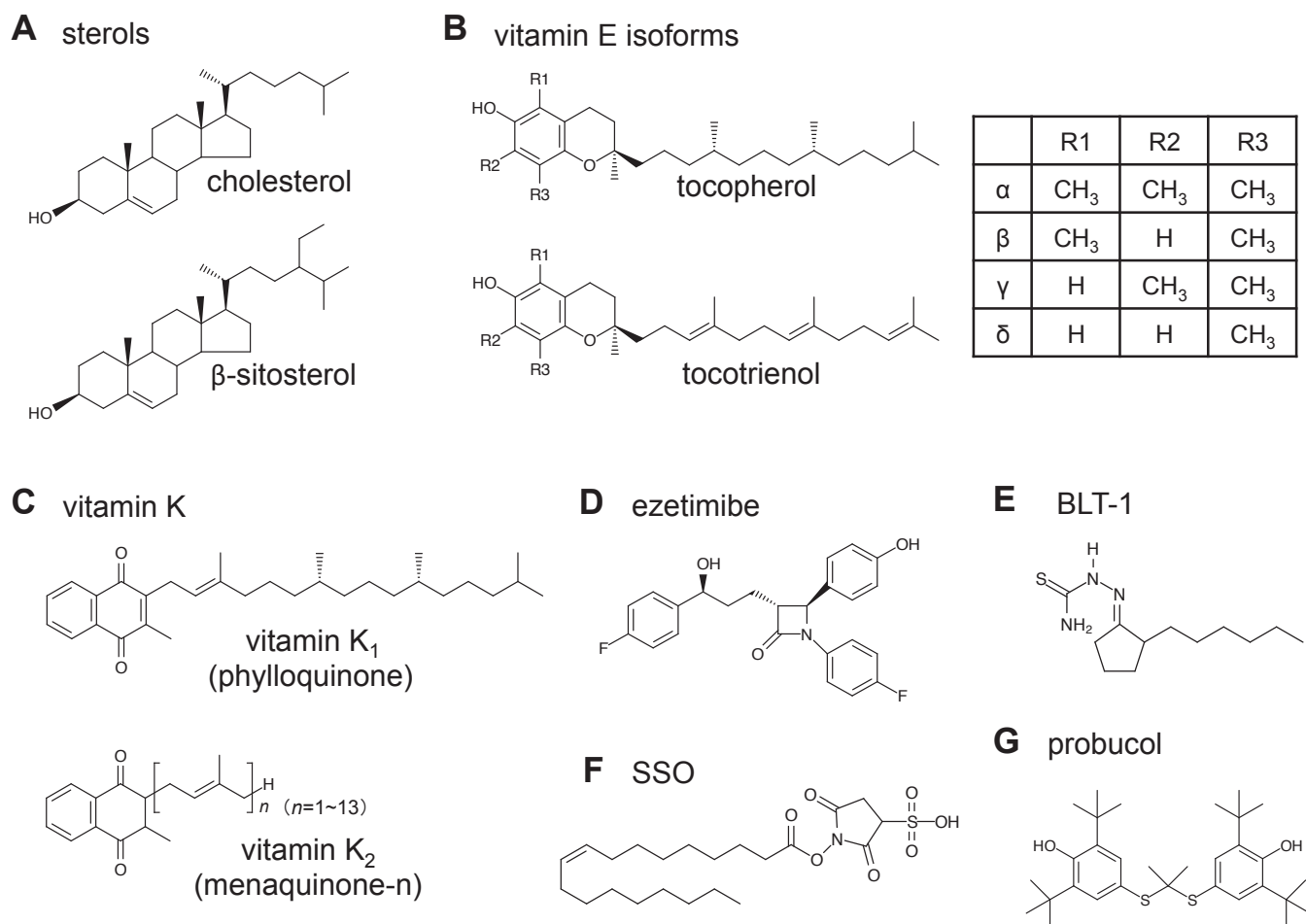

F SSO

G probucol

Fig. 1. Chemical structures of (A) sterols, (B) vitamin E isoforms, (C) vitamin K, (D) ezetimibe, (E) BLT-1, (F) SSO, and (G) probucol.

Niemann-Pick C1 Like 1

Niemann-Pick C1 like 1 (NPC1L1) is recognized as a key player in intestinal cholesterol absorption and a molecular target of ezetimibe. NPC1L1 was originally identified as a homologue of the Niemann-Pick C1 (NPC1) protein[17], which is involved in intracellular cholesterol trafficking from lysosomes to other organelles such as the endoplasmic reticulum and plasma membrane[18]. Deficiency of the NPC1 protein causes Niemann Pick disease type C, a genetic disorder characterized by lysosomal cholesterol accumulation resulting in neurodegeneration and premature death[19, 20]. Unlike NPC1 knockout (KO) mice, NPC1L1 KO mice appear healthy without severe phenotypes. However, in 2004, Altmann *et al.* found that intestinal cholesterol absorption in NPC1L1 KO mice was reduced to about 30% of that in wild-type (WT) mice, and the degree of this reduction was almost the same as that observed in ezetimibe-treated WT mice[9].

In addition, ezetimibe had little effect on the remaining level of intestinal cholesterol absorption in NPC1L1 KO mice. Based on these results and the fact that NPC1L1 is highly expressed on the brush border membrane of enterocytes in the proximal intestine, where cholesterol absorption primarily occurs[9], as well as *in vitro* observations that ezetimibe binds to the NPC1L1 protein[21, 22], NPC1L1 is now thought to be a central player in intestinal cholesterol absorption and a molecular target of ezetimibe. In this section, we summarize our recent findings on NPC1L1 function in addition to the drug-drug interaction between ezetimibe and warfarin.

NPC1L1-Mediated Sterol Absorption

Detailed analyses of NPC1L1 function were performed *in vitro* using NPC1L1-overexpressing Caco-2 (colorectal adenocarcinoma) cells[23]. Consistent with the physiological localization of NPC1L1, the introduced NPC1L1 protein was expressed on the apical
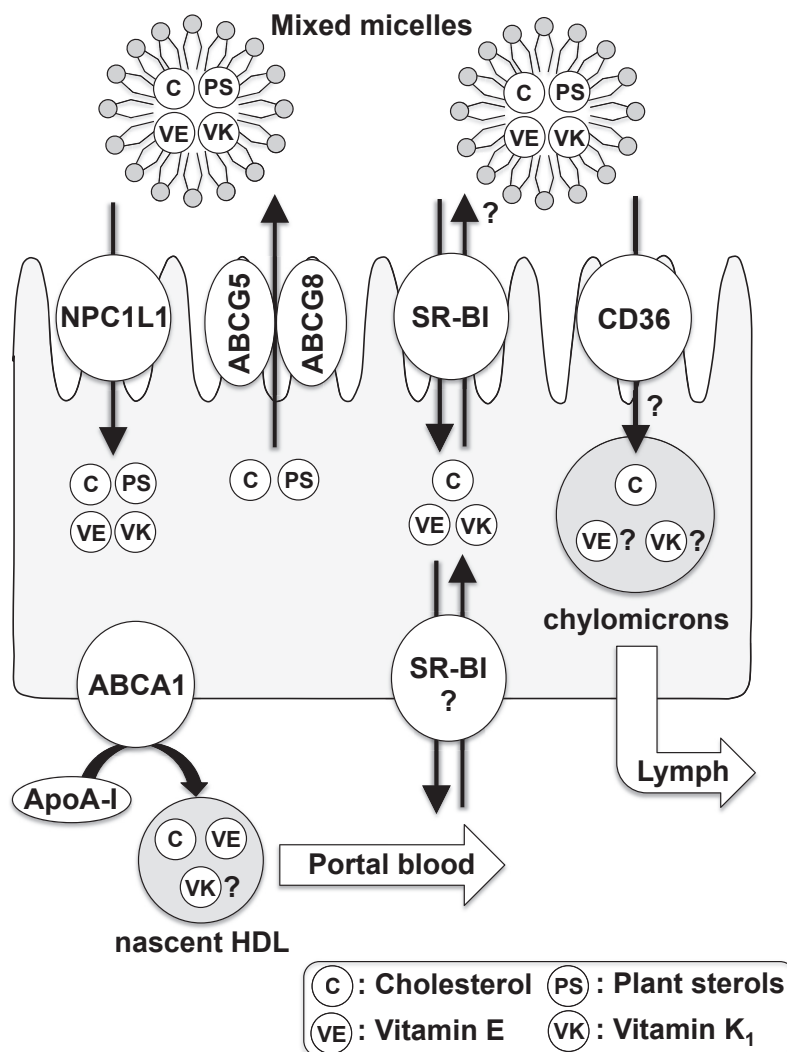
Fig. 2. Membrane proteins mediating the intestinal absorption of cholesterol, plant sterols, vitamin E, and vitamin K_1.

Recent research has revealed that several transmembrane proteins such as NPC1L1, ABCG5/G8, SR-B1, CD36, and ABCA1 play crucial roles in the intestinal absorption of cholesterol, plant sterols, vitamin E, and vitamin K_1.

membrane in Caco-2 cells. In addition, the cellular uptake of cholesterol dissolved in mixed micelles containing taurocholate and phosphatidylcholine was increased by NPC1L1 overexpression in Caco-2 cells. Moreover, this increase was inhibited by ezetimibe in a concentration-dependent manner. These *in vitro* results clearly indicate that NPC1L1 has micellar cholesterol uptake activity, which is sensitive to ezetimibe.

Interestingly, *in vitro* studies using NPC1L1-overexpressing Caco-2 cells also showed that micellar taurocholate could increase NPC1L1-mediated cholesterol uptake in a concentration-dependent manner[23]. By contrast, micellar phosphatidylcholine showed a negative correlation with cholesterol uptake

by NPC1L1. These results are consistent with *in vivo* observations that patients with a hereditary defect in bile acid synthesis exhibited a reduction in cholesterol absorption[24] and that cholesterol absorption was suppressed by phosphatidylcholine supplementation in humans[25]. Based on these findings, the effects of the micellar composition on NPC1L1 activity would be an important factor to control the efficiency of intestinal cholesterol absorption.

It has been reported that the absorption of plant sterols in NPC1L1 KO mice is lower than that in WT mice[26]. Consistently, our *in vitro* study demonstrated that β-sitosterol, a major dietary plant sterol (**Fig. 1A**), was taken up more efficiently in NPC1L1-overex-

pressing Caco-2 cells than in control cells, indicating that NPC1L1 has the ability to transport β-sitosterol in addition to cholesterol[23]. However, it should be noted that the NPC1L1-mediated uptake of β-sitosterol was approximately 30% of that of cholesterol. It has been reported that ~50% of dietary cholesterol is absorbed in the intestine compared to only 5%–15% of dietary plant sterols[27]. Our *in vitro* results indicate that the lower level of intestinal absorption of plant sterols might be due to the lower level of uptake of plant sterols by NPC1L1, in addition to the contribution of the well-known luminal backflux of sterols by the heterodimer of ATP-binding cassette transporter G5 and G8 (ABCG5/G8)[6, 8] (**Fig. 2**).

NPC1L1-Mediated Vitamin E Absorption

Given that fat-soluble vitamins, similar to cholesterol, are solubilized in mixed micelles and then absorbed in the small intestine, we assumed that some of these vitamins might be taken up by enterocytes via a shared pathway with cholesterol. Based on this hypothesis, α-tocopherol (a major isoform of vitamin E) (**Fig. 1B**) and retinol (vitamin A) uptake were first examined using NPC1L1-overexpressing Caco-2 cells[28]. Although ezetimibe-sensitive retinol uptake was not observed, α-tocopherol uptake was increased by the overexpression of NPC1L1 and this increase was significantly blocked by ezetimibe in a concentration-dependent manner. In addition, nonsynonymous variants of NPC1L1 identified in cholesterol low absorbers (A395V, G402S, R417W and G434R)[7] showed decreased transport activities for α-tocopherol and cholesterol compared to the WT, suggesting that the recognition and transport mechanisms for α-tocopherol are similar to those for cholesterol among the four variants and WT NPC1L1[29]. In accordance with *in vitro* observations, ezetimibe administration significantly inhibited the intestinal absorption of not only cholesterol but also α-tocopherol in Wistar rats, although the inhibitory effect on α-tocopherol absorption (approximately 35% reduction by 0.3 mg/kg ezetimibe) was less than that on cholesterol absorption (60%–80% reduction)[28, 30]. Together with observations that other isoforms of vitamin E such as γ-tocopherol, δ-tocopherol, and tocotrienols (**Fig. 1B**) could also be taken up via the ezetimibe-sensitive NPC1L1-mediated pathway[31-33], these results suggest that vitamin E is absorbed, at least partly, via the NPC1L1-dependent pathway.

Although ezetimibe inhibits vitamin E absorption *in vivo*, a clinical study has reported that serum concentrations of vitamin E were not significantly reduced after a 12-week administration of ezetimibe[15]. Based on the well-known fact that α-tocoph-

erol transfer protein (α-TTP), a cytosolic protein expressed in the liver, plays a key role in maintaining vitamin E homeostasis by regulating hepatic storage and trafficking of α-tocopherol[34, 35], the partial inhibition of vitamin E absorption by ezetimibe may not immediately cause vitamin E deficiency in humans. However, considering that humans cannot synthesize vitamin E and that it is therefore necessary to ingest this nutrient as part of the diet, the ezetimibe-mediated malabsorption of vitamin E should be taken into consideration as a potential risk for vitamin E deficiency.

NPC1L1-Mediated Vitamin K₁ Absorption

Vitamin K is an essential nutrient that facilitates blood coagulation by activating clotting factors such as prothrombin and factors II, VII, IX and X in the liver[36, 37]. Vitamin K antagonists such as warfarin are therefore clinically used to prevent thromboembolism. Dietary vitamin K is generally categorized into two forms: phylloquinone (vitamin K₁) and menaquinones (collectively referred to as vitamin K₂), which comprise 13 compounds classified based on the length of their side chain (menaquinone-1 to menaquinone-13) (**Fig. 1C**). Vitamin K₁ is enriched in green leafy vegetables, beans, and certain plant oils. On the other hand, most vitamin K₂ is produced by bacteria in the intestine. It has been reported that vitamin K₁ accounts for approximately 90% of dietary vitamin K[38].

Despite the importance of vitamin K, there is little information on the molecular mechanism of intestinal vitamin K₁ absorption. Based on the following three reasons, we hypothesized that NPC1L1 is a physiological vitamin K₁ importer in the small intestine. First, the intestinal absorption of vitamin K₁ depends on bile[39], similar to that of cholesterol and vitamin E. Second, vitamin K₁ is mainly absorbed in the upper intestine, where NPC1L1 is highly expressed[9, 40]. Third, the package insert for ezetimibe indicates a potential drug interaction between ezetimibe and warfarin that may enhance the anticoagulant effect of warfarin[41] (please see the next subsection for details). To determine whether vitamin K₁ is taken up via the NPC1L1-mediated pathway, *in vitro* vitamin K₁ uptake assays using NPC1L1-overexpressing Caco-2 cells were conducted[42]. The results showed that the cellular uptake of vitamin K₁ was significantly increased by NPC1L1 overexpression and that NPC1L1-mediated vitamin K₁ uptake was inhibited by ezetimibe in a concentration-dependent manner. In addition, *in vivo* acute vitamin K₁ absorption studies revealed that the intestinal absorption of vitamin K₁ in NPC1L1 KO mice was dramatically reduced to less than 30% of that in WT mice, which was similar to the intestinal
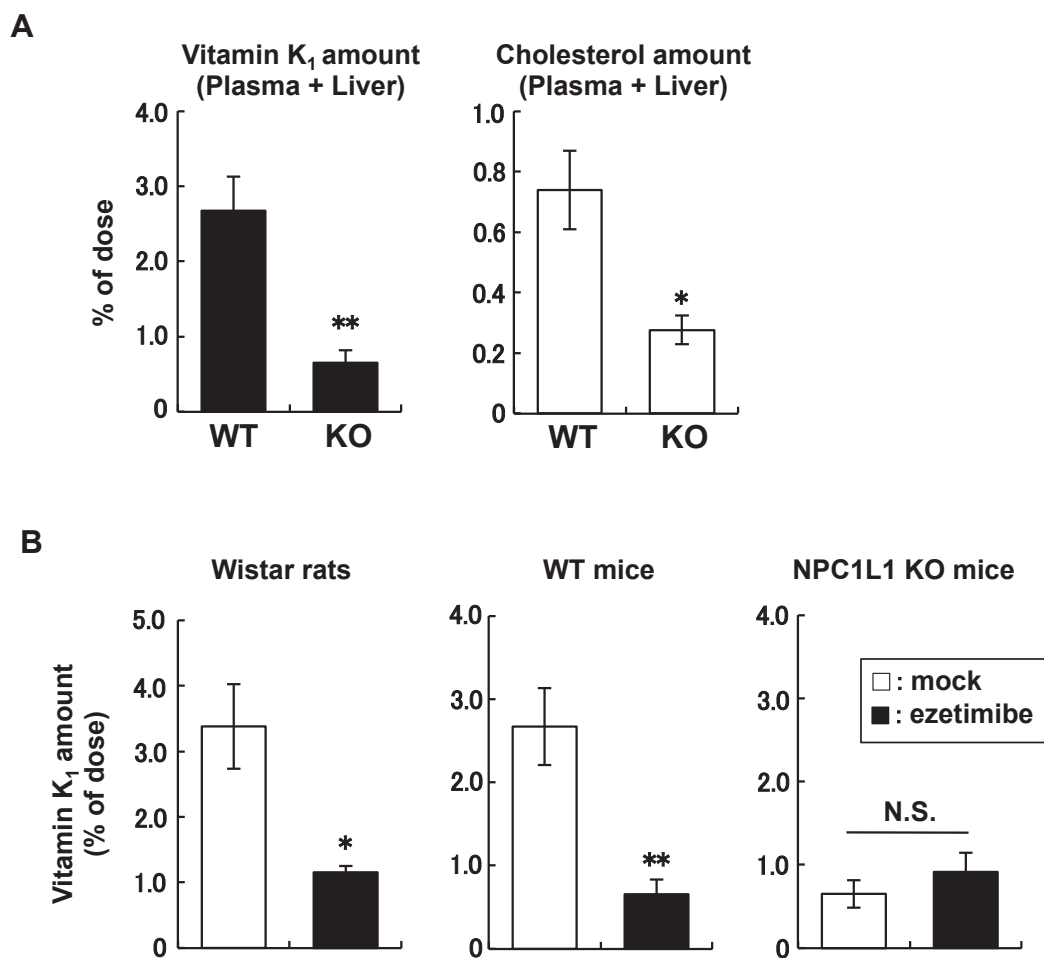
A

B

Fig. 3. Intestinal vitamin K_1 absorption in rodents.

(A) Intestinal absorption of vitamin K_1 and cholesterol was examined in wild-type (WT) mice and NPC1L1 knockout (KO) mice. Vitamin K_1 and [³H]cholesterol concentrations in the plasma and liver were examined 2 h after the intraduodenal administration of a vitamin K_1- or [³H]cholesterol-containing emulsion. (B) Vitamin K_1 absorption was examined in Wistar rats, WT mice, and NPC1L1 KO mice treated with or without ezetimibe (0.3 mg/kg for rats or 0.45 mg/kg for mice). Vitamin K_1 concentrations in the plasma and liver were examined 3 h (for rats) or 2 h (for mice) after the intraduodenal administration of a vitamin K_1-containing emulsion. Data are shown as the mean ± SEM ($n = 4 - 8$). **Significantly different by Student's t test ($p < 0.01$). *Significantly different by Student's t test ($p < 0.05$). N.S., not significantly different. (Modified and cited from reference 42).

cholesterol absorption results (**Fig. 3A**). Moreover, ezetimibe administration significantly inhibited vitamin K_1 absorption in Wistar rats and WT mice, whereas that in NPC1L1 KO mice was hardly affected by ezetimibe treatment (**Fig. 3B**). These results clearly indicate that the ezetimibe-sensitive NPC1L1-dependent pathway is primarily involved in intestinal vitamin K_1 absorption as well as cholesterol absorption.

Drug Interaction between Ezetimibe and Warfarin

Based on reports showing that anticoagulant activity is enhanced in patients taking warfarin in combination with ezetimibe[43], the ezetimibe package

insert warns that caution should be exercised when ezetimibe is co-administered with warfarin[41]. However, the mechanism of the warfarin-ezetimibe interaction was not clear. The results of vitamin K_1 absorption studies (**Fig. 3**), together with the fact that clotting factors are activated in the liver through the cyclic conversion of hepatic vitamin K (vitamin K cycle)[44], led to the hypothesis that the inhibition of vitamin K absorption by ezetimibe would apparently enhance the inhibitory effect of warfarin on the activation of clotting factors. To test this hypothesis, the effect of ezetimibe on anticoagulant activities and on hepatic vitamin K levels in Wistar rats treated with (or with-
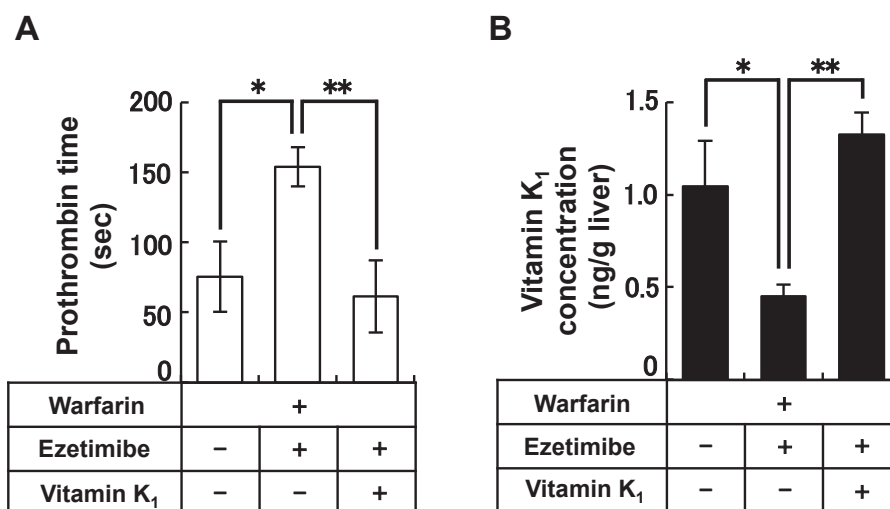
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B

Fig. 4. Drug interaction between ezetimibe and warfarin in Wistar rats.

(A) Prothrombin time and (B) hepatic vitamin K_1 concentrations of Wistar rats after 1 week of daily oral co-administration of warfarin (0.2 mg/kg per day), ezetimibe (1 mg/kg per day), and vitamin K_1 (1 μg/200 g body weight per day). Data are shown as the mean ± SEM ($n = 6-13$). **Significantly different by Mann-Whitney test with Bonferroni-Holm correction ($p < 0.01$). *Significantly different by Mann-Whitney test with Bonferroni-Holm correction ($p < 0.05$). (Modified and cited from reference 42).

out) warfarin was examined[42]. The anticoagulant effect of warfarin was reflected in the extension of prothrombin time. Consistent with a previous case report in humans[43], prothrombin time in rats co-treated with warfarin and ezetimibe (co-treatment group) was significantly longer than that in rats treated with warfarin alone (warfarin alone group) (**Fig. 4A**). Analysis of the hepatic vitamin K_1 level in these rats showed that the liver vitamin K_1 concentration in the co-treatment group was significantly lower than that in the warfarin alone group (**Fig. 4B**). In addition, vitamin K_1 rescue experiments revealed that restoration of the hepatic vitamin K_1 level by oral vitamin K_1 supplementation canceled the extension of prothrombin time observed in the co-treatment group (**Fig. 4**). Taken together with the observation that the co-administration of ezetimibe did not result in significant changes in the hepatic warfarin concentration in rats, these data suggest that the effect of the drug interaction between ezetimibe and warfarin is mediated by the decrease in the hepatic vitamin K level, which is caused by ezetimibe-mediated vitamin K_1 malabsorption (**Fig. 5**).

This drug-drug interaction mechanism was supported by our retrospective survey of clinical records at the University of Tokyo Hospital, showing that in more than 85% of warfarin-treated patients, the prothrombin time international normalized ratio (PT-INR) increased after starting the co-treatment with ezetimibe[42]. This result indicates that the ezetimibe-warfarin interaction occurs with high frequency in humans as a non-idiosyncratic reaction via the inhibition of vitamin K absorption. Hashikata *et al.* conducted further studies on this drug-drug interaction by retrospectively evaluating outpatients at the Kitasato University Hospital[45]. Interestingly, they revealed that the ezetimibe-warfarin interaction more frequently appeared in patients taking statins. It has been reported that the inhibition of cholesterol synthesis by statins increases intestinal cholesterol absorption via a physiological compensation process to maintain cholesterol homeostasis[46]. Indeed, Tremblay *et al.* showed that a 12-week atorvastatin treatment increased both the mRNA and protein levels of NPC1L1 in intestinal biopsy samples obtained from hyperlipidemic patients[47]. Based on these observations and our finding that NPC1L1 is a major vitamin K_1 importer in the intestine, it is likely that statin therapy increases ezetimibe-sensitive NPC1L1-mediated vitamin K_1 absorption and, therefore, that the drug interaction between ezetimibe and warfarin is strengthened in patients taking statins.

Class B Scavenger Receptors

Scavenger Receptor Class B Type I

Scavenger receptor class B type I (SR-BI) is a membrane protein and was initially identified as an
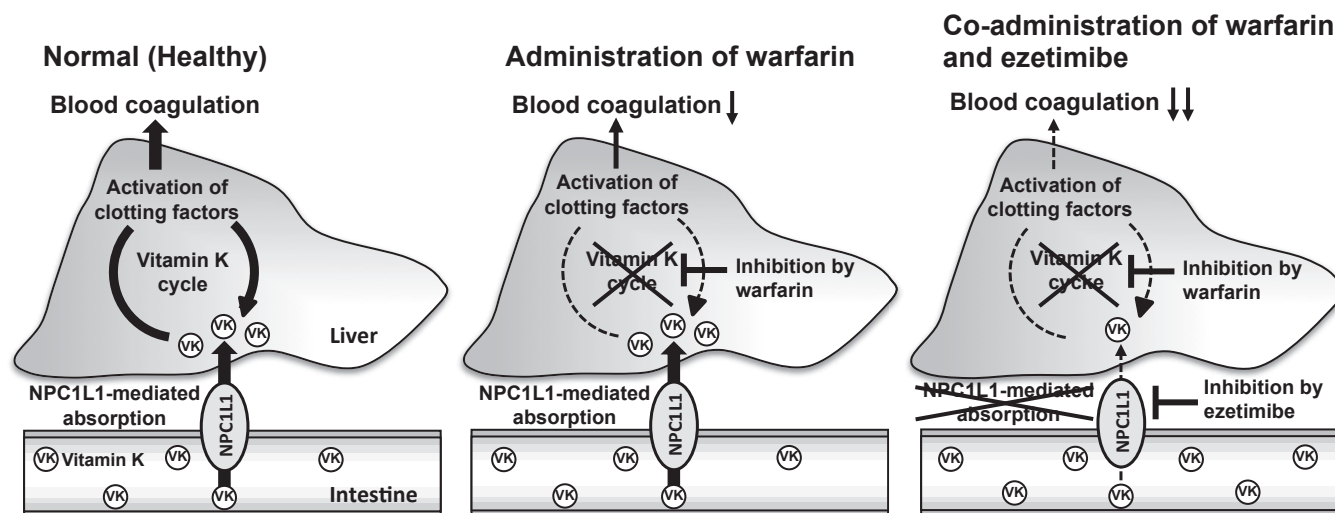
Fig. 5. Proposed mechanism of the drug interaction between ezetimibe and warfarin.

Vitamin K (VK) is absorbed by NPC1L1 in the intestine and then circulates through the vitamin K cycle in the liver. In this cycle, vitamin K activates clotting factors and regulates blood coagulation. When warfarin, an anticoagulant drug that inhibits the vitamin K cycle, and ezetimibe, an NPC1L1 inhibitor clinically used for dyslipidemia, are administered together, the anticoagulant effect of warfarin is apparently enhanced by the ezetimibe-related reduction in vitamin K absorption.

oxidized low-density lipoprotein (LDL) receptor[48], although it is currently known to be a high-density lipoprotein (HDL) receptor expressed on the basolateral membrane in the liver[49]. Given that SR-BI is expressed in several tissues and its cellular localization varies depending on cell type and cell culture conditions[50, 51], SR-BI is believed to have many physiological functions in addition to its well-characterized role in HDL metabolism.

In the small intestine, SR-BI is predominantly expressed on the brush border membrane of enterocytes[11, 52]. Based on *in vitro* results demonstrating that the uptake of micellar cholesterol by Caco-2 cells and that by brush border membrane vesicles prepared from rabbit enterocytes were effectively inhibited by SR-BI antibodies, SR-BI was assumed to be a receptor (or an acceptor) for cholesterol uptake[52]. Consistent with these *in vitro* results, Bietrix *et al.* reported that transgenic mice overexpressing SR-BI in the intestine showed significantly higher cholesterol absorption than WT mice, suggesting that SR-BI can take up cholesterol in the small intestine[11]. However, the physiological importance of SR-BI in cholesterol absorption is controversial because it has been reported that there is no significant difference in cholesterol absorption between WT and SR-BI KO mice[10]. Taken together with the fact that NPC1L1 mediates more than 70% of the cholesterol absorption in the small intestine[9], it is currently thought that SR-BI may be involved to some extent[11, 53] but is not physiologically essential for cholesterol absorption.

Similar to NPC1L1, SR-BI has been proposed to be involved in the intestinal absorption of fat-soluble vitamins. Reboul *et al.* demonstrated that vitamin E uptake in Caco-2 TC-7 cells was inhibited by both SR-BI antibodies and BLT-1 (**Fig. 1E**), a chemical inhibitor of lipid transport that acts via SR-BI[54]. Consistent with the *in vitro* results, the bioavailability of γ-tocopherol is significantly (2.7-fold) higher in SR-BI transgenic mice than in WT mice. These results suggest that SR-BI has vitamin E uptake activity both *in vitro* and *in vivo*. Additionally, it has been demonstrated that SR-BI has vitamin K_1 uptake activity[55]. *In vitro* vitamin K_1 uptake studies using Caco-2 TC-7 cells demonstrated that the apical uptake of vitamin K_1 was significantly blocked by both anti-SR-BI antibodies and BLT-1. In addition, SR-BI-overexpressing HEK293-T cells exhibited significantly enhanced vitamin K_1 uptake, which was sensitive to BLT-1 treatment. Consistent with these *in vitro* observations, SR-BI transgenic mice were found to have a higher content of vitamin K_1 in the proximal intestine mucosa 4 h after gavage with a vitamin K_1-enriched emulsion compared with WT mice. Moreover, the postprandial plasma vitamin K_1 concentration was also increased in SR-BI transgenic mice compared with WT mice. Together, these results indicate that SR-BI has the ability to import vitamin K_1. However, based on the result showing that BLT-1 treatment reduced only about 20% of vitamin K_1 uptake by intestinal grafts collected from WT mice[55] and the finding that more than 70% of vitamin K_1 absorbed in the intestine was

through NPC1L1-mediated pathway[42], the physiological contribution of SR-BI to vitamin K_1 absorption might not be very high. Additional studies with SR-BI KO mice are needed to better understand the physiological role of SR-BI in the absorption of vitamin E and vitamin K_1.

Although the significance of SR-BI in intestinal lipid absorption is still controversial, considering that SR-BI has bidirectional transport activity (not only uptake but also efflux) for lipids including cholesterol, vitamin E, and vitamin K_1[54-56], and that its cellular localization is dramatically altered by experimental conditions[50, 51], it is possible that the function of SR-BI may be dynamically altered in response to changes in physiological and pathological conditions (**Fig. 2**). Indeed, SR-BI occasionally expressed on the bile canalicular membrane has been reported to be involved in the biliary secretion of cholesterol[57] and α-tocopherol[58], rather than the (re)uptake of these lipids from bile. Further studies with animal models under several experimental conditions such as hyperlipidemic and lipid-deficient conditions may provide a new perspective on the physiological functions of SR-BI in the small intestine.

Cluster of Differentiation 36

Cluster of differentiation 36 (CD36), a membrane protein belonging to the scavenger receptor class B family, is expressed in various tissues, including in adipose tissue, the heart, and the small intestine[59]. To date, several studies have indicated that CD36 binds a number of lipids such as lipoproteins, cholesterol, and long-chain fatty acids and that CD36 can facilitate the cellular uptake of these lipids *in vitro*[60-63].

Given that CD36 is predominantly expressed on the brush border membrane of the proximal intestine[64], it has been assumed that CD36 might be involved in the apical uptake of dietary lipids in the small intestine. However, fecal lipid analyses showed that there were no significant differences in the intestinal absorption of fatty acids and cholesterol between WT and CD36 KO mice[12, 13]. This result suggests that CD36 in the small intestine is unlikely to be involved in the apical uptake of these lipids. However, based on the observation that the protein expression of NPC1L1 in the small intestine was significantly higher in CD36 KO mice compared with WT mice[14], it is also possible that CD36 deficiency was compensated by the up-regulation of NPC1L1, therefore leaving overall cholesterol absorption unchanged in CD36 KO mice.

Regarding the intestinal function of CD36, it has been also reported that CD36 KO mice exhibit deficiencies in the formation and secretion of chylomicrons, as evidenced by significantly less apolipoprotein B-48, a protein marker of chylomicrons, and smaller lipoprotein particles secreted into the lymph in CD36 KO mice compared with WT mice[13]. In accordance with this defect, CD36 KO mice exhibited a significant reduction in cholesterol and fatty acid (triglyceride) secretion into the lymph after a 6-h intraduodenal infusion with a lipid emulsion[13]. These findings indicate that CD36 in the small intestine is involved in the lymphatic secretion of lipids with chylomicrons. Although cholesterol secretion into the lymph decreased, there was no significant accumulation of cholesterol in the small intestine in CD36 KO mice[13]. This observation, together with the fact that overall cholesterol absorption was not reduced by CD36 deficiency[12, 13], implies that other pathways for cellular cholesterol secretion in the small intestine, such as ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux into the portal blood with HDL[65] (**Fig. 2**), might be enhanced in CD36 KO mice to compensate for the disrupted lymphatic secretion of cholesterol with chylomicrons.

Similar to SR-BI, CD36 has been reported to have the ability to import vitamin E and vitamin K_1. Recent *in vitro* studies showed that the cellular uptakes of γ-tocopherol and vitamin K_1 were significantly increased by CD36 overexpression in HEK293-T cells, and these increases were blocked by treatment with sulfo-*N*-succinimidyl oleate (SSO) (**Fig. 1F**), a chemical inhibitor of CD36[55, 66]. However, inconsistent with these *in vitro* results, *in vivo* acute absorption studies demonstrated that the plasma levels of γ-tocopherol and vitamin K_1 after force-feeding with an emulsion containing these vitamins did not decrease (but rather increase) in CD36 KO mice compared with WT mice[55, 66]. In addition, the contents of these vitamins in the intestinal mucosa after the gavage were similar between the two groups of mice. The increase in intestinal NPC1L1 expression[14] and the disrupted secretion of chylomicrons in CD36 KO mice[13] seem to be key factors to clarify the discrepancy between the *in vitro* and *in vivo* observations. Therefore, further studies considering the associations of CD36 with NPC1L1 and/or chylomicrons are needed to clarify the physiological role of CD36 in the absorption of fat-soluble vitamins as well as cholesterol.

ATP-Binding Cassette Transporter A1

ABCA1 is a key player in the biogenesis of HDL, which is responsible for reverse cholesterol transport, a physiological process for eliminating excess cholesterol from peripheral tissues by returning it to the liver[67].

The loss of ABCA1 function causes Tangier disease, which is characterized by the accumulation of cholesterol (esters) throughout the body due to HDL deficiency, resulting in an increased risk of atherosclerosis and cardiovascular events in patients with Tangier disease[3-5]. ABCA1 is expressed on the basolateral membrane in several tissues, including in the liver and small intestine, and mediates the ATP-dependent efflux of cellular cholesterol and phospholipids to apolipoprotein A-I (apoA-I), which functions as a lipid acceptor to form nascent HDL[67].

Recent studies with ABCA1 KO mice revealed that ABCA1 is involved in the regulation of the plasma and/or tissue levels of fat-soluble vitamins in addition to cholesterol and phospholipids. Indeed, plasma concentrations of vitamin E are extremely low in ABCA1 KO mice (undetectable levels) compared with WT mice (3.4 mg/dL)[68]. In addition, the hepatic vitamin K_1 concentration in ABCA1 KO mice is also reduced to approximately 20% of that in WT mice, which causes a bleeding tendency in ABCA1 KO mice[68]. These observations raise the possibility that ABCA1 can transport the fat-soluble vitamins as well as cholesterol and phospholipids. Indeed, Oram *et al*. demonstrated that apoA-I-dependent α-tocopherol efflux was absent in fibroblasts derived from patients with Tangier disease and that ABCA1 overexpression in baby hamster kidney cells (BHK cells) significantly increased α-tocopherol efflux to apoA-I[69]. Given that the conditioned media collected from ABCA1-overexpressing BHK cells did not promote α-tocopherol efflux from control (empty vector-transfected) BHK cells, the increase in α-tocopherol efflux in ABCA1-overexpressing cells was likely due to the direct transport of α-tocopherol by ABCA1 rather than being secondary to the increase in the amount of HDL particles caused by ABCA1 overexpression[69]. In addition, it has been reported *in vitro* that ABCA1 in hepatocyte is involved in the efflux of α-tocopherol to apoA-I, and this efflux is inhibited by probucol (**Fig. 1G**), which inactivates ABCA1 by inhibiting binding to apoA-I[70]. Given that the dietary administration of probucol reduced the plasma concentrations of α-tocopherol as well as HDL cholesterol in mice[70], ABCA1-mediated α-tocopherol secretion from peripheral tissues (especially from the liver) does occur *in vivo*. These data, together with the recent finding that vitamin E can be exported to not only apoA-I (nascent HDL) but also more mature HDL via the ATP-binding cassette transporter G1-dependent pathway[71], indicate that transporter-mediated vitamin E efflux to HDL particles plays important roles in the whole-body regulation of vitamin E.

In addition to hepatic α-tocopherol secretion,

the intestinal absorption of dietary vitamin E can influence the plasma concentrations of this nutrient. It has been reported that the intestinal absorption of vitamin E (γ-tocopherol) was significantly lower in ABCA1 KO mice than in WT mice[72]. Taken together with the fact that ABCA1 is expressed on the basolateral membrane in the small intestine[73], these results suggest that ABCA1 is responsible for the secretion of vitamin E into portal blood with intestinal HDL, and this pathway, as well as the well-known pathway of vitamin E secretion into lymph with chylomicrons, is significantly involved in vitamin E absorption[74] (**Fig. 2**).

As for vitamin K, there is no direct evidence whether ABCA1 mediates vitamin K transport. *In vitro* studies will be needed to reveal whether ABCA1 has vitamin K efflux activity. In addition, to reveal the mechanism(s) behind the observed reduction in the hepatic vitamin K_1 concentration in ABCA1 KO mice[68], further studies to analyze vitamin K_1 concentrations in other tissues and the efficiency of intestinal vitamin K_1 absorption in ABCA1 KO mice are necessary and important.

Conclusions and Future Perspectives

In this review, membrane proteins that have the potential to transport dietary lipids such as cholesterol, vitamin E, and vitamin K_1 were discussed. Intestinal lipid absorption is regulated by multiple processes, including apical uptake, apical backflux, basolateral efflux with HDL, and lymphatic secretion with chylomicrons (**Fig. 2**). Interestingly, despite the differences in chemical structure among cholesterol, vitamin E, and vitamin K_1, the membrane transport processes of these lipids are very similar. Indeed, as descried in this review, most of the intestinal cholesterol transporters also have the ability to transport vitamin E and vitamin K_1. Vitamin E acts as an antioxidant, potentially preventing the occurrence of cardiovascular events[2]. Vitamin K inhibits the development of arteriosclerosis by activating the matrix Gla protein, which suppresses vascular calcification[1, 75]. Based on this data, it seems reasonable that cholesterol transporters are also involved in the intestinal absorption of these vitamins in order to prevent the harmful effects of excess cholesterol on the body. In addition, these findings provide new insights into the development and/or the clinical use of drugs for controlling plasma cholesterol levels. To date, several types of drugs whose molecular targets are cholesterol transporters have been developed or are under development for the treatment of dyslipidemia[76, 77]. It is important to consider that treatment with these drugs may also

affect the behavior of fat-soluble nutrients such as vitamin E and vitamin K, which may cause unexpected adverse effects or unpredictable drug-drug interactions. Further analyses of the association between the intestinal absorption of cholesterol and that of fat-soluble nutrients are necessary for the development of safer drugs and for appropriate pharmacotherapy.

Discrepancies between *in vitro* and *in vivo* data have been frequently observed in studies on intestinal lipid transporters, and such discrepancies have made it difficult to uncover the physiological functions of these proteins. One of the main reasons for these discrepancies is that compensation systems are working *in vivo* to maintain lipid homeostasis. In order to understand the complicated but well-balanced systems for intestinal lipid absorption, it is important to clarify not only the individual functions (abilities) of lipid transporters but also the associations among the systems' components. From this point of view, further studies using *in silico* methods such as a systems biological approach[78] will be helpful to elucidate the tissue-level networks of lipid transporters and related molecules and will contribute to a better understanding of the molecular mechanisms involved in the regulation of intestinal lipid absorption.

Acknowledgement

Conflict of Interest

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