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Title

Inhibitory effect of ezetimibe can be prevented by an administration interval of 4 h between α-tocopherol and ezetimibe

Abstract

Tocopherol is used not only as an ethical drug but also as a supplement. In 2008, Narushima et al. reported that α-tocopherol is partly transported via an intestinal cholesterol transporter, Niemann-Pick C1-Like 1 (NPC1L1). Ezetimibe, a selective inhibitor of NPC1L1, is administered for a long time to inhibit cholesterol absorption and there is a possibility that absorption of α-tocopherol is also inhibited by ezetimibe. In this study, we investigated the influence of ezetimibe on the absorption of α-tocopherol with single administration and long-term administration. We also examined an approach to avoid its undesirable consequence. α-Tocopherol (10 mg/kg) and ezetimibe (0.1 mg/kg) were administered to rats, and the plasma concentration profiles of α-tocopherol and tissue concentrations were investigated. The plasma concentration of α-tocopherol was decreased by the combination use of ezetimibe in the case of concurrent single administration. On the other hand, inhibition of the absorption of α-tocopherol was prevented by an administration interval of 4 h. In a group of rats with administration for 2 months with a 4-h interval, not only the plasma concentration but also the liver concentration was increased compared with those in a group with concurrent combination intake of α-tocopherol and ezetimibe. The absorption of α-tocopherol was inhibited by ezetimibe. The inhibitory effect

of ezetimibe can be prevented by an administration interval of 4 h, though ezetimibe is a medicine of enterohepatic circulation. Attention should be paid to the use of ezetimibe and components of NPC1L1 substrates such as α-tocopherol.

Key words: α-tocopherol; ezetimibe; absorption; NPC1L1; intestine.

Introduction

The small intestine is an important tissue for absorption of necessary components from digested food and medicine and as a barrier against foreign substances to maintain homeostasis. Cholesterol homeostasis is a highly regulated balance of de novo synthesis, dietary cholesterol absorption, and biliary clearance and excretion [1,2], and excess cholesterol is a risk for the development of various diseases such as arteriosclerosis. Cholesterol is present as mixed micelles formed by bile salts and phospholipids in the intestinal lumen. Intestinal cholesterol absorption begins with the micellar solubilization of both dietary cholesterol and biliary cholesterol in the lumen of the small intestine [3]. In 2004, Altmann et al. identified Niemann-Pick C1 Like 1 (NPC1L1) as an apically localized sterol transporter in the small intestine [4]. Ezetimibe (Zetia®, Merck & Co., Inc.), an inhibitor of NPC1L1, is a widely used medicine to inhibit the absorption of cholesterol from the diet for patients with hypercholesterolemia [5]. NPC1L1 protein has 13 predicted transmembrane domains and extensive N-linked glycosylation sites located within the extracellular loops and it contains a sterol-sensing domain (SSD) [6,7]. It has been predicted that the substrates of NPC1L1 have sterol domains and it has been thought that NPC1L1 transports substances that have a sterol structure.

In 2008, Narushima et al. reported that α-tocopherol, which does not have a sterol structure, was partly transported via NPC1L1 [8]. Tocopherol acetate is used as an ethical drug for treating vitamin E deficiency and for improving peripheral circulatory disturbance [9]. In addition, α-tocopherol is used not only as a food component but also as a supplement. In a

clinical setting, patients usually take many kinds of drugs, foods or supplements at the same time. Drug-drug interactions and food-drug interactions would increase the risk of adverse events. Generally, ezetimibe is taken once a day for long-term treatment of hypercholesterolemia. In patients with hypercholesterolemia who take ezetimibe, α-tocopherol taken daily as an ethical drug or supplement may not be absorbed. However, the influence of ezetimibe remains unclear. To avoid these potential undesirable events, it is important to investigate in detail the influence of long-term administration of ezetimibe on the absorption of α-tocopherol.

We therefore performed an *in vivo* absorption study using rats and investigated the effects of short-term and long-term administration of ezetimibe on the absorption of tocopherol. The results suggested that the absorption of α-tocopherol is inhibited by ezetimibe administered at the same time. On the other hand, inhibition of the absorption of α-tocopherol can be prevented when the timing of administration of ezetimibe is delayed after the intake of α-tocopherol.

Materials and Methods

Chemicals and reagents

α-Tocopherol and ezetimibe ((4-fluorophenyl)-(3R)-[3-(4-fluorophenyl)-(3S)-hydroxypropyl]-4S-(4-hydroxyphenyl)-2-azetidinone) (Zetia®) were purchased from Wako Pure Chemical (Osaka, Japan) and Merck & Co., Inc. (New Jersey, United States), respectively. Other reagents were purchased from Wako Pure Chemical unless otherwise noted. All reagents were of the highest grade available and used

without further purification.

Animals

Male Wistar rats, 6 weeks old (160-180 g in weight), were obtained from Jla (Tokyo, Japan). The housing conditions were the same as those described previously [10]. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals”.

Preparation of the formulation for administration of α-tocopherol

Zetia® (containing 10 mg of ezetimibe/tablet) was reduced to a powder in order to dissolve dimethyl sulfoxide (DMSO) and to prepare a solution of 10 mg/ml. Because of the cytotoxicity of DMSO, this solution was diluted (final concentration of DMSO: 1%) to administer 0.1 mg/ml of ezetimibe solution at the dose of 0.1 mg/kg weight (0.1 ml/kg weight). The concentration of ezetimibe was based on a previous report [11]. On the other hand, α-tocopherol was dissolved in olive oil to administer a solution of 10 mg/ml at the dose of 10 mg/kg weight (1 ml/kg weight). The dose and preparation of α-tocopherol solution were based on a previous report of Abuasal et al. [12].

Absorption study using Wistar rats

After acclimation for about 1 week, rats were randomly divided into 3 groups: control, α-tocopherol and α-tocopherol+ezetimibe groups. The rats were fasted for 12-16 h before the experiments. Saline, α-tocopherol and α-tocopherol+ezetimibe were administered in liquid solution orally to the control, α-tocopherol and α-tocopherol+ezetimibe groups, respectively. In the α-tocopherol+ezetimibe group, ezetimibe was administered at the same timing of administration of α-tocopherol or at 1 h and 4 h after administration of α-tocopherol. The rats were anesthetized by intraperitoneal (i.p.) injection of 50 mg/kg sodium pentobarbital. Plasma samples were obtained at designated times as described previously [13] with some modification. Experimental rats were killed at a designated time after administration of α-tocopherol, and tissue samples were excised at that time. The liver, spleen and kidney were removed rapidly, washed with saline, and weighed. Then the organs were homogenized with 1 ml distilled water/g tissue using a Potter-Elvehjem homogenizer with 20 strokes. The intestine was opened to expose the epithelium to lines and the mucosa was obtained by gentle scraping with a glass slide. The mucosa was homogenized with 1 ml distilled water per 1 cm intestine using a Potter-Elvehjem homogenizer with 20 strokes. Plasma samples and tissue samples (homogenate) were kept at -20°C and -80°C, respectively, until assay.

Analytical procedure

Analysis of α-tocopherol was carried out as described in a previous report [14] with some modification. The concentration of α-tocopherol was determined using an HPLC system

equipped with an L-6200 pump, an L-7300 column oven and an F-1050 fluorescence spectrophotometer (HITACHI, Tokyo, Japan). One hundred μl of a sample with 100 μl of 0.1 M Na_2HPO_4 buffer and 200 μl of methanol with 1 μg/ml δ-tocopherol as an internal standard were added. Then 1,100 μl of n-hexane/dichloromethane (4/1, v/v) was added and the mixture was shaken vigorously for 1.5 min. After centrifugation at 800 × g for 10 min, 900 μl of the organic layer was taken and evaporated to dryness under a nitrogen gas stream. The residue was dissolved in 200 μl of mobile phase for HPLC injection. The column for HPLC was an Inertsil® ODS-4 (3 mm in inside diameter × 150 mm) (GL Sciences Inc., Tokyo, Japan). A mobile phase containing methanol/distilled water (98/2, v/v) was used. Column temperature and flow rate were 30°C and 0.4 ml/min, respectively. The light excitation and emission wavelengths for detection were 298 and 325 nm, respectively. Fifteen μl of a sample was injected into the HPLC system.

Data analysis

To analyze pharmacokinetics of α-tocopherol, the area under the curve (AUC) was calculated by the trapezoidal rule. Student's t-test was used to determine the significance of the differences between two group means. Statistical significance among means of more than two groups was determined by one-way analysis of variance (ANOVA) followed by the Turkey-Kramer test. Data are expressed as means with standard deviation (S.D.). Statistical significance was defined as $p<0.05$.

Results

Plasma and tissue concentrations of α-tocopherol after single oral administration

Plasma concentration data for α-tocopherol were used in this study after confirming that the concentrations before oral administration were almost the same (2.0-5.0 μg/ml). In the first part of this study, the plasma concentrations of α-tocopherol with ezetimibe and without ezetimibe were investigated for up to 24 h after oral administration (Figure 1A). It was found that the plasma concentration of α-tocopherol was decreased by administration of ezetimibe at the same timing. The value of AUC was then calculated with these concentration profiles. The value was significantly decreased by administration of ezetimibe (Table 1). In addition, we investigated the tissue distribution of α-tocopherol at designated times up to 24 h after oral administration (Figure 2A). It was clear that a large amount of α-tocopherol remained in the intestinal mucosa up to 4 h. The liver concentration of α-tocopherol was gradually increased from 2 h after oral administration. On the other hand, the spleen concentration was almost all the same as that at 0 h. The results suggested that α-tocopherol was absorbed from the small intestine up to 4 h after administration in the setting of a single administration.

Influence of administration timing of ezetimibe on absorption of α-tocopherol

Based on the results showing that administered α-tocopherol was absorbed up to 4 h, we then focused on an approach to prevent the inhibitory effect of ezetimibe. We hypothesized that a delay in the timing of administration of ezetimibe would prevent the inhibitory effect of

ezetimibe on absorption of α-tocopherol. The plasma concentrations of α-tocopherol were investigated in the conditions of ezetimibe being administered at 1 h and 4 h after administration of α-tocopherol (Figure 1B). We chose the intervals of 1 h and 4 h in this experiment because a large amount of α-tocopherol remained in the small intestine at 1 h after administration and it was decreased to almost the same level as the control (0 h) level at 4 h after administration (Figure 2A). We confirmed that the concentrations of α-tocopherol in all groups (α-toc, α-toc+EZE, α-toc+EZE 1-h interval and α-toc+EZE 4-h interval) were almost the same before its administration. The value of AUC was significantly increased by administration after a 4-h interval compared with when both substances were administered at the same time (Table 1). On the other hand, the value of AUC was not altered in the group with an administration interval of 1 h compared with that of the same timing.

In addition, we investigated the concentrations of α-tocopherol in tissues (small intestine and liver) after administration of ezetimibe at the same timing (Figure 2B). The concentration of α-tocopherol in the small intestine at 2 h after oral administration of α-tocopherol without ezetimibe was 2.89 ± 1.89 μg/mg protein, whereas the concentration in the small intestine with ezetimibe was 0.97 ± 1.89 μg/mg protein. There was no significant difference between the concentrations in the small intestine in the two groups. On the other hand, the liver concentration at 2 h after oral administration with ezetimibe (2.80 ± 0.96 μg/mg protein) was significantly decreased compared to that without ezetimibe (6.54 ± 0.65 μg/mg protein). There were no significant differences in the concentrations of α-tocopherol in the small intestine and liver at 8 h

after administration between the groups with and without ezetimibe.

Tissue distribution of α-tocopherol after long-term administration and inhibitory effect of ezetimibe on its absorption

Ezetimibe is generally taken once a day by patients with hypercholesterolemia over a long duration. We confirmed that the plasma concentration profile of α-tocopherol after single oral administration was not altered by ezetimibe orally administered for 2 weeks (Figure 3). It was shown that the AUC value was 92.15 ± 17.33 μg × h/ml and there was no significant difference between the AUC value and that of the α-tocopherol group (Table 1).

Next, we investigated the long-term inhibitory effect of ezetimibe on absorption of α-tocopherol. In the long-term administration, α-tocopherol and/or ezetimibe or saline were administered every day. Tissue samples (liver, spleen and kidney) were excised after 2 months. The concentrations of α-tocopherol in the kidney and spleen were almost the same in all groups for 2 months (Figure 4). The liver concentration of α-tocopherol in the α-tocopherol group was significantly increased compared with that in the saline group. In the α-tocopherol+ezetimibe group, the liver concentration was significantly decreased compared with that in the α-tocopherol group. Compared with that in the α-tocopherol+ezetimibe group, the liver concentration was significantly increased in the α-tocopherol→ezetimibe 4-h interval group, whereas it is not increased in the α-tocopherol→ezetimibe 1-h interval group (Figure 4).

Discussion

Oral delivery is generally the most desirable means for administration of supplements and drugs mainly because of consumer or patient acceptance, convenience in administration and cost-effective manufacture. Absorption of components from the gastrointestinal tract is one of the important determinants of oral bioavailability. Since patients usually take some kinds of drugs and supplements at the same time, potential drug-drug interactions involving transporters can often occur and such interactions may directly affect the therapeutic safety and efficacy.

A change in metabolic clearance of a drug, particularly via cytochrome P450-mediated metabolism, has been considered to be the cause of many clinically important drug interactions [15-17]. Recently, it has been recognized that changes in the activity of not only chelate formation but also drug transporters may also influence the absorption of administered drugs from the intestine [18-20]. In this study, we focused on one of the potential drug-drug or food-drug interactions of the intestine, particularly that involving intestinal cholesterol transporter NPC1L1.

NPC1L1 is essential for the intestinal absorption of cholesterol and is recognized as a pharmacological target of ezetimibe [4,21], a cholesterol absorption inhibitor clinically used for treatment of hypercholesterolemia. In addition to cholesterol, some phytosterols and other lipid nutrients have been shown to be transported via NPC1L1 into enterocytes [22,23], although its recognition of substrates has not been fully clarified. In patients with hypercholesterolemia who take ezetimibe, not only these lipid nutrients but also α-tocopherol as an ethical drug or

supplement that are taken daily may not be absorbed. To avoid potential undesirable events, we investigated in detail the influence of long-term administration of ezetimibe on the absorption of α-tocopherol.

It has been reported that NPC1L1 is involved in the intestinal absorption of α-tocopherol [8], and we confirmed that the absorption of α-tocopherol was significantly inhibited by ezetimibe (Figure 1A, Table 1). The average AUC value of α-tocopherol was decreased by only 25%, but the plasma concentration profile showed that the absorption of orally administered α-tocopherol was almost completely inhibited. The results suggested that the absorption of α-tocopherol taken as a drug or supplement was inhibited by ezetimibe taken at the same time. In an uptake assay using NPC1L1-overexpressed Caco-2 cells, Narushima et al. found that the uptake of cholesterol and α-tocopherol was reduced to approximately 50% by ezetimibe [8]. The K_i values for cholesterol and α-tocopherol uptake in their study were 4.9 ± 0.7 μM and 11.0 ± 3.6 μM, respectively. These results suggested that NPC1L1 contributes greatly to α-tocopherol absorption. It was also demonstrated that estimated peak concentration time of α-tocopherol was about 4 h after oral administration, whereas Abuasal et al. reported that it was about 8 h. There are some differences in the kind of rats and dissolved oil for preparation in our study and the study by Abuasal et al., though the dose and fasting time were almost the same [12].

In addition, a large amount of α-tocopherol remained in the small intestine at 1 h after administration and it was decreased to almost the same level as the control (0 h) level at 4 h after administration (Figure 2A). Distribution of α-tocopherol to the liver was observed from 2 h after

administration. We confirmed that the liver concentration at 2 h after oral administration with ezetimibe was significantly decreased compared with that without ezetimibe (Figure 2B). The results suggested that α-tocopherol was well absorbed from the intestine until about 4 h after oral administration. From these results, we hypothesized that a 4-h interval may prevent the interaction between ezetimibe and α-tocopherol. We confirmed that the concentrations of α-tocopherol in all groups (α-toc, α-toc+EZE, α-toc+EZE 1-h interval and α-toc+EZE 4-h interval) were almost the same before its administration (Figure 1). When ezetimibe was administered 4 h after administration of α-tocopherol, absorption of α-tocopherol was not inhibited. The absorption of α-tocopherol was almost the same as that of α-tocopherol after single administration. On the other hand, the inhibited absorption of α-tocopherol did not recover even when ezetimibe was administered 1 h after administration of α-tocopherol (Figure 1B, Table 1). The results suggested that the administration of α-tocopherol and ezetimibe with a 4-h interval can prevent the inhibition of α-tocopherol absorption.

Ezetimibe for patients with hypercholesterolemia and α-tocopherol for therapeutics and supplementation are generally taken for a long time. The influence of long-term administration of ezetimibe and α-tocopherol has remained unclear. We therefore investigated the influence when both were administered to rats for 2 months to determine plasma and tissue concentrations of α-tocopherol. Trough blood samples were collected once a week about 24 h after the last administration based on the results of single administration of α-tocopherol. At first, we investigated the plasma concentration profile of α-tocopherol after repeated administration of

ezetimibe for 2 weeks. Blood samples collection was started about 24 h after the last ezetimibe administration. We confirmed that the plasma concentration profile of α-tocopherol after single oral administration was not altered by ezetimibe orally administered for 2 weeks (Figure 3). The AUC value was calculated to be 92.15 ± 17.33 μg × h/ml. There was no significant difference between the AUC value and that of the α-tocopherol group (Table 1). Takada et al. reported the ezetimibe and ezetimibe-glucuronide concentrations in rats after 1 week of administration [24]. Their results suggested that ezetimibe and ezetimibe-glucuronide were not greatly accumulated in the liver and other tissues, though their study design was slightly different from our study design (dose and period of ezetimibe administration). Our results are generally consistent with these previous reports.

In long-term administration, the concentration of α-tocopherol would be almost the same throughout the experiments. On the other hand, α-tocopherol was accumulated in the liver after 2 months of administration (Figure 4), being consistent with previous reports [25,26]. The accumulation of α-tocopherol in the liver was inhibited by ezetimibe administered at the same time. In the group in which ezetimibe was administered 4 h after administration of α-tocopherol, the inhibition of α-tocopherol accumulation in the liver by ezetimibe was prevented. In addition, the inhibition of absorption of α-tocopherol was not prevented in the group in which ezetimibe was administered 1 h after α-tocopherol administration (Figure 4). These results suggested that the undesirable interaction between α-tocopherol and ezetimibe can be prevented when patients with hypercholesterolemia take ezetimibe more than 4 h after intake of α-tocopherol even if both

are taken for the same period. To apply these results to humans, we estimated the interval time of dosing to avoid the interaction in humans by the stripping method. Data for serum concentrations in humans reported by Yoshikawa et al. were used [27]. Results of analysis by the stripping method indicated that absorption of α-tocopherol was probably up to 10.6 h in humans. These data suggested that absorption of α-tocopherol in humans is slow and that a longer interval is needed to avoid the interactions in humans than in rats (probably about 11 h or more).

In clinical trials of long-term administration of ezetimibe, it was shown that plasma LDL (low-density lipoprotein) cholesterol level was decreased [28,29]. These results suggested that the active site of ezetimibe in the body was not only in the intestine and but also in the liver. In humans, the level of cholesterol would be decreased by up-regulation of the LDL receptor in the liver and increase of uptake to the liver even though ezetimibe is taken only once a day, and inhibition of absorption from the small intestine is not maintained whole day. In rodents, NPC1L1 is mostly expressed in the intestine, particularly in the upper portion of the intestine [4]. The cholesterol-lowering effect of ezetimibe in this study with rats may be different from that in humans even with long-term administration.

It has been shown that the plasma level of α-tocopherol was slightly decreased with dietary intake of α-tocopherol as vitamin E in patients with primary hypercholesterolemia [30]. The level or homeostasis of α-tocopherol is probably maintained by α-tocopherol transfer protein (α-TTP) in the liver [31,32]. Thus, there would be no serious problem of interaction in the case of dietary intake of α-tocopherol. However, there is a possibility of an undesirable interaction in the

case of supplementation or a therapeutic dose of α-tocopherol in addition to dietary intake. We may be able to clarify the relationship between doses and levels in the body by performing a clinical study with careful attention to this relationship.

Our results also suggest that α-tocopherol administered to rats disappears earlier than it does in humans since rats have no gallbladder and bile is constantly secreted to the bile duct. Ezetimibe is a medicine of enterohepatic circulation, and ezetimibe glucuronide has a stronger inhibitory effect on cholesterol absorption than does ezetimibe [5,32]. Their concentrations in the intestine have not been fully investigated yet. Although we focused on absorption of α-tocopherol to avoid food-drug or drug-drug interactions in this study, our approach could apply to other NPC1L1 substrates and have some clinical significance. It has also been reported that it is possible to inhibit the absorption of other fat-soluble vitamins and lipid nutrients such as some carotenoids including β-carotene and lutein in coexistence with α-tocopherol [23,31,33,34]. In addition to passive diffusion, mechanisms of intestinal absorption of α-tocopherol involve not only NPC1L1 but also scavenger receptor class B type 1 (SR-B1) in the intestinal apical membrane [9,14,31,34]. Further investigations to obtain evidence of the relation between these concentrations and their effect in the active site and how to avoid undesirable drug-drug or drug-food and food-food interactions are in progress.

Conclusion

The intestinal absorption of α-tocopherol was inhibited by ezetimibe administered at the same

time. The inhibitory effect of ezetimibe could be prevented by an administration interval of 4 h, though ezetimibe is a medicine of enterohepatic circulation and is taken once a day for a long time. On the other hand, inhibition of the absorption of α-tocopherol was not be prevented when ezetimibe was administered 1 h after administration of α-tocopherol. Patients with hypercholesterolemia who take ezetimibe should pay attention to intake timing of supplements or other medicines of NPC1L1 substrates such as α-tocopherol.

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Figure legends

Figure 1. Plasma concentration profile of α-tocopherol in rats after oral administration of α-tocopherol or α-tocopherol+ezetimibe (A). Difference in plasma concentration profile of α-tocopherol with difference in timing of orally administered α-tocopherol and ezetimibe in rats (B).

(A) α-Tocopherol (10 mg/kg) and ezetimibe (0.1 mg/kg) (α-tocopherol+ezetimibe group only) were orally administered to rats and blood samples were collected at 2, 4, 6, 8, 10, 12, 20 and 24 h after administration. Each point represents the mean with S.D. of 6-7 measurements. (B) Ezetimibe (0.1 mg/kg) was administered 1 or 4 h after α-tocopherol (10 mg/kg) administration to rats. Blood samples were collected at 2, 4, 6, 8, 10, 12, 20 and 24 h after administration of α-tocopherol. Each point represents the mean with S.D. of 6-9 measurements.

Figure 2. Tissue accumulation of α-tocopherol without ezetimibe (A) or with ezetimibe (B) after oral administration to rats.

(A) α-Tocopherol (10 mg/kg) was orally administered to rats, and the small intestine, liver and spleen were taken 1, 2, 4 or 8 h after administration. Mucosal homogenate and tissue homogenate were prepared and the concentrations were measured. The concentration of α-tocopherol was corrected by the weight of the tissue. Each column represents the mean with S.D. of 3 measurements. (B) Ezetimibe (0.1 mg/kg) and α-tocopherol (10 mg/kg) were administered to rats at the same timing. The small intestine, liver and spleen were taken 2 or 8 h after administration.

Mucosal homogenate and liver homogenate were prepared and the concentrations were measured. The concentration of α-tocopherol was corrected by the weight of the tissue. Each column represents the mean with S.D. of 3 measurements.

Figure 3. Plasma concentration profile of α-tocopherol in rats after oral administration of ezetimibe for 2 weeks.

α-Tocopherol (10 mg/kg) was orally administered to rats after administration of ezetimibe for 2 weeks and blood samples were collected at 2, 4, 6, 8, 10, 12, 20 and 24 h after administration. Each point represents the mean with S.D. of 6 measurements.

Figure 4. Difference in tissue accumulation of α-tocopherol with difference in timing of orally administered α-tocopherol and/or ezetimibe to rats for 2 months.

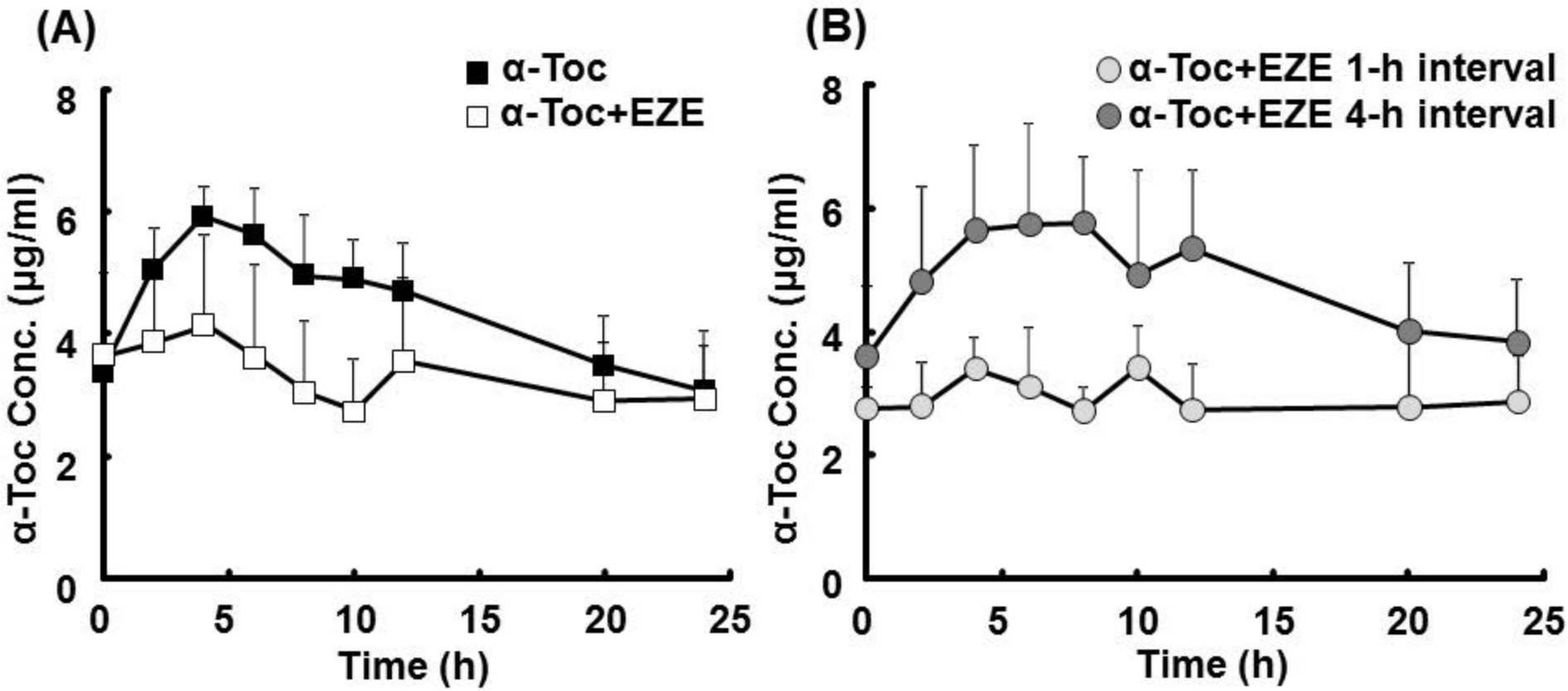
α-Tocopherol (10 mg/kg) and ezetimibe (0.1 mg/kg) were orally administered to rats at the same time or with a delay in the timing of their administration for 1 h or 4 h. Tissue homogenate was prepared with the addition of saline at 1 ml per 1 g tissue. The concentration of α-tocopherol was then measured. The concentration of α-tocopherol was corrected by the weight of the tissue. Each column represents the mean with S.D. of 6-7 measurements. *; significantly different from the α-Toc group at $p<0.05$. †; significantly different from the α-Toc+EZE group at $p<0.05$.

Table 1. AUC of α-tocopherol orally administered with or without ezetimibe

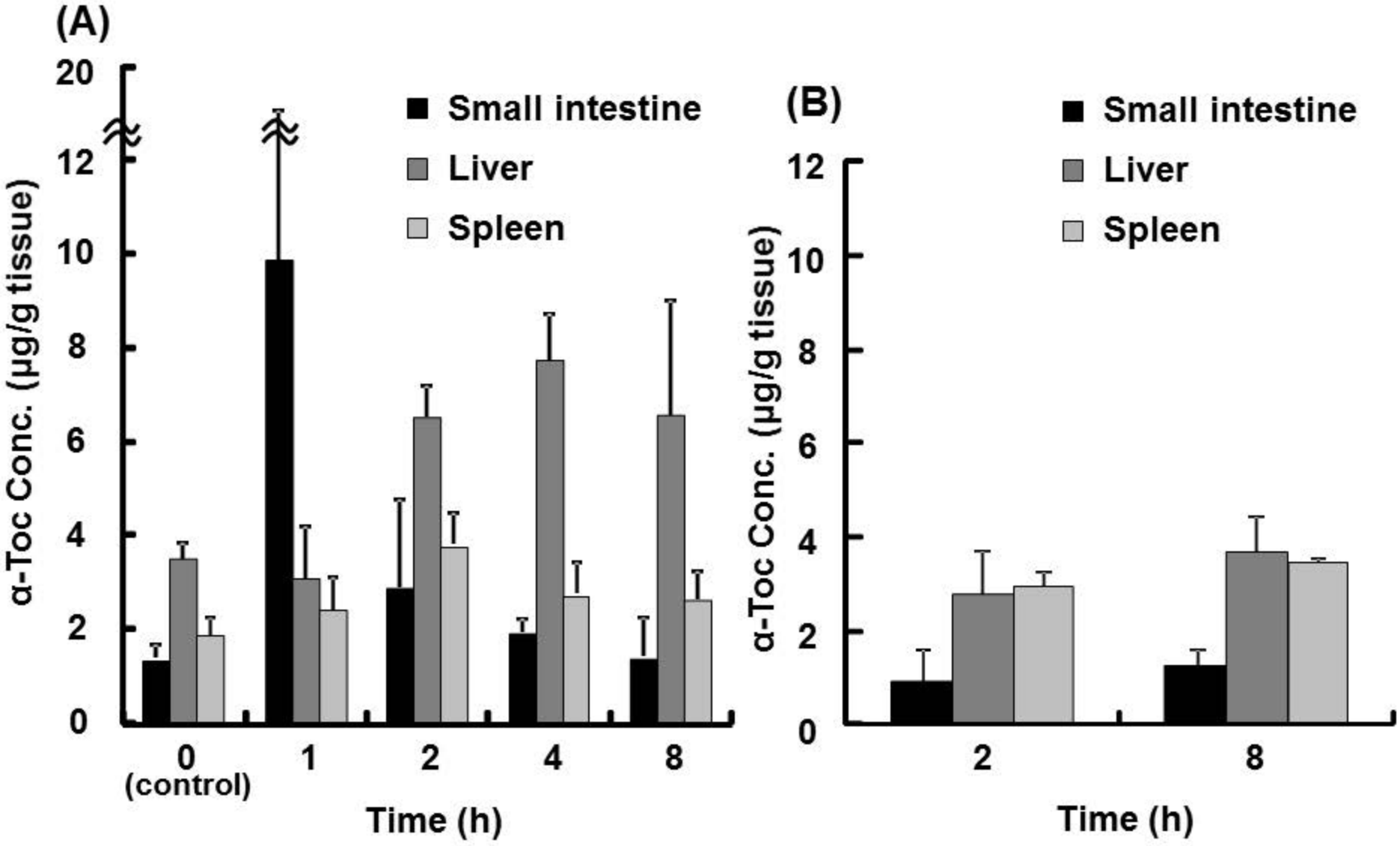
	AUC (μg×h/ml)
α-Toc	106.53 ± 11.22
α-Toc+EZE	76.23 ± 29.60 *
α-Toc+EZE 1-h interval	70.10 ± 13.58
α-Toc+EZE 4-h interval	116.22 ± 19.27 †

Each AUC value was calculated by the trapezoidal rule from data in Figure 1. Each value represents the mean ± S.D. of 6-9 measurements. *; significantly different from the α-Toc group at $p<0.05$. †; significantly different from the α-Toc+EZE group at $p<0.05$.

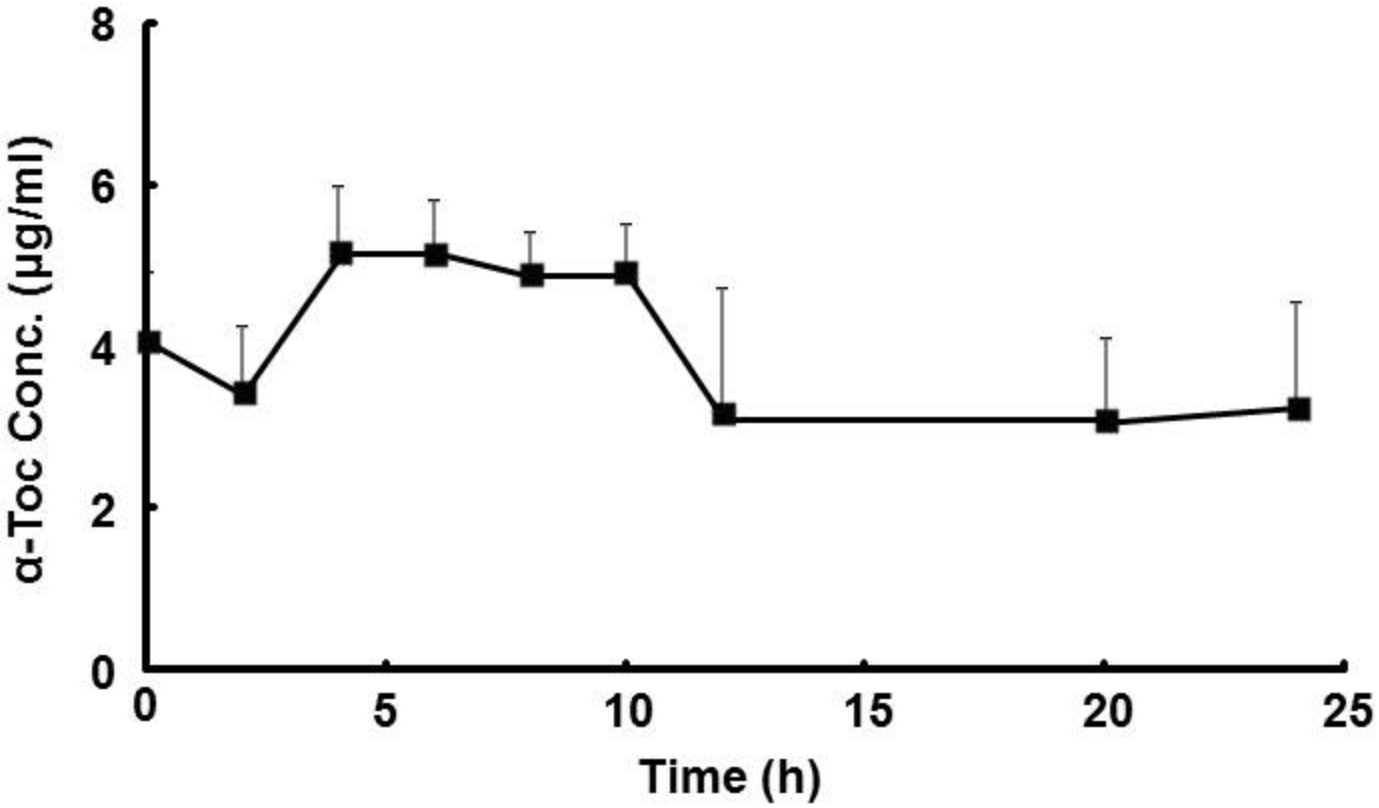
Nashimoto S. Figure 1.



Nashimoto S. Figure 2.



Nashimoto S. Figure 3.



Nashimoto S. Figure 4.

