Effect of δ-Tocopherol on Mice Adipose Tissues and Mice Adipocytes Induced Inflammation

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Abstract: The study aim was to evaluate the potential anti-inflammatory effects of vitamin E analogs, especially α-tocopherol and δ-tocopherol. We used male C57BL/6JJcl mice, which were divided into four groups: the control (C), high-fat and high-sucrose diet (H), high-fat and high-sucrose diet+α-tocopherol (Ha) and high-fat and high-sucrose diet+δ-tocopherol (Hd) groups. The mice were fed for 16 weeks. To the high-fat and high-sucrose diet, 800 mg/kg of α-tocopherol or δ-tocopherol was added more. The final body weight was significantly higher in the H group than in the C group. On the other hand, the final body weight was drastically lower in the Ha group and Hd group than in the H group. However, the energy intake was not significantly different among all groups. Therefore, we assumed that α-tocopherol and δ-tocopherol have potential anti-obesity effect. Besides, inflammatory cytokine gene expression was significantly higher in the epididymal fat of the H group than in the C group. These results showed that inflammation was induced by epididymal fat of mice fed a high-fat and high-sucrose diet for 16 weeks. Unfortunately, addition of α-tocopherol or δ-tocopherol to the diet did not restrain inflammation of epididymal fat. Investigation of the anti-inflammatory effects of α-tocopherol or δ-tocopherol in co-cultured 3T3-L1 cells and RAW264.7 cells showed that δ-tocopherol inhibited increased gene expression of the inflammatory cytokines, IL-1β, IL-6, and iNOS. These results suggest that an anti-inflammatory effect in the δ-tocopherol is stronger than that in the α-tocopherol in vitro. We intend to perform an experiment by in vivo sequentially in the future.

Key words: δ-tocopherol, vitamin E analogs, anti-inflammation, anti-obesity, mice adipose tissue, mice adipocytes

1 Introduction

In Japan, one of three adult men is obese, which has been a long-term social problem. Obesity describes a condition in which an individual has not only overweight but also excessive body fat. It has been shown previously that monocyte chemoattractant protein-1 (MCP-1) gene expression increases in enlarged mesenteric adipose tissue with long-term obesity, which is followed by macrophage infiltration and activation1). Furthermore, inflammatory factors are produced form excessive infiltration of macrophages into adipose tissue. Finally, the inflammatory reaction in adipose tissue becomes increasingly aggravated. Acceleration of such chronic inflammation is thought to contribute to the onset of lifestyle-related disease2).

Vitamin E is a fat-soluble vitamin present in vegetable oils and nuts and has eight different naturally occurring forms: four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ-, and δ-tocotrienol). These forms differ in the number and position of methyl groups on the chroman ring. Tocopherols have saturated tails, whereas tocotrienols have three double bonds in their phytyl tails. It is widely known that the main function of vitamin E is its anti-oxidative effect3). However, it has been recently reported that vitamin E also has antiangiogenic4), hypcholes-terolemic5), non-alcoholic steatohepatitis improvement6,7), natriuretic hormone8,9), and anti-obesity effects10,11). These effects have been reported not only for α-tocopherol but also for other analogs.

Orally administered vitamin E analogs are absorbed in the small intestine. Reportedly, the absorption by intestinal cells of α-tocopherol is not significantly different from that of γ-tocopherol12). After uptake into intestinal cells, tocoph-
Erol analogs are secreted into chylomicrons. Subsequently, chylomicron remnants, which are analogs incorporated into the liver, are discriminated into \( \alpha \)-tocopherol and non-\( \alpha \)-tocopherol by \( \alpha \)-tocopherol transfer protein (\( \alpha \)-TTP), which reportedly binds to \( \alpha \)-tocopherol preferentially in rat liver\(^{10}\) and humans\(^{14}\). In rat liver, biodiscrimination by \( \alpha \)-TTP is related to the bioavailability of each tocopherol, and the relative affinities of \( \alpha \)-TTP to \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \)-tocopherols are 100\%, 38\%, 9\%, and 2\%, respectively, in rat primary hepatocytes and rat liver\(^{15}\). This \( \alpha \)-TTP catalyzes the secretion of vitamin E analogs via a novel non-Golgi-mediated pathway in rat liver cells; then, \( \alpha \)-tocopherol is preferentially incorporated into very-low-density lipoprotein and transported to various tissues by lipoprotein\(^{16}\). Either excess \( \alpha \)-tocopherol or non-\( \alpha \)-tocopherols, such as \( \gamma \)-tocopherol, \( \delta \)-tocopherol, or tocotrienols, is rapidly metabolized and excreted in the urine\(^{17}\) or bile\(^{18}\). However, we recently reported that \( \delta \)-tocopherol easily accumulates into the adipose tissue of mice\(^{19}\). Therefore, it is possible that \( \delta \)-tocopherol has a novel function in adipose tissue. The study aim was to determine if \( \delta \)-tocopherol has an anti-inflammatory effect in the adipose tissue and adipocytes of mice.

2 Experimental Procedures

2.1 Exp.1 Effects of vitamin E analogs on the adipose tissue of mice fed a high-fat and high-sucrose diet for 4 months

2.1.1 Materials

In this study, \( \alpha \)-tocopherol and \( \delta \)-tocopherol were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). A portion of vitamin E was donated by Mitsubishi Chemical Foods Inc. (Tokyo, Japan). Sepasol\(^{8}\)-RNA I Super\((\text{in vitro})\) and Sepasol\(^{8}\)-RNA II Super G\((\text{in vivo})\) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). The High Capacity RNA-to-cDNA Kit and Taqman\(^{8}\) Gene Expression Assays were purchased from Thermo Fisher Scientific K.K. (Applied Biosystems, Tokyo, Japan).

2.1.2 Experimental procedure

All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals at Kanagawa Institute of Technology.

We used male C57BL/6Jcl strain mice (3 weeks old, \( n = 24 \)), which were purchased from CLEA Japan, Inc. and housed individually in plastic cages in an environment controlled at 23°C ± 2°C and 55% ± 5% humidity, with a 12 h/12 h light/dark cycle. Initially, the mice were fed a basic diet for 1 week to allow them to adapt to the new environment. To avoid differences, the mice were then divided according to their average weight into four groups: the control\(\text{C}(n = 7)\), high-fat and high-sucrose\(\text{H}(n = 6)\), high-fat and high-sucrose diet + \( \alpha \)-tocopherol\(\text{Ha}(n = 6)\), and high-fat and high-sucrose diet + \( \delta \)-tocopherol\(\text{Hd}(n = 5)\) groups. Table 1 presents the diet composition of each group. The feed and water were supplied \textit{ad libitum} for 16 weeks. The mice fasted for 16-h and then sacrificed under isoflurane anesthesia, after which the arterial blood and tissue samples were taken for analysis.

2.1.3 Measurement of triglyceride (TG) concentration in mice plasma

The plasma concentration of triglyceride (TG) in mice measured by using a Triglyceride E-test wako (FUJIFILM Wako Pure

| Group                                    | C | H | Ha | Hd |
|------------------------------------------|---|---|----|----|
| Cornstarch                               | 400| 84| 84 | 84 |
| Vitamin free-casein                      | 200| 200| 200| 200|
| \( \alpha \)-Cornstarch                   | 132| 28| 28 | 28 |
| Sucrose                                  | 100| 312| 312| 312|
| Lard                                     | 0 | 208| 208| 208|
| Vitamin E-deficient stripped corn oil    | 70 | 70 | 70 | 70 |
| Cellulose                                | 50 | 50 | 50 | 50 |
| Mineral mix (AIN-93G)                    | 35 | 35 | 35 | 35 |
| Vitamin E-deficient vitamin mix          | 10 | 10 | 10 | 10 |
| L-Cystine                                | 3 | 3 | 3 | 3 |
| \( t \)-Buthylhydroquinone               | 0.014| 0.014| 0.014| 0.014|
| \( \alpha \)-tocopherol                   | 0.05| 0.05| 0.85| 0.05|
| \( \delta \)-tocopherol                   | 0 | 0 | 0 | 0.8|
| Total energy(kcal/kg diet)               | 3958| 4998| 4998| 4998|

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2.1.4 mRNA analysis of mice adipose tissue

Total RNA was extracted by using Sepasol®-RNA I Super G solution. The RNA amount and purity were measured at 260 and 280 nm with a NanoDrop Q5000 spectrophotometer (Tommy Seiko Co., Ltd., Tokyo, Japan). The total RNA was reverse-transcribed into cDNA by using a High Capacity RNA-to-cDNA Kit. The mRNA expression of each gene was measured by using the 7500 Fast Real-Time PCR system (Applied Biosystems, Thermo Fisher Scientific K.K., Tokyo, Japan) and Taqman®Gene Expression Assays. GAPDH was used as the housekeeping gene.

Table 2 presents the Assay IDs and RefSeqs of the mouse primers used for the quantitative real-time PCR. The difference between the Ct values of the sample and GAPDH were calculated, and the logarithm of this difference for each sample was taken as the measured value.

| Gene | AssayID          | RefSeq       |
|------|------------------|--------------|
| Tnf  | Mm99999068_ml    | NM_013693.2  |
| Ccl2 | Mm00441242_ml    | NM_011333.3  |
| Il6  | Mm00446190_ml    | NM_038689.2  |
| Il1b | Mm00434228_ml    | NM_008361.3  |
| Nos2 | Mm00440502_ml    | NM_010927.3  |
| Gapdh| Mm99999915_g1    | NM_008084.2  |

Assay ID and reference sequence number (RefSeq) of primer probe mixtures used in TaqMan® Gene Expression Assays (Applied Biosystems).

2.2 Exp.2 Anti-inflammatory effects of vitamin E analogs on co-culture of 3T3-L1 adipocytes and RAW264.7 cells

2.2.1 Materials

Mouse 3T3-L1 preadipocytes were obtained from the American Type Culture Collection CL-173 (Manassas, VA, USA), and RAW264.7 macrophages from DS Pharma Biomedical Co., Ltd. (Osaka, Japan). RIPA buffer was purchased from Nacalai Tesque Inc. (Kyoto, Japan). The rabbit anti-NF-κB antibody and anti-GAPDH antibody were purchased from Cell Signaling (Tokyo, Japan). In addition, 10% Mini-PROTEAN TGX Precast Gels, Tris/Glycine/SDS Buffer, Trans-Blot® Turbo™ Transfer Pack, 10× Tris-buffered saline, 0.05% Tween 20 solution, Clarity™ Western ECL substrate and goat anti-rabbit horseradish peroxidase conjugate were purchased from BIO-RAD (Tokyo, Japan).

2.2.2 Cell culture

Mouse 3T3-L1 adipocytes and RAW264.7 macrophages were maintained in high-glucose Dulbecco’s modified Eagle’s medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin and streptomycin, followed by incubation at 37°C in humidified 5% CO2. After passage, the 3T3-L1 adipocytes were cultured in 12-well plates (1.0×10^6 cells/well). Confluent 3T3-L1 adipocytes were incubated with 1 μg/mL insulin, 0.25 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine for 12 days in high-glucose DMEM containing 10% FBS. The medium was replaced with fresh medium every 2 days during differentiation induction. However, the medium was replaced with fresh medium contained insulin only after the second.

2.2.3 Treatment of the co-culture with tocopherol analogs

After differentiation induction for 12 days, the 3T3-L1 adipocytes were treated with new serum-free medium for 2 h, then followed by α-tocopherol or δ-tocopherol in serum-free medium at final concentrations of 10 μM for 24 h. Control cells were treated with dimethyl sulfoxide only. RAW264.7 macrophages (2.0×10^5 cells/well) were seeded onto 12-well plates and cultured with the differentiated 3T3-L1 adipocytes in serum-free medium for 12 h. The control cells were mixed with 3T3-L1 adipocytes and RAW264.7 macrophages cultured separately.

2.2.4 mRNA analysis

A method similar to that described in 2.1.4 was used for the mRNA analysis for culture cells. However, total RNA was extracted by using Sepasol®-RNA II Super solution.

2.2.5 Western blot analysis

Cells were recovered with phosphate-buffered saline and lysed with RIPA buffer. The cellular lysate was incubated on ice for 15 min and then centrifuged for 10 min at 10,000 × g at 4°C. Sample solutions were applied to 10% Mini-PROTEAN TGX Precast Gels and separated on a Tris/glycine/SDS buffer. The proteins were then transferred onto polyvinylidene difluoride membrane of a Trans-Blot® Turbo™ Transfer Pack Transfer System. The membranes were blocked with 5% skimmed milk in Tris-buffered saline.
with 0.05% Tween 20 solution (1×TTBS) at room temperature for 40 min and then incubated overnight at 4°C with the primary antibodies (NF-κB at 1:1000 and GAPDH at 1:1000). After washing the membranes three times with 1×TTBS, they were incubated with the secondary antibody at a concentration of 1:2000 for 1 h at room temperature. They were then washed three times with 1×TTBS, treated with reagents in Clarity™ Western ECL substrate, and detected with the Universal Hood II system (BIO-RAD, Tokyo, Japan).

2.3 Statistical analysis
The data are presented as mean ± SD. Differences were evaluated by using one-way ANOVA followed by Turkey HSD post hoc test and were considered statistically significant at \( p < 0.05 \). The analysis was performed using IBS SPSS Statistics 21 (IBM Corp., Armonk, NY, USA).

3 Results
3.1 Exp.1 Effects of vitamin E analogs on the adipose tissues of mice fed a high-fat and high-sucrose diet for 4 months

3.1.1 Body weight gain of each group for 4 months
Figure 1 shows the body weight gain of each group every week. Average body weight was significantly higher in the H group than in the C group from the experiment start second week to the 16th week. On the other hand, average body weight was markedly lower in the Ha group than in the H group from the experiment start 4th week to the 16th week. Moreover, average body weight was drastically lower in the Hd group than in the H group from the experiment start 9th week to the 16th week. Therefore, we found that \( \alpha \)-tocopherol and \( \delta \)-tocopherol intake can reduce body weight gain of mice fed a high-fat and high-sucrose diet.

3.1.2 Final body weights, food intake, energy intake and each tissue weight in each group (Table 3)
The final body weight was significantly higher in the H group than in the C group from the experiment start 9th week to the 16th week. Therefore, we found that \( \alpha \)-tocopherol and \( \delta \)-tocopherol intake can reduce body weight gain of mice fed a high-fat and high-sucrose diet.

![Fig. 1 Body weight gain of each group for 4 months. The data are presented as mean ± SDs (n = 5-7). Statistical analysis was performed by one-way ANOVA, followed by Tukey’s HSD post-hoc test (* \( p < 0.05 \) (C vs H), \( ^{*} p < 0.05 \) (H vs Ha), \( ^{*} p < 0.05 \) (H vs Hd)) every week.]

Table 3 Final body weight, Food intake and each tissue weight.

|                          | C    | H    | Ha   | Hd   |
|--------------------------|------|------|------|------|
| Final body weight (g)    | 35.4±2.1<sup>a</sup> | 46.7±2.5<sup>b</sup> | 40.9±2.3<sup>a</sup> | 41.2±4.8<sup>b</sup> |
| Food intake (g/day)      | 3.5±0.5<sup>a</sup>  | 2.9±0.1<sup>b</sup>  | 2.7±0.2<sup>b</sup>  | 2.8±0.3<sup>b</sup>  |
| Energy intake (kcal/day) | 14.0±1.7      | 14.2±0.5      | 13.7±0.8      | 13.9±1.6      |
| Liver (g)                | 1.1±0.1<sup>a</sup> | 1.6±0.3<sup>b</sup> | 1.2±0.1<sup>b</sup> | 1.4±0.4<sup>b</sup> |
| Liver (g/100g body weight)| 3.3±0.2      | 3.6±0.6      | 3.0±0.2      | 3.4±0.7      |
| Perirenal fat (g)        | 0.6±0.3<sup>a</sup> | 1.2±0.2<sup>b</sup> | 1.0±0.3<sup>b</sup> | 1.0±0.3<sup>b</sup> |
| Perirenal fat (g/100g body weight)| 1.8±0.7      | 2.6±0.4      | 2.6±0.6      | 2.5±0.6      |
| Epididymal fat (g)       | 1.4±0.5<sup>a</sup> | 2.4±0.3<sup>b</sup> | 2.3±0.6<sup>b</sup> | 2.1±0.6<sup>b</sup> |
| Epididymal fat (g/100g body weight)| 4.0±1.1      | 5.3±0.8      | 5.7±1.4      | 5.3±1.2      |

<sup>1</sup> Values are mean ± SDs, n=5-7
<sup>2</sup> Different superscript letters are significantly different by one-way ANOVA, followed by Turkey’s HSD post-hoc test (\( p < 0.05 \))
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3.1.2 Group Weight

The final body weight was significantly higher in the H group than in the C group. However, the final body weight was drastically lower in the Ha group and Hd group than in the H group. On the other hand, food intake was significantly lower in the H, Ha and Hd group than in the C group. However, there were no significant differences in energy intake among any of the group. The liver weight was markedly higher in the H group than in the C group. But there were not significant differences among the H, Ha and Hd groups. However, the liver weight tended to be lower in the Ha group than in the H group. The adipose tissue weight (perirenal fat and epididymal fat) was significantly higher in the H group than in the C group, but the differences were not significant among H, Ha and Hd groups.

3.1.3 Concentration of TG in mice plasma

The plasma TG concentration was significantly higher in the H group than in the C group. On the other hand, the plasma TG concentration tended to be lower in the Ha group than in the H group (p = 0.055). Similar results were observed in the Hd group (p = 0.075) (Fig. 2). These results indicated that α-tocopherol and δ-tocopherol potentially can improve the lipid metabolism in the body.

3.1.4 Gene expression of each inflammatory cytokine and chemokine in the epididymal fat of mice

Figure 3 shows gene expression of each inflammatory cytokine and chemokine in the epididymal fat of mice. There were no significant differences in Il1b and Il6 in epididymal fat among any of the group (Figs. 3A and 3B). However, the Ccl2 and Tnf in the epididymal fat were significantly higher in the H group than in the C group (Figs. 3C and 3D). These results showed that inflammation was induced in the adipose tissue of mice under this breeding condition. However, vitamin E intake was not able to inhibit the inflammation.

Fig. 2  Effect of vitamin E analogs on plasma triglyceride concentration in mice fed a high-fat and high-sucrose diet for 16 weeks. The data are presented as mean ± SDs (n = 5-7). Statistical analysis was performed by one-way ANOVA, followed by Tukey’s HSD post-hoc test (***p < 0.01).

Fig. 3  Effects of vitamin E analogs on gene expression of inflammatory cytokine in epididymal fat of mice fed a high-fat and high sucrose diet for 16 weeks. (A) Il1b, (B) Il6, (C) Ccl2, and (D) Tnf. The data are presented as mean ± SDs (n = 5-7). Statistical analysis was performed by one-way ANOVA, followed by Tukey’s HSD post-hoc test (***p < 0.01).
3.2 Exp.2 Anti-inflammatory effects of vitamin E analogs on co-culture of 3T3-L1 adipocytes and RAW264.7 cells in vitro

3.2.1 Gene expression of each inflammatory cytokine and chemokine in the co-culture of 3T3-L1 adipocytes and RAW264.7 cells in vitro

Figure 4 shows gene expression of each inflammatory cytokine and chemokine in the co-culture of 3T3-L1 adipocytes and RAW264.7 cells. The *Il1b* and *Il6* expression levels were significantly higher in the co-culture group than in the control group, but these increases were significantly attenuated by treatment with the δ-tocopherol (Figs. 4A and 4B). On the other hand, The *Ccl2* in the co-culture group was drastically higher than that in the control group. However, vitamin E addition was not able to reduce the inflammation (Fig. 4C). Besides, *Nos2* expression level in the co-culture group was remarkably higher than that in the control group, however this increase was significantly inhibited by treatment with the α-tocopherol and δ-tocopherol (Fig. 4D). This finding showed that α-tocopherol and δ-tocopherol had an anti-inflammatory effect on inflammation induced by co-culture of 3T3-L1 adipocytes and RAW264.7 cells in vitro.

3.2.2 Protein expression of NF-κB in the co-culture of 3T3-L1 adipocytes and RAW264.7 cells in vitro

As α-tocopherol and δ-tocopherol were able to inhibit the inflammation of co-culture of 3T3-L1 adipocytes and RAW264.7 cells, we examined the protein expression of NF-κB, a transcriptional factor of IL-6, IL-1β and iNOS. The expression level of the NF-κB protein was significantly higher in the co-culture group than in the control group. However, there was no significant differences in NF-κB protein expression between co-culture group and vitamin E groups (Fig. 5).
4 Discussion

We investigated about the anti-inflammatory effects of vitamin E analogs on mice adipocytes and mice adipose tissues. We fed a high-fat and high-sucrose diet to mice with α-tocopherol or δ-tocopherol fed simultaneously. The results showed that the final body weight was significantly higher in the H group than in the C group. However, the final body weight was drastically lower in the Ha and Hd groups than in the H group. On the other hand, there were no significant differences in the average food intake and average energy consumptions among H, Ha and Hd groups (Table 3). Moreover, the plasma concentration of TG was drastically higher in the H group than in the C group but tended to be lower in the Ha and Hd groups (Fig. 2). Summarizing these results, we assumed that weight reduction occurred as a result of tocopherol intake and depend on the influence of lipid metabolism in the body. However, there was no significant differences in each tissue weight among all groups. We presumed that the unevenness of individual data influenced this result. In the future, examination of the effects of tocopherol on body-weight reduction due to tocopherol appears to be a critical issue.

Regarding the effects of vitamin E analogs on body weight reduction, Zhao et al. reported the effect of γ-tocotrienol on body-weight reduction of mice fed a high-fat diet. They found that the body-weight gain of mice fed a high-fat diet + γ-tocotrienol was significantly lower than that of mice fed a high-fat diet. They assumed that the body-weight reduction in the high-fat diet + γ-tocotrienol group depend on the reduction of liver and adipose tissue weight. Additionally, Fukui et al. reported that final body weight was significantly lower in the high-fat diet with tocotrienol mixture group than in the high-fat diet group. Moreover, Wong et al. reviewed the relationship between vitamin E and metabolic syndrome. In that review, most of the studies examined the relationship of vitamin E to tocotrienol, and few studies examined tocopherol. Hence, body-weight reduction due to tocopherol appears to be a novel finding, but the underlying mechanism associated with tocopherol was not clear in this study. In the future, we would like to continue examining the weight loss effect of tocopherol.

Regarding the inflammatory mechanism in mice adipose tissue, MCP-1 (alias: Ccl2) and TNF-α (alias: Tnfα) gene expression in epididymal fat were significantly higher in the H group than in the C group (Figs. 3A and 3B). Yang et al. also reported that TNF-α and MCP-1 gene expression in epididymal fat of mice fed a high-fat and high-sucrose diet for 12 weeks were higher than that of mice fed a control diet, which is the same result as in the present study. The above results support the idea that inflammation is induced in the adipose tissue of mice fed a high-fat and high-sucrose diet for 4 months without the enforced method. Therefore, it can serve as a lifestyle-related disease mouse model. However, there were no significant differences in IL-1β (alias: Il1b) and IL-6 (alias: Il6) in epididymal fat among any of the group (Figs. 3A and 3B). Accordingly, it is guessed that the influence on the gene expression may be different by a kind of inflammatory cytokine under this breeding condition of mice. Moreover, α-tocopherol or δ-tocopherol intake was also not able to inhibit the inflammation. Therewith, we performed the experiment using co-culture cells to determine if vitamin E analogs show an anti-inflammatory action in the adipocytes in vitro. The IL-1β and IL-6 gene expressions were drastically higher in co-culture group than in control group. On the other hand, two gene expressions were significantly lower in the δ-tocopherol than in the co-culture group (Figs. 4A and 4B). Moreover, we found that the iNOS (alias: Nos2) gene expression in the α- and δ-tocopherol groups were significantly lower than in the co-culture group (Fig. 4C). In this study, IL-1β, IL-6, and MCP-1 were common genes which we investigated by both examination (in vivo and in vitro). However, three gene expressions were different between in vitro and in vivo. We assume that this result depends on the difference in the delivery of tocopherol to cell or tissue. From these results, we suggested that δ-tocopherol has an anti-inflammatory action in mice adipocytes in vitro. We examined the protein expression of NF-κB because it is a common transcription factor of IL-6, IL-1β and iNOS, and its protein expression was dramatically higher in co-culture group than in the control group, but there were no significant differences among co-culture group, α-tocopherol group, and δ-tocopherol group (Fig. 5). NF-κB is an important transcription factor in the gene expression of inflammatory cytokine. NF-κB is usually associated with IκB in cytosol, but IκB deviates from NF-κB because phosphorylated NF-κB moves into a nucleus when signal transmission of inflammation starts. Shen et al. reported that δ-tocotrienol shows an anti-inflammatory effect in macrophage cells stimulated by lipopolysaccharides because δ-tocotrienol inhibited the nuclear translocation of NF-κB. Husain et al. also reported that NF-κB p65 binding to DNA in the nucleus was significantly decreased by γ- and δ-tocotrienol relative to that by vehicle, but it had no effect in α-tocotrienol and α-tocopherol. These results showed that among the vitamin E analogs, some show no anti-inflammatory effect for α-tocopherol, which was the same as the result of Husain et al. Therefore, we concluded that δ-tocopherol has a potential an anti-inflammatory effect because it inhibited the IL-1β, IL-6, and iNOS expressions, which are a target gene related to inflammation. The present study also showed an anti-inflammatory effect for δ-tocopherol, which has not previously been reported. Unfortunately, we were not able to elucidate the mechanism underlying this anti-inflammatory effect of δ-tocopherol. In
the future, we would like to investigate other transcription factors and signal transmission before the transcription factor. We did not observe an anti-inflammatory effect in the in vivo experiment in this study, but an anti-inflammatory effect of δ-tocopherol was clearly shown in vitro. We assumed that the difference between in vivo and in vitro depended on the difference in amount of δ-tocopherol in the adipocytes. We previously reported that δ-tocopherol is easy to accumulate in the epididymal fat of mice, but the amount is very few. Moreover, the δ-tocopherol level in the epididymal fat was significantly lower in a high-fat and high-sucrose diet group than that in the control group. Therefore, we speculated that the δ-tocopherol level is very few in epididymal fat of mice even though our study showed that δ-tocopherol accumulated in the epididymal fat of mice. In the future, we plan to repeat the experiment in mice bred longer than 4 months or in mice fed >800 mg/kg vitamin E in their diet.

5 Conclusion

The high-fat and high-sucrose diet fed to mice for 4 months in this study clearly induced inflammation in the adipose tissue, but there was no anti-inflammatory effect of vitamin E in vivo. On the other hand, we found that δ-tocopherol significantly suppressed upregulation of gene expression of inflammatory cytokines (IL-1β, IL-6, and iNOS) in co-culture with 3T3-L1 adipocytes and RAW264.7 cells. On the other hand, α-tocopherol remarkably inhibited upregulation of iNOS gene expression only. Therefore, we suggested that an anti-inflammatory effect in the δ-tocopherol is stronger than that in the α-tocopherol in vitro. We consider that we perform in vivo examination sequentially in the future.

Contributions

C. K. accomplished conceptualization, draft of experiment, resources, writing-review and editing. H. T. performed investigation and data curation mainly. Y. Y. performed formal analysis of data and writing-figures. T. N. performed investigation together. M. N., R. T.-Y., and C. T.-M. performed validation and methodology. All authors approved the manuscript.

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