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The enhancement of the oral bioavailability of γ-tocotrienol in mice by γ-cyclodextrin inclusion☆

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Abstract

Cyclodextrin (CD) is widely used in the pharmaceutical and nutritional fields to form an inclusion complex with lipophilic compounds for the improvement of their aqueous solubility, stability and diffusibility under physiological conditions. In this study, we investigated the effect of the γ-tocotrienol (γT3) inclusion complex with CD on its oral bioavailability. Five-week-old C57BL6 mice were fed a vitamin E-free diet for 28 days, followed by the oral administration of 2.79 mg of γT3-rich fraction (TRF) extracted from rice bran or the equivalent dose (14.5 mg) of a CD inclusion complex with TRF (TRF/CD). The levels of γT3 in sequentially collected plasma were determined by LC-MS/MS. The pharmacokinetic study revealed that the plasma concentrations of γT3 were increased and peaked at 6 or 3 h after the oral administration of TRF or TRF/CD, respectively (C_{max} values of 7.9±3.3 or 11.4±4.5 μM, respectively). The area under the curve of plasma γT3 concentration also showed a 1.4-fold increase in the group administered with TRF/CD compared with the TRF-only group. Furthermore, the mice that had received the TRF/CD tended to reduce the endotoxin shock induced by injection with lethal amounts of *Escherichia coli* lipopolysaccharide, compared with the mice that had received TRF alone. Taken together, our results suggest that the CD inclusion improved γT3 bioavailability, resulting in the enhancement of γT3 physiological activity, which would be a useful approach for the nutrition delivery system.

Keywords: γ-Tocotrienol; γ-Cyclodextrin; Inclusion complex; Oral bioavailability

1. Introduction

Vitamin E is one of the most promising lipophilic antioxidants, consisting of eight naturally occurring forms known as tocopherols and tocotrienols. Tocopherols carry a saturated phytyl group that is derived from homogentisic acid and phytyl pyrophosphate, whereas tocotrienols are thought to arise from the condensation of homogentisic acid and geranylgeranyl pyrophosphate [1]. Tocopherols and tocotrienols can be subdivided into four isomers (α, β, γ and δ) relating to the numbers and the position of the methyl groups on their chromanol ring (Table 1). The major source of vitamin E is plant-derived oils. Tocotrienols are the major vitamin E component of palm oil. Significant amounts are also found in barley, oats and rice bran [2,3]. α-Tocotrienol is the predominant form of tocotrienol in oat (*Avena sativa* L.) and barley. β-Tocotrienol is the major form of tocotrienol found in hulled and dehulled wheats [4]. Rice bran oil, a byproduct of the rice milling industry, is a major natural source of γ-tocotrienol (γT3) and is believed to be a healthy vegetable oil, especially in Asian countries [5].

Since tocotrienols differ from tocopherols by the presence of three *trans* double bonds in the hydrocarbon tail, tocotrienols are thought to assume a unique conformation. Therefore, members of the natural vitamin E family possess overlapping and unique functional properties that could be due to their structural difference. Among these, tocotrienols, but not tocopherols, have been linked to additional beneficial therapeutic properties that include antithrombotic and neuroprotective activities. Tocotrienols and tocopherols have also been linked to the ability to inhibit the proliferation of breast cancer cells and that to lower serum cholesterol levels when administered in the diet of chickens, swine, rats and hypercholesterolemic humans [6–12]. Moreover, recent studies also revealed their beneficial biological properties, such as anti-angiogenesis and anticancer activities [13]. Therefore, tocotrienols are one of the attractive groups, not only as a functional food constituent but also as a clinical therapeutic agent.

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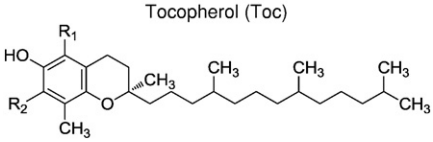
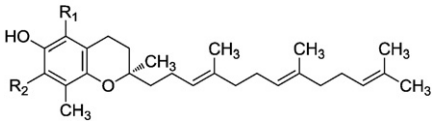
Table 1
Chemical structures and contents of TRF used in this study

Name and structure	Symbol	R_1	R_2	Percentage in TRF	Percentage in TRF/CD
Tocopherol (Toc) 	α-Toc	CH_3	CH_3	0.5	0.1
	β-Toc	CH_3	H	0.4	0.1
	γ-Toc	H	CH_3	2.8	0.6
	δ-Toc	H	H	0.5	0.1
Tocotrienol (T3) 	αT3	CH_3	CH_3	1.8	0.3
	βT3	CH_3	H	0.4	0.1
	γT3	H	CH_3	65.0	12.0
	δT3	H	H	4.6	1.3

Cyclodextrins (CDs) are polysaccharides built from six to eight (α=6, β=7, γ=8) D-glucose units. The α-CD, β-CD and γ-CD are widely used natural CDs. The D-glucose units are covalently linked at the carbon atoms C_1 and C_4 to form a macrocycle [14,15]. CDs can generally form inclusion complexes with a number of lipophilic substances and thus have been used for improving their water solubility, stability, diffusibility and bioavailability [16,17]. Previous studies have shown that the bioavailability of coenzyme Q_{10} or curcumin was improved by CD inclusion [18,19]. Among the three natural CDs, γ-CD shows the highest water solubility and is the only form digested in the intestinal tract. Therefore, much attention has recently been given to the usage of γ-CD as a "host" molecule of the inclusion complex in oral bioavailability experiments and as a promising nutrition delivery system.

The aim of this study was to examine the effect of γT3 inclusion with γ-CD on its bioavailability. A T3-rich fraction (TRF) was prepared by extraction from rice bran, which is a readily available and abundant source of γT3. We performed pharmacokinetic analyses of the plasma levels of γT3 in mice after the oral administration of TRF or a TRF inclusion complex with γ-CD (TRF/CD). We further evaluated the effects of oral TRF and TRF/CD on endotoxin shock induced by the administration of *Escherichia coli* lipopolysaccharide (LPS). Our results demonstrate that the improvement of the oral bioavailability of TRF by γ-CD inclusion leads to the enhancement of potential TRF bioactivities.

2. Materials and methods

2.1. Materials

TRF (Oryza tocotrienol 72), γ-CD and TRF/CD were provided by Oryza Oil & Fat Chemical Company (Aichi, Japan) and Cyclochem (Kobe, Japan). TRF contained vitamin E as follows: 0.5% α-tocopherol (α-Toc), 0.4% β-tocopherol, 2.8% γ-tocopherol, 0.5% δ-tocopherol, 1.8% α-tocotrienol, 0.4% β-tocotrienol, 65.0% γT3 and 4.6% δ-tocotrienol (Table 1). γT3 was provided by Eisai Food & Chemicals (Tokyo, Japan). Vitamin E-free diet was purchased from Funabashi Farm (Chiba, Japan), prepared on the basis of the diet of rodents for experiments determined by the American Institute of Nutrition (AIN) [20] and contained 20% (w/w) vitamin-free casein, 15% corn starch, 25% sucrose, 25% glucose, 5% cellulose, 5% vitamin E-free corn oil, 3.5% vitamin E-free AIN76 vitamin mix, 1% AIN93 mineral mix, 0.3% DL-methionine and 0.2% choline bitartrate. Vitamin E-free corn oil was obtained from Tama Biochemical (Tokyo, Japan). 2,2,5,7,8-Pentamethyl-6-chromanol (PMC) and *E. coli* LPS were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Animal experimentation

All animal experiments were approved by the local animal ethics committee and performed according to guidelines for the care and use of laboratory animals at the University of Shizuoka. Four-week-old male C57BL6 mice were purchased from Japan SLC (Hamamatsu, Japan). All mice were housed in plastic cages (four or five mice per cage) with free access to drinking water and a pelleted basal diet (CE-2, CLEA, Tokyo, Japan) under controlled humidity conditions (55%±5%), light (12 h/12 h light/dark cycle) and temperature (23°C±1°C). Following quarantine for 1 week, the mice were divided according to their body weight into four groups and then fed a vitamin E-free diet starting on Day 0 (Fig. 1). Initial body weight (Day 0) was 19.7±0.6 g, and final body weight (Day 28) was 24.8±1.0 g. The mice were deprived of food for 3 h before and after every oral administration of the test compounds. The following equivalent doses of TRF and γ-CD were used for oral administration on Days 28, 30 and 32: TRF, 2.79 mg in 200 μl of corn oil; TRF/CD, 14.5 mg in 200 μl of corn oil; γ-CD, 11.71 mg in 200 μl of corn oil; or corn oil, 200 μl as control. Amounts of infused total tocotrienols were 2.00 and 1.99 mg per mouse in TRF and TRF/CD, respectively. Similarly, amounts of infused γT3 were 1.81 and 1.74 mg per mouse in TRF and TRF/CD, respectively. For the analyses of γT3 in plasma samples by LC-MS/MS, ~30 μl of tail vein blood samples was collected sequentially into heparinized tubes containing 1 mg of ascorbic acid after the oral administration of the test compounds.

2.3. Standard and sample preparation for LC-MS/MS

The sample preparation was performed according to the method of Yap *et al.* [21] with a slight modification. Briefly, a 10 μl aliquot of plasma was deproteinized by adding 500 μl of a mixture of acetonitrile/tetrahydrofuran (3:2, v/v) containing 250 pmol PMC as an internal standard. The mixture was created using a vortex for 2.5 min and centrifuged at 13,000*g* for 20 min, and then an aliquot (100 μl) of the supernatant was subjected to LC-MS/MS analysis. A standard curve was constructed in the range of 0.1–10 pmol per 10 μl injection of γT3 with PMC (5 pmol). Concentrations of γT3 in the samples were determined by interpolation from the standard curve regression line.

2.4. LC-MS/MS analysis of γT3 in mice plasma samples

LC-MS/MS analyses using nanospace SI-1 (Shiseido, Tokyo, Japan) and API2000 (Applied Biosystems, Forester City, CA, USA) were performed with an electrospray ionization device running in a positive ionization mode. Aliquots (10 μl) of prepared samples were subjected to LC-MS/MS analyses. LC separation was performed with a 3.5-μm Waters X-Bridge C18 column (2.1×150 mm; Waters) at 40°C, using solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol). The flow rate was 0.2 ml/min, and the linear gradient program was used as follows: 80% B at 0 min, 100% B at 5–30 min and 80% B at 31–45 min. The MRM transition for the following compounds was monitored: γT3, *m/z* 411.5/191.1; α-Toc, *m/z* 431.5/165.2; and PMC, *m/z* 221.3/165.1.

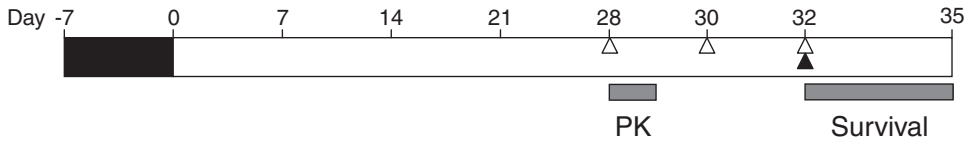


Fig. 1. Experimental protocol. (■) Basal diet. (□) Vitamin E-free diet. (△) Oral administration of the test sample. (▲) LPS (60 mg/kg ip). Gray bars represent the experimental periods of blood collection for pharmacokinetic analysis (PK) and survival analysis (Survival), as indicated.

2.5. Calculation of pharmacokinetic parameters

Pharmacokinetic parameters were calculated from the time course of the plasma γT3 concentration. The maximum plasma γT3 concentration (C_{max}), time to the maximum plasma γT3 concentration (T_{max}) and elimination half-life ($T_{1/2}$) were calculated from the parameters estimated using the one-compartment model [22]. The area under the plasma γT3 and α-Toc concentration–time curve from 0 to 24 h (AUC_{0-24}) was calculated using the trapezoidal formula [23].

2.6. Effects on endotoxin shock induced by LPS

Six hours after the oral administration of the test compounds on Day 32, a single dose of LPS (60 mg/kg) was intraperitoneally administered into C57BL6 mice to induce endotoxin shock. The mice were observed for 72 h.

2.7. Statistical analysis

The resulting values are expressed as the mean±S.E.M. of the indicated number of observations. Statistical analysis for comparing difference was performed using Student's *t* test. The differences in the Kaplan–Meier survival curves were evaluated by the log-rank test.

3. Results

3.1. LC-MS/MS analyses of γT3 in mice plasma

To examine the effect of γT3 inclusion with γ-CD on its bioavailability, we performed LC-MS/MS analysis to determine the levels of γT3 in mice plasma. Standard solutions with a concentration range of γT3 from 0 to 10 pmol containing 5 pmol PMC as an internal standard were prepared in duplicate. A linear calibration curve for γT3 was obtained with a correlation coefficient of R^2=0.9997 (Fig. 2A). The detection limit determined as a signal-to-noise ratio greater than 10 was found to be approximately 0.01 pmol concentration of injected γT3. Fig. 2B shows typical chromatograms obtained by LC-MS/MS analyses of γT3 and PMC in mouse plasma samples. γT3 and PMC were eluted at 12.7 and 4.8 min, respectively. When γT3 (500 pmol) and PMC (250 pmol) added to 10 μl of mouse plasma were analyzed under the present conditions, the recovery rates of γT3 and PMC were 64.5%±2.5% and 85.3%±5.8% (n=5), respectively. The repeatability of the measurements was excellent, with coefficients of variation for γT3 and PMC being about 0.08% and 0.14%, respectively.

3.2. Pharmacokinetic analysis of γT3 in mice plasma

To evaluate the bioavailability of γT3, we performed pharmacokinetic studies of γT3 levels in mouse plasma. Mice were fed a vitamin E-free diet for 28 days to reduce their endogenous vitamin E levels, and then TRF or TRF/CD was orally administered (Fig. 1). The body weights before the oral administration of the test compounds were not significantly different among the groups. As shown in Fig. 3A, plasma γT3 levels were significantly increased after the first oral administration on Day 28 of TRF and TRF/CD. The C_{max} and AUC values in mice that received TRF were 7.9±3.3 μM and 47.7±14.6 μM*h, respectively. The C_{max} and AUC values in mice that were administered TRF/CD were about 1.4-fold greater than those given TRF alone (C_{max} and AUC values were 11.4±4.5 μM and 68.1±18.3 μM*h, respectively) (Fig. 3A and B). The T_{max} values in mice that received TRF and TRF/CD were 6 and 3 h, respectively. $T_{1/2}$ in the TRF/CD group was also longer than that in the TRF-only group. In addition, we detected the trace amounts of vitamin E including γT3 and α-Toc before the oral administration of TRF or TRF/CD, which could be derived from standard diet fed before starting the vitamin E-free diet. Although it was not statistically significant (Student's *t* test, P>.05), we also observed the increase of AUC values for α-Toc in the TRF/CD group (Fig. 3B). These effects were possibly due to the stabilization of TRF in the intestinal tract, resulting in the acceleration of the absorption rate.

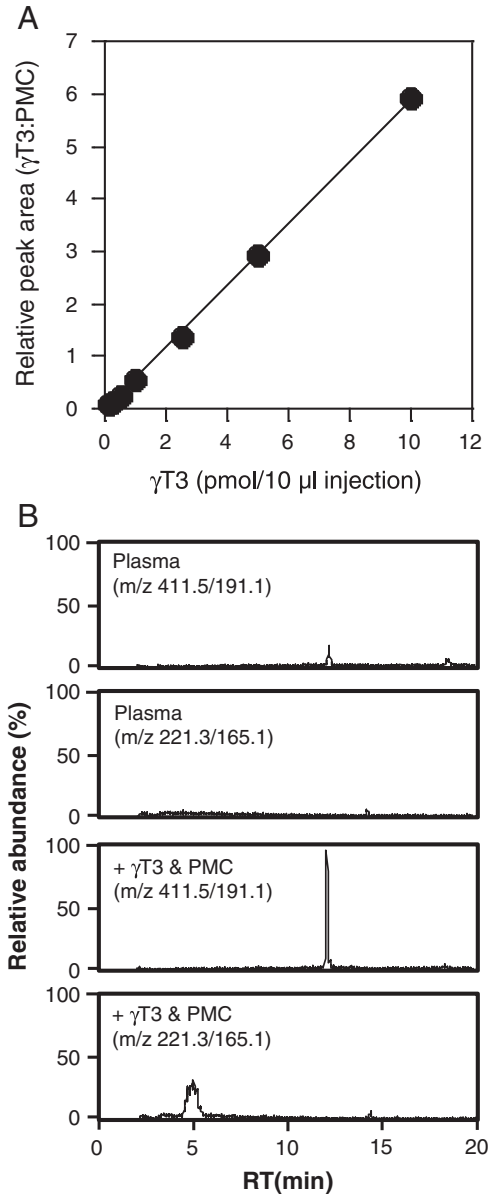


Fig. 2. LC-MS/MS analyses of plasma γT3. (A) Calibration curve for γT3 detected by LC-MS/MS. Analytes were prepared by 0–500 pmol γT3 dissolved in 500 μl of acetonitrile/tetrahydrofuran (3:2, v/v) containing 250 pmol PMC and subjected to LC-MS/MS analyses. Peak area ratios obtained by monitoring the ion transition for m/z 411.5/191.1 for γT3 and m/z 221.3/165.1 for PMC as internal standard were plotted. Data are mean values (n=2). (B) Typical chromatograms obtained by monitoring the ion transition for γT3 (m/z 411.5/191.1) and PMC (m/z 221.3/165.1) in mouse control plasma (no addition) and γT3 and PMC-added mouse plasma are shown.

3.3. Effects of TRF and TRF/CD on LPS-induced mortality

In order to further confirm the improvement of TRF bioavailability *in vivo*, we examined the effects of TRF and TRF/CD on endotoxin (LPS)-induced mortality in mice. Mice orally administered corn oil (control), TRF, TRF/CD or CD on Days 28, 30 and 32 were subjected to a single intraperitoneal injection of LPS (60 mg/kg body weight) 6 h after the last dose of the test compound. As shown in Fig. 4, LPS-treated control mice (which received an oral administration of vitamin E-free corn oil) started to die at 6 h, and all mice died within 42 h after the LPS injection. On the other hand, 20% of mice in the TRF-administered group survived by the end of the 72 h observation. Interestingly, more mice administered TRF/CD were resistant to

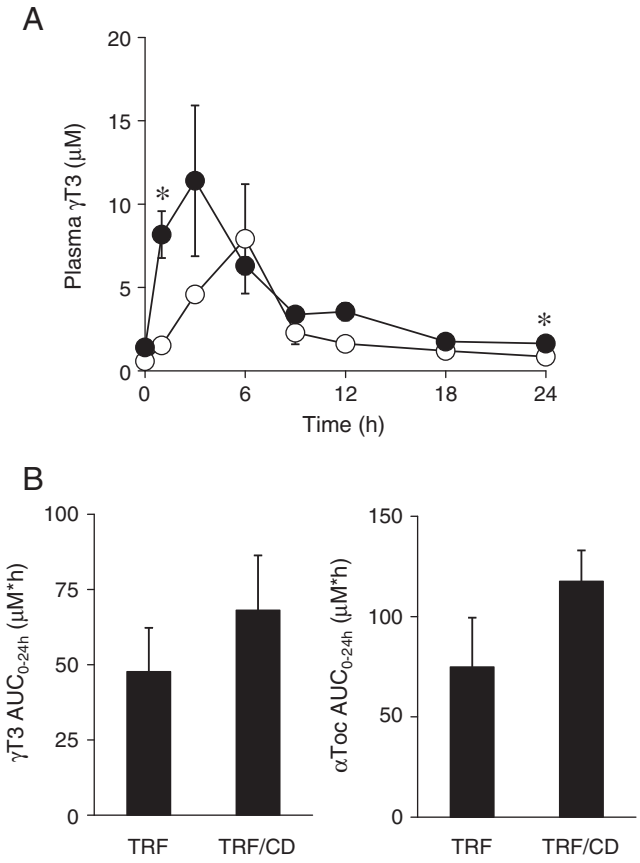


Fig. 3. Plasma γT3 concentrations after the oral administration of TRF or TRF/CD. (A) C57BL6 mice (n=3 in each group) fed a vitamin E-free diet for 24 days were orally administered three times every other day with equivalent doses of TRF (○) or TRF/CD (●). Plasma samples sequentially collected after the first oral administration on Day 28 were prepared and subjected to LC-MS/MS analyses for γT3. (B) $AUC_{0-24\ h}$ values of γT3 and α-Toc were calculated as described in Materials and methods. Data are expressed as the mean±S.E.M. (n=3). Significance determined using Student's t test is expressed as follows: $^{*}P<.05$ TRF/CD- vs. TRF-administered mice.

endotoxin shock, and 36% of mice survived until the end of observation, although this protective effect was not statistically significant when compared with LPS-treated control (log-rank test, $P>.05$). In contrast, no protective effect on the LPS-induced shock was observed in the CD-administered group. In addition, the mice that received TRF, TRF/CD or CD without LPS exhibited no adverse effects (data not shown).

4. Discussion

In this study, we compared the bioavailability of γT3 when given as a TRF inclusion complex with CD with that administered as a free form dissolved in corn oil. The C_{max} and AUC values for plasma γT3 levels were markedly greater in mice administered TRF/CD orally than those given TRF alone (Fig. 3). Moreover, TRF/CD-administered mice tended to be more resistant against the lethal effects of LPS-induced endotoxin shock than those given TRF alone (Fig. 4). These effects could be attributed, at least in part, to the elevated level of plasma TRF when given as an inclusion complex with CD. Our preliminary experiments strongly support this assumption since TRF used in this study exhibited anti-inflammatory effects, such as inhibition of iNOS induction and NO production in LPS/IFNγ-treated mouse macrophage-like RAW264.7 cells (unpublished results). Thus, our results suggest that the *in vivo* bioactivity of TRF was improved by an oral administration of a novel TRF inclusion complex with CD.

On the other hand, the bioavailability of orally administered tocotrienols was relatively inferior to that of α-Toc [1]. In the intestine, all forms of vitamin E are taken up with micelles. Additionally, in our experimental conditions, mice were deprived of food for 3 h before and after an oral administration of the test compounds. Plasma concentrations of γT3 at 3 and 6 h (before and after restart of feeding) were 11.4 and 6.3 μM in the TRF/CD group and 4.6 and 7.9 μM in the TRF group, respectively. During the experimental period of 3–6 h, it was observed that γT3 absorption, especially in the TRF group, could be accelerated by bile acids secreted upon refeeding, resulting in the delay of the T_{max} value (6 h) compared with that of TRF/CD (3 h). However, our results showed that the half-life of γT3 ($T_{1/2}$) in the plasma of mice given TRF/CD was about 1.8-fold greater than that of the TRF-administered mice, although the stability of absorbed γT3 derived from TRF/CD or TRF appears not to be different between the two groups. This may be due to the fact that γT3 given as a TRF inclusion complex with CD was possibly stable in the digestive tract, and γT3 was incorporated into cyclomicron and then released into lymph gradually and continuously from the CD complex. Tocotrienols and tocopherols are then absorbed into the bloodstream from the mesentery. A previous report provides supporting information that TRF/CD enhances intestinal absorption of tocotrienol by improving its solubility and stability in the gastrointestinal tract [24]. Therefore, the elevated levels of C_{max} and AUC and the shortened T_{max} (rapid absorption) of γT3 in the TRF/CD group may be attributed not only to the stability of the inclusion complex but also to the slow release rate of γT3 from TRF/CD in the intestinal tract.

γ-CD can be rapidly and essentially digested by human salivary and pancreatic amylases, and the digest is absorbed in the human small intestine, unlike α-CD and β-CD, which are generally recognized to be nondigestible [25–28]. However, it is interesting to note that orally administered γ-CD can provide a blunted postprandial increase in plasma glucose and insulin, which is more similar to that of slowly digestible carbohydrates rather than rapidly digestible ones [28]. Therefore, nutritional products can be formulated with γ-CD to provide diabetics or other relevant individuals with a nutrition source because the inclusion complex delivers a blunted postprandial glycemic response. In addition, γ-CD does not represent a hazard to human health in detailed and reassuring toxicity studies [29,30]. It can be well tolerated in up to 20% of the diet without any adverse effects [31]. Moreover, γ-CD possesses the huge advantage of having a larger internal cavity, which can trap larger molecules that cannot be trapped by α-CD and β-CD [32]. Furthermore, γ-CD has a noncoplanar and more flexible structure, which gives it a much higher solubility than α-CD and β-CD [32]. When the γ-CD inclusion complex with coenzyme Q_{10} was orally administered to healthy human volunteers, C_{max} and $AUC_{0-24\ h}$ were significantly increased to 121% and 111%, respectively [33]. On the basis of these properties, γ-CD can be used as a carrier and a stabilizer for many bulky guests, such as lipid-soluble vitamins, polyunsaturated fatty acids, sensitive colors and the unique flavors from herbs, spices, fruits, etc. Therefore, γ-CD has much wider applications in many industries, especially in the food and pharmaceutical industries.

Naturally occurring vitamin E, consisting of tocopherols and tocotrienols, is a well-known lipophilic antioxidant. Tocotrienols have slightly higher antioxidant activity than tocopherols in membranes [2,34] and possess neuroprotective, anticancer and cholesterol-lowering properties that are often not exhibited by tocopherols. These additional effects of tocotrienols in mammalian cells can be partially explained by their influence on signal transduction and gene expression and in particular by affecting the mevalonate–cholesterol biosynthesis pathway in a non-antioxidant manner [1,35]. The possible beneficial effects of vitamin E supplementation have been studied with regard to many diseases, including atherosclerosis, other

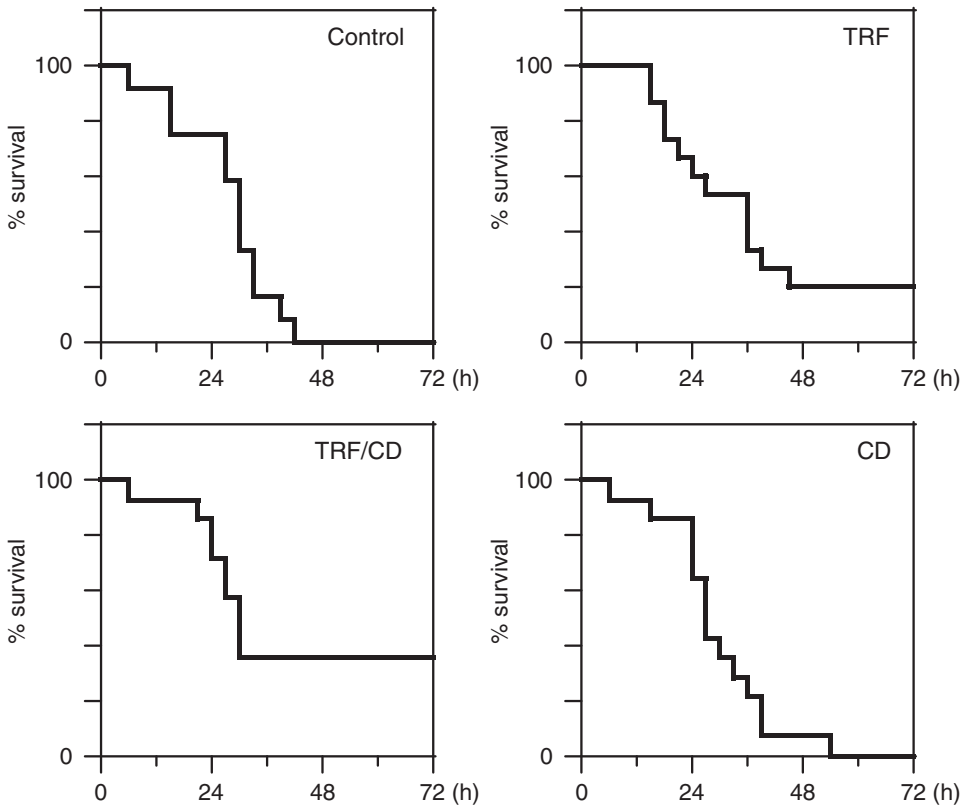


Fig. 4. The effects of TRF or TRF/CD on endotoxin (LPS)-induced mortality. C57BL6 mice were given a single intraperitoneal dose of LPS (60 mg/kg body weight) at 6 h after the oral administration of corn oil (control, n=12) or corn oil containing TRF (n=14), TRF/CD (n=15) or CD (n=12) on Day 32. Mice were observed for 72 h after LPS injection.

cardiovascular diseases, certain types of cancer and a number of neurodegenerative diseases. As many of these diseases are caused, aggravated or associated with inflammatory events, vitamin E can interfere with inflammation by scavenging reactive oxygen and nitrogen species, modulating the synthesis of inflammatory lipid mediators and/or influencing signal transduction and the production of specific cytokines, chemokines and other inflammatory molecules [36,37]. Moreover, several lipid malabsorption syndromes associated with low efficiency of vitamin E uptake clearly can be prevented and in some situations reversed by supplemental vitamin E [38].

In conclusion, our results demonstrate that CD inclusion improved the oral bioavailability of γT3, resulting in the enhancement of γT3 physiological activity. Therefore, we conclude that the vitamin E inclusion complex with γ-CD would exhibit beneficial effects on several intractable chronic diseases associated with inflammation and oxidative stress.

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References

[1] Sen CK, Khanna S, Roy S. Tocotrienols: vitamin E beyond tocopherols. Life Sci 2006;78:2088–98.
[2] Sen CK, Khanna S, Roy S. Tocotrienols in health and disease: the other half of the natural vitamin E family. Mol Aspects Med 2007;28:692–728.
[3] Zingg JM. Vitamin E: an overview of major research directions. Mol Aspects Med 2007;28:400–22.
[4] Panfili G, Fratianni A, Irano M. Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. J Agric Food Chem 2003;51:3940–4.
[5] Sugano M, Koba K, Tsuji E. Health benefits of rice bran oil. Anticancer Res 1999;19: 3651–7.
[6] Qureshi AA, Pearce BC, Nor RM, Gapor A, Peterson DM, Elson CE. Dietary alpha-tocopherol attenuates the impact of gamma-tocotrienol on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in chickens. J Nutr 1996;126:389–94.
[7] Qureshi AA, Bradlow BA, Brace L, Manganello J, Peterson DM, Pearce BC, et al. Response of hypercholesterolemic subjects to administration of tocotrienols. Lipids 1995;30:1171–7.
[8] Qureshi AA, Qureshi N, Hasler-Rapacz JO, Weber FE, Chaudhary V, Crenshaw TD, et al. Dietary tocotrienols reduce concentrations of plasma cholesterol, apolipoprotein B, thromboxane B2, and platelet factor 4 in pigs with inherited hyperlipidemias. Am J Clin Nutr 1991;53:1042S–6S.
[9] Qureshi AA, Qureshi N, Wright JJ, Shen Z, Kramer G, Gapor A, et al. Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (PalmVitee). Am J Clin Nutr 1991;53:S1021–6.
[10] Qureshi AA, Burger WC, Peterson DM, Elson CE. The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J Biol Chem 1986;261: 10544–50.
[11] Theriault A, Chao JT, Wang Q, Gapor A, Adeli K. Tocotrienol: a review of its therapeutic potential. Clin Biochem 1999;32:309–19.
[12] Nesaretnam K, Stephen R, Dils R, Darbre P. Tocotrienols inhibit the growth of human breast cancer cells irrespective of estrogen receptor status. Lipids 1998;33: 461–9.
[13] Miyazawa T, Shibata A, Sookwong P, Kawakami Y, Eitsuka T, Asai A. Antiangiogenic and anticancer potential of unsaturated vitamin E (tocotrienol). J Nutr Biochem 2009;20:79–86.
[14] Szejtli J. Medicinal applications of cyclodextrins. Med Res Rev 1994;14: 353–86.
[15] Stella VJ, Rajewski RA. Cyclodextrins: their future in drug formulation and delivery. Pharm Res 1997;14:556–67.
[16] Uekama K, Hirayama F, Irie T. Cyclodextrin drug carrier systems. Chem Rev 1998;98:2045–76.
[17] Thompson DO. Cyclodextrins — enabling excipients: their present and future use in pharmaceuticals. Crit Rev Ther Drug Carrier Syst 1997;14:1–104.
[18] Prosek M, Butinar J, Lukanc B, Fir MM, Milivojevic L, Krizman M, et al. Bioavailability of water-soluble CoQ_{10} in beagle dogs. J Pharm Biomed Anal 2008;47:918–22.

[19] Yadav VR, Suresh S, Devi K, Yadav S. Effect of cyclodextrin complexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat colitis model. AAPS PharmSciTech 2009;10:752–62.
[20] American Institute of Nutrition. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 1997;107: 1340–8.
[21] Yap SP, Julianto T, Wong JW, Yuen KH. Simple high-performance liquid chromatographic method for the determination of tocotrienols in human plasma. J Chromatogr B Biomed Sci Appl 1999;735:279–83.
[22] Gibaldi M, Perrier D. Pharmacokinetics. 2nd ed. Drugs and the pharmaceutical sciences. New York: Marcel Dekker; 1982.
[23] Chiou WL. Critical evaluation of the potential error in pharmacokinetic studies of using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. Journal of Pharmacokinet Biopharm 1978;6:539–46.
[24] Ikeda S, Uchida T, Ichikawa T, Watanabe T, Uekaji Y, Nakata D, et al. Complexation of tocotrienol with gamma-cyclodextrin enhances intestinal absorption of tocotrienol in rats. Biosci Biotechnol Biochem 2010;74:1452–7.
[25] Marshall JJ, Miwa I. Kinetic difference between hydrolyses of gamma-cyclodextrin by human salivary and pancreatic alpha-amylases. Biochim Biophys Acta 1981;661:142–7.
[26] Kondo H, Nakatani H, Hiromi K. In vitro action of human and porcine alpha-amylases on cyclomalto-oligosaccharides. Carbohydr Res 1990;204:207–13.
[27] De Bie AT, Van Ommen B, Bar A. Disposition of [^{14}C]gamma-cyclodextrin in germ-free and conventional rats. Regul Toxicol Pharmacol 1998;27:150–8.
[28] Lai CS, Chow JM, Wolf BW. (2005) Methods of using gamma cyclodextrin to control blood glucose and insulin secretion. US Patent 20050215523 A1.
[29] Waalkens-Berendsen DH, Smits van Prooije AE, Bar A. Embryotoxicity and teratogenicity study with gamma-cyclodextrin in rabbits. Regul Toxicol Pharmacol 1998;27:172–7.
[30] Waalkens-Berendsen DH, Verhagen FJ, Bar A. Embryotoxicity and teratogenicity study with gamma-cyclodextrin in rats. Regul Toxicol Pharmacol 1998;27: 166–71.
[31] Munro IC, Newberne PM, Young VR, Bar A. Safety assessment of gamma-cyclodextrin. Regul Toxicol Pharmacol 39 Suppl 2004;1:S3–S13.
[32] Li Z, Wang M, Wang F, Gu Z, Du G, Wu J, et al. gamma-Cyclodextrin: a review on enzymatic production and applications. Appl Microbiol Biotechnol 2007;77: 245–55.
[33] Terao K, Nakata D, Fukumi H, Schmid G, Arima H, Hirayama F, et al. Enhancement of oral bioavailability of coenzyme Q_{10} by complexation with γ-cyclodextrin in healthy adults. Nutr Res 2006;26:503–8.
[34] Suzuki YJ, Tsuchiya M, Wassall SR, Choo YM, Govil G, Kagan VE, et al. Structural and dynamic membrane properties of alpha-tocopherol and alpha-tocotrienol: implication to the molecular mechanism of their antioxidant potency. Biochemistry 1993;32:10692–9.
[35] Packer L, Weber SU, Rimbach G. Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. J Nutr 2001;131:S369–73.
[36] Reiter E, Jiang Q, Christen S. Anti-inflammatory properties of alpha- and gamma-tocopherol. Mol Aspects Med 2007;28:668–91.
[37] Singh U, Devaraj S, Jialal I. Vitamin E, oxidative stress, and inflammation. Annu Rev Nutr 2005;25:151–74.
[38] Meydani M, Koga T, Ali S. Vitamin E deficiency. Encyclopedia of life sciences. Chichester: John Wiley & Sons; 2003. Available from: http://www.els.net/. [doi: 10.1038/npg.els.0006101].