JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 67–71
©2001 The Authors
Received May 17, 2000
Accepted August 29, 2000
ISSN 0022-3573

Pharmacokinetics and bioavailability of α-, γ- and δ-tocotrienols under different food status

S. P. Yap, K. H. Yuen and J. W. Wong

Abstract

We have investigated the pharmacokinetics and bioavailability of α-, γ- and δ-tocotrienols under fed and fasted conditions in eight healthy volunteers.

The volunteers were administered a single oral dose of mixed tocotrienols (300 mg) under fed or fasted conditions. The bioavailability of tocotrienols under the two conditions was compared using the parameters peak plasma concentration (C_{max}), time to reach peak plasma concentration (T_{max}) and total area under the plasma concentration–time curve ($AUC_{0-\infty}$).

A statistically significant difference was observed between the fed and fasted logarithmic transformed values of C_{max} ($P < 0.01$) and $AUC_{0-\infty}$ ($P < 0.01$) for all three tocotrienols. In addition, the 90% confidence intervals for the ratio of the logarithmic transformed $AUC_{0-\infty}$ values of α-, γ- and δ-tocotrienols under the fed state over those of the fasted state were found to lie between 2.24–3.40, 2.05–4.09 and 1.59–3.81, respectively, while those of the C_{max} were between 2.28–4.39, 2.31–5.87 and 1.52–4.05, respectively. However, no statistically significant difference was observed between the fed and fasted T_{max} values of the three homologues. The mean apparent elimination half-life ($t_{\frac{1}{2}}$) of α-, γ- and δ-tocotrienols was estimated to be 4.4, 4.3 and 2.3 h, respectively, being between 4.5- to 8.7-fold shorter than that reported for α-tocopherol. No statistically significant difference was observed between the fed and fasted $t_{\frac{1}{2}}$ values. The mean apparent volume of distribution (Vd/f) values under the fed state were significantly smaller than those of the fasted state, which could be attributed to increased absorption of the tocotrienols in the fed state.

Introduction

Tocotrienol is a form of naturally occurring vitamin E that is present in palm oil. It possesses the general structural features of an aromatic chromanol head and a 16-carbon hydrocarbon tail. The α-, β-, γ- and δ-homologues are determined by the number and position of methyl substituents in the chromanol nucleus. In recent years, studies have examined the biological and health effects of tocotrienols for their cholesterol lowering effect (Qureshi et al 1991, 1995), anticancer and tumour suppressive activities (Goh et al 1994; Nesaretnam et al 1998), antioxidant properties (Kooyenga et al 1997; Serbinova et al 1992) and anti-aggregation of blood platelets (Mahadevappa et al 1991). Despite the growing interest in tocotrienols, there is a paucity of information on their absorption and disposition. In studies using thoracic duct-cannulated rats, Ikeda et al (1996) demonstrated that, as well as α-tocopherol, α-, δ- and γ-tocotrienols were transported via the lymphatic system after oral absorption. They also reported that α-tocotrienol was preferentially absorbed compared with δ-, γ-tocotrienols and α-tocopherol. Hayes et al (1993)

School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Minden, Penang, Malaysia

S. P. Yap, K. H. Yuen, J. W. Wong

Correspondence: K. H. Yuen, School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Minden, Penang, Malaysia. Email: khyuen@usm.my

Acknowledgement: We wish to acknowledge the kind support of Hovid Pte. Ltd, Malaysia.

reported that tocotrienols were transported by chylomicrons and would disappear from the plasma during chylomicron clearance. They also reported that in fasting humans, the plasma tocotrienol concentration was not increased significantly after tocotrienol supplementation, whereas the platelet concentration of δ-tocotrienol was doubled. However, none of those studies described the kinetics nor the bioavailability of α-, γ- and δ-tocotrienols, especially under different food status.

The aim of the present study was to determine the disposition of the tocotrienols and also the influence of food status on their bioavailability.

Protocol

The protocol for the study was approved by a Joint School of Pharmaceutical Sciences, USM – General Hospital Penang Committee on Bioavailability Studies. Volunteers were given information on the drug and nature of the study in advance of the trial.

Eight adult healthy male volunteers (age 22–47 years, mean = 33, s.d. = 9; weight 50–79 kg, mean = 68, s.d. = 9) participated in a two-period, two-sequence study after providing written informed consent. All volunteers refrained from taking drug and vitamin preparations one week before and during the study period. During the first trial period, after fasting for a minimum of 12 h overnight, all volunteers were administered 300 mg of mixed tocotrienols orally in the form of four soft gelatin capsules (Tocovid) with 150 mL water. Food and drinks were withheld for at least 4 h after dosing. Standard meals consisting of chicken with rice were given at 4 and 10 h after administration and water was given freely. After a one-week wash-out period all volunteers received the same dose of the preparation after taking a standard high fat breakfast according to the recommendations of USP23-NF18, Supplement 5.

Blood samples (5 mL) were withdrawn into heparinized vacutainers via an in-dwelling cannula placed in the anticubital vein before and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 18 and 24 h after dosing. The plasma was separated by centrifugation for 20 min at 2000 *g* and transferred to separate glass containers to be kept frozen at –20°C until analysis.

Materials and Methods

Materials

Tocotrienol standard kit was purchased from Merck (Darmstadt, Germany). All of the other solvents used were either of analytical reagent grade or of HPLC grade and were purchased from Merck and Ajax Chemicals (Auburn, Australia).

Product studied

Tocovid (batch no: PD2689, manufacturing date: July 1998, expiry date: July 2001) was supplied by Hovid Pte. Ltd (Malaysia). Each capsule contained an assayed amount of 41.6 mg γ-tocotrienol, 21.8 mg α-tocotrienol, 10.7 mg δ-tocotrienol and 34.8 int. units d-α-tocopherol.

Analysis of α-, γ- and δ-tocotrienols

Plasma α-, γ- and δ-tocotrienols were determined using a high-performance liquid chromatography (HPLC) method reported by Yap et al (1999).

Data and pharmacokinetics analysis

The bioavailability of the tocotrienols under fed and fasted conditions was compared using the pharmacokinetic parameters peak plasma concentration (C_{max}), time to reach peak plasma concentration (T_{max}) and total area under the plasma concentration–time curve ($AUC_{0\text{-}\infty}$), which were estimated from the plasma concentration–time data. C_{max} and T_{max} were obtained directly from the plasma concentration values (Weiner 1981). $AUC_{0\text{-}\infty}$ was calculated by adding the area from time zero to the last detectable sampling time t ($AUC_{0\text{-}t}$; calculated by the trapezoidal formula) and the area from time t to infinity ($AUC_{0\text{-}\infty}$; calculated by dividing the last measurable plasma drug concentration with the elimination rate constant (k_e)). In all cases, the $AUC_{t\text{-}\infty}$ was found to be less than 20% of the $AUC_{0-\infty}$. The k_e was estimated from the terminal slope of the individual plasma concentration–time curves after logarithmic transformation of the plasma concentration and application of linear regression (Gibaldi & Perrier 1982). The elimination half-life ($t_{\frac{1}{2}}$) was calculated from the quotient $\ln 2/k_e$, and the apparent volume of distribution (Vd/f) as Dose/($AUC_{0\text{-}\infty} \cdot k_e$). The lag time of absorption (t_{lag}) was determined by extrapolating the initial ascending portion of the plasma concentration–time curves to the time axis.

Statistical analysis

The values of C_{max}, $AUC_{0-\infty}$, k_e, t_{lag} and Vd/f obtained under fasted or fed conditions were analysed using an analysis of variance procedure appropriate for a randomized block design. The C_{max} and $AUC_{0-\infty}$ values were logarithmic transformed before analysis. The T_{max}

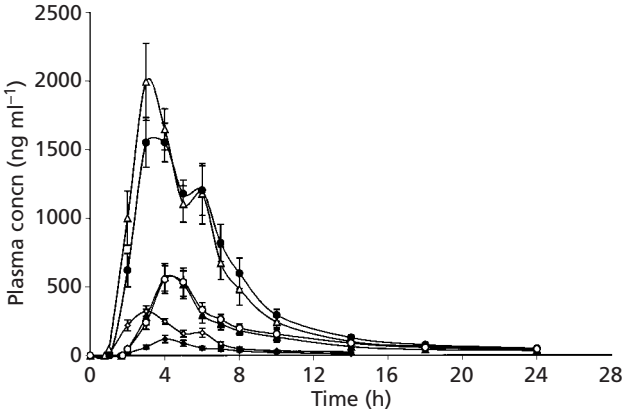

Figure 1 Plasma concentration–time profiles (mean ± s.e.m., n = 8) of α-, γ- and δ-tocotrienol after oral administration of a single dose of 300 mg mixed tocotrienols. ●, α-Tocotrienol with food; ○, α-tocotrienol without food; △, γ-tocotrienol with food; ▲, γ-tocotrienol without food; ◇, δ-tocotrienol with food; ◆, δ-tocotrienol without food.

Table 1 Pharmacokinetic parameters of α-tocotrienols after oral administration of 300 mg mixed tocotrienols with or without food.

Parameter	Fasted	Fed
Maximum concn (C_{max}; ng mL^{-1})	578.0 ± 298.8	1828.8 ± 309.9*
Time to maximum concn (T_{max}; h)	4.3 ± 0.7	4.4 ± 1.4
Volume of distribution (Vd/f; L)	226.3 ± 99.5	59.5 ± 16.0*
Elimination rate constant (k_e; h^{-1})	0.147 ± 0.055	0.166 ± 0.037
$AUC_{0-\infty}$ (h ng mL^{-1})	3448.2 ± 1192.5	9512.1 ± 2047.5*
Lag time of absorption (t_{lag}; h)	1.7 ± 0.6	1.0 ± 0.4**

$AUC_{0-\infty}$ is the area under the plasma concentration–time curve from time zero to infinity. Values are means ± s.d. (n = 8). *$P < 0.01$, **$P < 0.05$ compared with fasted values.

Table 2 Pharmacokinetic parameters of γ-tocotrienols after oral administration of 300 mg mixed tocotrienols with or without food.

Parameter	Fasted	Fed
Maximum concn (C_{max}; ng mL^{-1})	580.3 ± 288.5	2134.8 ± 685.7*
Time to maximum concn (T_{max}; h)	4.3 ± 0.7	3.8 ± 1.2
Volume of distribution (Vd/f; L)	522.3 ± 406.2	118.0 ± 56.0**
Elimination rate constant (k_e; h^{-1})	0.155 ± 0.030	0.169 ± 0.047
$AUC_{0-\infty}$ (h ng mL^{-1})	3275.1 ± 1244.4	9490.7 ± 1633.1*
Lag time of absorption (t_{lag}; h)	1.7 ± 0.6	0.8 ± 0.4**

$AUC_{0-\infty}$ is the area under the plasma concentration–time curve from time zero to infinity. Values are means ± s.d. (n = 8). *$P < 0.01$, **$P < 0.05$ compared with fasted values.

Table 3 Pharmacokinetic parameters of δ-tocotrienols after oral administration of 300 mg mixed tocotrienols with or without food.

Parameter	Fasted	Fed
Maximum concn (C_{max}; ng mL^{-1})	137.7 ± 60.9	341.8 ± 92.0*
Time to maximum concn (T_{max}; h)	3.9 ± 0.4	3.3 ± 0.5
Volume of distribution (Vd/f; L)	433.0 ± 208.5	130.2 ± 58.4*
Elimination rate constant (k_e; h^{-1})	0.303 ± 0.139	0.300 ± 0.126
$AUC_{0-\infty}$ (h ng mL^{-1})	581.6 ± 288.3	1433.4 ± 321.4*
Lag time of absorption (t_{lag}; h)	1.6 ± 0.7	0.9 ± 0.5

$AUC_{0-\infty}$ is the area under the plasma concentration–time curve from time zero to infinity. Values are means ± s.d. (n = 8, except for fasted values n = 7). *$P < 0.01$ compared with fasted values.

values were analysed using the Wilcoxon Signed Rank Test for paired samples. A statistically significant difference was considered at $P < 0.05$.

Results and Discussion

The mean plasma concentration vs time profiles of α-, γ- and δ-tocotrienols under fed or fasted conditions are presented in Figure 1. It was apparent from the plots that the plasma levels of all three tocotrienols increased markedly when dosed with food. Also, a secondary peak was observed in the plasma profiles of α-, γ- and δ-tocotrienols in the fed state. This secondary peak could be due to entero-hepatic cycling of these compounds under the fed condition and merits further investigation. Peak plasma concentrations for α-, γ- and δ-tocotrienols were achieved between 3–5 h. Thereafter, there was an initial rapid decline followed by a more gradual decline in the plasma concentrations of all three tocotrienols. A biphasic nature of the decline was more apparent when the plasma concentrations were plotted on a logarithmic scale (not shown), being suggestive of a multi-compartment pharmacokinetic model.

The mean values of C_{max}, $AUC_{0-\infty}$ and T_{max} for α-, γ- and δ-tocotrienols obtained under fed or fasted conditions are given in Tables 1, 2 and 3, respectively. In accord with the plasma profiles, the mean C_{max} and $AUC_{0-\infty}$ values of all three tocotrienols for the fed state

were higher compared with the values of the fasted state. In all cases a statistically significant difference was observed between the fed and fasted logarithmic transformed values of both parameters ($P < 0.01$). The 90 % confidence intervals for the ratio of the logarithmic transformed C_{max} values of α-, γ- and δ-tocotrienols under the fed state over those of the fasted state were found to lie between 2.28–4.39, 2.31–5.87 and 1.52–4.05, respectively, while those of $AUC_{0-\infty}$ were between 2.24–3.40, 2.05–4.09 and 1.59–3.81, respectively, suggesting a more than 2-fold increase in the extent of absorption of the tocotrienols under the fed state. In comparison, no statistically significant difference ($P > 0.05$) was observed between the fed and fasted values of T_{max} for all three tocotrienols. Thus, it appeared that when administered with food only the extent of absorption of the tocotrienols was significantly increased and not the rate.

In one of the subjects dosed when fasted, δ-tocotrienol was not measurable in the plasma samples. It was observed that the absorption of the tocotrienols tended to be more variable when administered in the fasted state, as evidenced by the larger coefficient of variation (C.V.) values in the parameters $AUC_{0-\infty}$ and C_{max}, calculated using the relationship, s.d./mean $\times$ 100 %. Under the fasted state, the C.V. values of $AUC_{0-\infty}$ for α-, γ- and δ-tocotrienols were 34.6, 38.0 and 49.6, respectively, while for C_{max} they were 51.7, 49.7 and 44.2, respectively. The corresponding C.V. values in the fed state for the parameter $AUC_{0-\infty}$ were 21.5, 17.2 and 22.4, respectively, while for C_{max} they were 16.9, 32.1 and 26.9, respectively, all being relatively smaller compared with those of the fasted condition. Thus, it was apparent that the absorption of the tocotrienols was markedly increased and was also more consistent when taken with food.

As shown in Tables 1, 2 and 3, a lag time of absorption was observed with the tocotrienols in the fed and fasted states. This may be related to the absorption mechanism of the fat-soluble vitamins in general, which requires emulsification by bile salts before transportation across the gut mucosa (Kayden & Traber 1993). A high fat diet causes stimulation of bile secretion, which may thus explain the increased absorption of tocotrienols in the fed state, and also a faster onset in their absorption. It can be seen that the absorption lag times of all the tocotrienols were reduced in the fed state (although a statistical significant difference was not observed between the fed and fasted values of δ-tocotrienol) (Tables 1, 2 and 3).

The mean numerical values of the pharmacokinetic parameters k_e and Vd/f, of α-, γ- and δ-tocotrienols under the fed and fasted conditions are also shown in Tables 1, 2 and 3. The mean fed and fasted values of k_e for all the three homologues were similar and were not significantly different ($P > 0.05$). The mean k_e value for δ-tocotrienol, estimated to be 0.302 h^{-1}, was larger than that of α- and γ-tocotrienols (0.157 and 0.162 h^{-1}, respectively). Thus, the values for the apparent elimination half-life ($t_{\frac{1}{2}}$) of the tocotrienols calculated from the quotient $\ln 2/k_e$ were approximately 2.3, 4.4 and 4.3 h, respectively. A review of the literature revealed that no such values on the disposition of tocotrienols have been reported to date. These values are many times smaller compared with the $t_{\frac{1}{2}}$ of α-tocopherol, with reported values of about 20 h (Bateman & Uccellini 1985; Julianto et al 2000). Thus, the current twice-daily dosing recommended by the manufacturer appears to be appropriate, as it will not lead to undesirably high plasma levels due to accumulation on repeated usage as a supplementation.

The apparent volume of distribution (Vd/f) values for all three tocotrienols, calculated from the relationship Dose/($AUC_{0-\infty} \cdot k_e$) were found to be significantly reduced ($P < 0.05$) in the fed state compared with the fasted state. This discrepancy was attributed to the increase in bioavailability of the tocotrienols when dosed to fed subjects, resulting in a larger $AUC_{0-\infty}$ value. Nevertheless, the values (fed and fasted) appeared to be relatively big, which may be indicative of either incomplete absorption or extensive redistribution from the blood or both.

Conclusion

In conclusion, food was found to increase the onset as well as the extent of absorption of α-, γ- and δ-tocotrienols by more than two folds. In addition, their apparent elimination half-life was found to be relatively short, being much shorter than that of α-tocopherol. Thus, the twice daily dosing recommended by the manufacturer appeared appropriate and would not lead to undesirably high levels due to accumulation on repeated dosing.

References

Bateman, N. E., Uccellini, D. A. (1985) Kinetics of D-α-tocopherol in a water soluble base in man. *J. Pharm. Pharmacol.* **37**: 728–729

Gibaldi, M., Perrier, D. (1982) *Pharmacokinetics*. 2nd edn, Marcel Dekker, New York, pp 145–149

Goh, S. H., Hew, N. F., Norhanom, A. W., Yadav, M. (1994) Inhibition of tumour promotion by various palm-oil tocotrienols. *Int. J. Cancer* **57**: 529–531

Hayes, K. C., Pronczuk, A., Liang, J. S. (1993) Differences in the plasma transport and tissue concentration of tocopherols and tocotrienols: observations in humans and hamsters. *Proc. Soc. Exp. Biol. Med.* **202**: 353–360

Ikeda, I., Imasato, Y., Sasaki, E., Sugano, M. (1996) Lymphatic transport of α-, γ- and δ-tocotrienols and α-tocopherol in rats. *Int. J. Vitam. Nutr. Res.* **66**: 217–221

Julianto, T., Yuen, K. H., Mohammad Noor, A. (2000) Improved bioavailability of vitamin E with self-emulsifying formulation. *Int. J. Pharm.* **200**: 53–57

Kayden, H. J., Traber, M. G. (1993) Absorption, lipoprotein transport and regulation of plasma concentrations of vitamin E in humans. *J. Lipid Res.* **34**: 343–358

Kooyenga, D. K., Gellar, M., Watkins, T. R., Gapor, A., Diakoumakis, E., Bierenbaum, M. L. (1997) Palm oil antioxidants: effects in patients with hyperlipidemia and carotid stenosis – 2 year experience. *Asia Pacific J. Clin. Nutr.* **6**: 72–75

Mahadevappa, V. G., Sicilia, F., Holub, B. J. (1991) Effect of tocotrienol derivatives on collagen and ADP-induced human platelet aggregation. Proc. 1989 Int. Palm Oil Conference – Nutrition and Health Aspect of Palm Oil, PORIM, Kuala Lumpur, Malaysia, pp 36–38

Nesaretnam, K., Stephen, R., Dils, R., Darbre, P. (1998) Tocotrienols inhibit the growth of human breast cancer cells irrespective of estrogen receptor status. *Lipids* **33**: 461–469

Qureshi, A. A., Qureshi, N., Hasler-Rapacz, J. O., Weber, F. E., Chaudhary, V., Crenshaw, T. D., Gapor, A., Ong, A. S. H., Chong, Y. H., Peterson, D., Rapacz, J. (1991) Dietary tocotrienols reduce concentrations of plasma cholesterol, apolipoprotein B, thromboxane B_2 and platelet factor 4 in pigs with inherited hyperlipidemias. *Am. J. Clin. Nutr.* **53**: 1042S–1046S

Qureshi, A. A., Bradlow, B. A., Brace, L., Manganello, J., Peterson, D. M., Pearce, B. C., Wright, J. J. K., Gapor, A. Elson, C. E. (1995) Response of hypercholesterolemic subjects to administration of tocotrienols. *Lipids* **30**: 1171–1177

Serbinova, E., Khwaja, S., Catudioc, J., Ericson, J., Torres, Z., Gapor, A., Kagan, V., Packer, L. (1992) Palm oil vitamin E protects against ischemia/reperfusion injury in the isolated perfused langendorff heart. *Nutr. Res. Suppl.* **1**: S203–S215

Weiner, D. L. (1981) In: Buncher, C. R., Tsay, J. Y. (eds) *Statistics in the Pharmaceutical Industry*. Marcel Dekker Inc., New York, pp 205–229

Yap, S. P., Julianto, T., Wong, J. W., Yuen, K. H. (1999) Simple high-performance liquid chromatographic method for the determination of tocotrienols in human plasma. *J. Chromatogr. B* **735**: 279–283