Lipidaemic effects of tocotrienols, tocopherols and squalene: studies in the hamster

HT Khor and DY Chiang

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Syrian Golden hamsters have been widely used as an experimental model for the investigation of the aetiology and development of atherosclerosis and cardiovascular disease. The responses of the hamster to dietary fat manipulations are generally similar to that observed in humans. The lipidaemic effect of a tocotrienol and tocopherol mixture from palm oil on human trials has not been consistent. In this study, the lipidaemic effect of tocotrienols and tocopherols were investigated by using pure tocotrienols (that were made from palm oil) and pure commercial tocotrienols and squalene. A palm oil tricaprylin fraction (POTG), free of all unpalatable components, was used in diet study. In the present feeding experiment, tocotrienols added at 162 ppm to POTG (POTG-T) significantly (P<0.05) lowered serum total cholesterol (TC) level as compared to that of the POTG group; but the serum LDL-C, HDL-C and TG levels of the POTG-T group were not significantly lower than that of the POTG group (P>0.05). Increasing the level of tocotrienol supplementation in the diet (POTG-T3H) appeared to raise rather than reduce the serum TC, LDL-C and HDL-C levels as compared to that of POTG-T3 group. This observation that lower level of tocotrienol supplementation appeared to exhibit stronger hypolipidaemic effect than a higher level of tocotrienol supplementation is interesting; but its explanation is yet forthcoming. When tocopherols were supplemented at 72 ppm in the POTG diet it was observed that the serum TC, LDL-C and HDL-C levels were all somewhat increased compared to that of the POTG group. These results suggest that tocotrienols and tocopherols may have opposite cholesterolemic effects in the hamster, and further experiments need to clarify the mode of action of these vitamin E isomers. Our second series of experiments the cholesterolemic effects of tocotrienols and tocopherols were studied in the presence of squalene, a key intermediate in the cholesterol synthesis pathway and a commercial cholesterol lowering agent. Squalene supplementation diet at 0.1% in the diet (POTG-S) significantly (P<0.05) serum TC level when compared to that of the POTG group. The LDL-C, HDL-C and TG levels appeared to be lowered by the squalene supplementation also but the differences between the POTG-S and POTG groups were not statistically significant (P>0.05). When tocopherols were added to the squalene-containing POTG diets, the serum TC and LDL-C levels were further reduced (P>0.01) when compared to that of the POTG and POTG-S groups. The HDL-C and TG levels were not affected by tocotrienol or tocopherol supplementation in the presence of squalene. These results indicate that in the presence of squalene and squalene POTG exhibit hypocholesterolemic action whereas tocopherols may have a hypercholesterolemic effect in the hamster.

Key words: Plasma lipids, vitamin E, tocotrienols, squalene

Introduction

Epidemiological studies reveal that plasma vitamin E level is inversely correlated with coronary artery disease2. All the above studies focus on tocopherol, the most widely distributed form of vitamin E in nature.

Tocotrienols, another form of vitamin E, were found to be present in limited amounts in vegetable oils as the richest source of tocotrienols in nature3,4. A tocotrienol concentrate, trade-name Palmivite, was prepared from palm oil and capislated; each capsule contains about 40 mg of tocotrienols and 20 mg of tocopherols. Human trials on the above tocopherol-tocotrienol-emulsified capsules with normal and hypercholesterolemic subjects yielded inconsistent results5,6. The discrepancies in the above observations are not yet explained; however, one obvious difference in the above trials was the dosages used in the experiments. It appeared that experiments with lower dosages of Palmivite tended to give positive hypocholesterolemic effect whereas higher dosages of Palmivite tended to give neutral effects. In order to study the cholesterolemic effect of tocotrienols and tocopherols separately we have isolated pure palm oil tocotrienols from palm oil fatty distillate, a by-product of the palm oil refining process. The tocotrienols were isolated and bunched separately and were obtained from commercial sources. The tocotrienols, tocopherols and squalene were added to the dietary fat, palm oil tricaprylin, isolated from all unpalatable components from palm oil. The Hamster was used as the experimental model because it has many similar features of lipid metabolism as that seen in humans including the responses to dietary lipid manipulations. Hamsters have been extensively used in studies of lipid metabolism in relation to atherosclerosis and cardiovascular disease7,8.

Materials and methods

Animals and diets

Male Syrian hamsters of body weights ranging from 100 to 150 g were obtained from the animal Research Centre, University of Malaya. They were provided with light and dark cycle. All groups were given cholesterol-free, high fat (20%, w/w) semisynthetic diets supplemented either with tocotrienols, tocopherols or squalene. Tocotrienols were isolated from palm oil fatty distillate by solvent extraction and column chromatography9. The tocotrienols were chromatographically pure (99.9%) by HPLC and Capillary GLC and were composed of α-tocotrienol (43%), γ-tocotrienol (50%) and δ-tocotrienol (7%). Tocopherols (containing mainly α-tocopherol) and squalene were obtained from commercial sources (Sigma Chemical Co., St. Louis, USA). The general formats of the semi-synthetic diet is given in Table I. The animals were fed these experimental diets for 45 days. Water was given ad libitum and diets were given daily. At the end of the experimental period, the animals were fasted overnight. Blood and liver were taken while the animals were under ether anesthesia. Serum was prepared by centrifugation at 2500 rpm for 10 minutes at room temperature. Serum and liver were stored at -20°C until analysis.

Table 1. Composition of the semisynthetic diet

<table>
<thead>
<tr>
<th>Ingredients</th>
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<tbody>
<tr>
<td>Cornflour</td>
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<td>DL-methionine</td>
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<td>Vitamin mix</td>
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</tr>
</tbody>
</table>

*palm oil triglycerides isolated from all unpalatable matters from commercial palm oil.

Experimental protocols

Study 1. Effect of dietary tocotrienols and tocopherols on serum and liver lipids. Four groups of hamsters were isolated from palm oil containing POTG diet. The second group was fed on selenized semisynthetic diet containing POTG supplemented with 162 ppm of tocotrienol, and the third group was fed on semisynthetic diet containing POTG supplemented with 1000 ppm of tocotrienols.

Study 2. Effect of squalene, tocotrienols and tocopherols in serum and liver lipids. Four groups of hamsters were used. Group 1 was fed on a control semisynthetic diet which contained POTG as the dietary fat. Group 2 was fed on the control semisynthetic diet supplemented with 0.1% of squalene. Group 3 was fed on the control semisynthetic diet supplemented with 0.1% of squalene and 162 ppm of tocotrienol diet. Group 4 was fed on the control semisynthetic diet supplemented with 0.1% of squalene and 72 ppm of tocopherols.

Analysis of serum lipids

Serum total cholesterol, HDL-cholesterol and TG levels were analysed by enzymic methods using Sigma diagnostic kits (Sigma Chemical Co., St. Louis, USA). LDL-cholesterol level was determined from the supernatant of the HDL fraction according to that described by Pearse et al. Analysis of liver lipids

Liver lipids were extracted with chloroform-methanol (2:1, v/v) as described by Folch et al. Liver lipids were fractionated into neutral and polar lipid fraction on a solidified Florisil column. The polar lipid fraction were then separated into different lipid classes on HPLC using a 10 s silica column and quantified with a evaporative light scattering detector.

Results

The hamsters grew well and appeared healthy on these semisynthetic diets and no significant difference in body weights was evident at the end of the feeding period. Supplementation of the POTG diet with different levels of tocotrienols and tocopherols had significant effect on the serum lipids. As shown in Figure 1, the serum total cholesterol (TC), LDL-C and HDL-C levels were somewhat elevated, though not significantly, when the hamsters received a diet supplemented with 72 ppm of tocotrienols (POTG-T) as compared to the control, POTG group which received tocopherol supplementation. When tocotrienols were supplemented at 162 ppm the serum TC, LDL-C and HDL-C levels were lower as compared to that of the control POTG group, but statistical significance was only achieved only between TC differences of the control (POTG) and tocotrienol-treated (POTG-T) groups. When the level of tocotrienol supplementation was raised from 162 ppm to 1000 ppm in the diet, the serum TC, LDL-C and HDL-C levels were further reduced but on the contrary somewhat raised as compared to the POTG-T3 group.

Figure 1. Effect of tocopherol and tocotrienol supplementation on the serum lipid levels in the hamster.

Supplementation with either tocopherols or tocotrienols appeared to lower the serum TG level as compared with the control group but due to variability in TG values among groups there was no statistically significant effect. There also appeared to be no difference in the serum HDL-C levels.

Supplementation of the diets with tocopherols and tocotrienols produced no effect on the liver total lipids. Similarly tocotrienol supplementation neither caused a reduction in liver TC levels when compared to the control (Figure 2). The slight elevation in liver TC in POTG-T group was feed by tocopherol and tocotrienol supplementation (Figure 2). The other liver lipids such as diacylglycerols (DAG), monoacylglycerols (MAG) and free fatty acids (FFA) were not much affected.

Figure 2. Effect of tocopherol and tocotrienol supplementation on liver lipids in cholesterol-fed and trilysic lipid levels in the hamster.

The effect of squalene supplementation (0.1%) to a cholesterol-free, high fat semi-synthetic diet on serum lipids is shown in Figure 3. These results show that squalene supplementation significantly lowered (P<0.05) serum TC level as compared to the control and the squalene-supplemented groups; the HDL-C, LDL-C and TG level also appeared to be somewhat lower as compared to the control POTG group. When tocotrienols (162 ppm) were added to the squalene-supplemented diet the serum TC, LDL-C and HDL-C (P<0.01) levels were further lowered as
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Syrian Golden hamsters have been widely used as an experimental model for the investigation of the aetiology and development of atherosclerosis and cardiovascular disease. The responses of the hamster to dietary fat manipulations are very similar to that observed in humans. The lipidaemic effect of a tocotrienol and tocopherol supplement from palm oil on human trials has not been consistent. In this study, the cholesterol-lowering effect of tocotrienols and tocopherols were demonstrated by using pure tocotrienols (that were isolated from palm oil with a natural ratio) and pure commercial tocopherols and squalene. A palm oil tritrylphenol fraction (POTG), free of all unsaponifiable matters, was used in a diet of a different feeding experiment. Tocotrienols added at 162 ppm to POTG (POTG-T3L) significantly (P<0.05) lowered serum total cholesterol (TC) level as compared to that of the POTG group; but the serum LDL-C, HDL-C and TG levels of the POTG-T3L group were significantly lower than that of the POTG group (P<0.05). Increasing the level of tocotrienol supplementation in the diet (POTG-T3H) appeared to raise rather then reduce the serum TC, LDL-C and HDL-C levels as compared to that of POTG-T3L. This observation that lower level of tocotrienol supplementation appeared to exhibit stronger hypolipidaemic effect than a higher level of tocotrienol supplementation is interesting; but its explanation is not yet forthcoming. When tocopherols were supplemented at 72 ppm to the POTG diet it was observed that the serum TC, LDL-C and HDL-C levels were all somewhat increased when compared to that of the POTG group. These results suggest that tocotrienols and tocopherols may have opposite cholesterol-lowering effects in the hamster, and further experiments need to clarify the mode of action of these vitamin E isomers. In our second series of experiments the cholesterol-lowering effects of tocotrienols and tocopherols were studied in the presence of squaene, a key intermediate in the cholesterol synthesis pathway and a commercial cholesterol lowering agent. Squaene supplementation at 1.0% of diet (P<0.05) serum TC level when compared to that of the POTG group. The LDL-C, HDL-C and TG levels of POTG and TG levels appeared to be lowered by the squaene supplementation also but the difference between the POTG-SQ and POTG groups were not statistically significant (P>0.05). When tocopherols were added to the squaene-containing POTG diets, the serum TC and LDL-C levels were further reduced (P<0.01) when compared to that of the POTG and POTG-SQ groups. The HDL-C and TG levels were not affected by tocotrienol or tocopherol supplementation in the presence of squaene. These results indicate that in the presence of squaene and POTG exhibit cholesterol-lowering action whereas tocopherol may have a hypolipidaemic effect in the hamster.

Key words: Plasma lipids, vitamin E, tocotrienols, squalene

Introduction
Epidemiological studies reveal that plasma vitamin E level is inversely correlated with cardiovascular disease. All the above studies focus on tocopherol, the most widely distributed form of vitamin E in nature.

Tocotrienols, another form of vitamin E, were found to be present in palm oil and other vegetable oils, as the richest source of tocotrienols in nature. A tocotrienol concentrate, trade-named Palmitinol, was prepared from palm oil and capsaicin; which contains about 40 mg of tocotrienols and 20 mg of tocopherols. Human trials on the above tocopherol-tocotrienol-emulsions capsules with normal and hypercholesterolemic subjects yielded inconsistent results. The discrepancies in the above observations are not yet explained; however, one obvious difference in the above trials was the doses used in the experiments. It appeared that experiments with lower doses of Palmitinol tended to give positive hypolipidaemic effect whereas higher doses of Palmitinol gave to neural effect.

In order to study the cholesterol-lowering effect of tocotrienols and tocopherols separately we have isolated pure palm oil tocotrienols from palm oil fatty distillate, a by-product of the palm oil refining process. Tocotrienols and squalene were obtained from commercial sources. The tocotrienols, tocopherols and squalene were added to the dietary fat, palm oil tristerylglycerol, isolated free of all unsaponifiable components from palm oil. The Hamster was used as the experimental model because it has many similar features of lipid metabolism as that seen in humans including the responses to dietary lipid manipulations. Hamsters have been extensively used in studies of lipid metabolism in relation to atherosclerosis and cardiovascular disease.

Materials and methods
Animals and diet
Male Syrian hamsters of body weights ranging from 100 to 150 g were obtained from the Research Centre, University of Malaya only. They were fed a diet of approximately equal body weights and housed individually in stainless cages in a temperature-regulated, 25 ± 2°C, and light-controlled room with a light cycle of 12h:12h and 5 days to 3 weeks of age. All animals were examined, and those of similar age and weight were included in each dietary group. The animals were fed the experimental diets for 45 days. Water was given ad libitum and diets were given daily. At the end of the 45-day experimental period, the animals were fasted overnight. Blood and liver were taken while the animals were under anaesthesia. Serum was prepared by centrifugation at 2500 rpm for 10 minutes at room temperature. Serum and liver were stored at −20°C until analysis.

Table 1. Composition of the semi-synthetic diets

<table>
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<td>Cellulose</td>
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</table>
* palm oil tritrylphenol fraction free of all unsaponifiable matters from commercial palm oil.

Experimental protocols
Study 1. Effect of dietary tocotrienols and tocopherol on serum and liver lipids. Four groups of hamsters were isolated from palm oil fed hamsters. One group was fed on dietary containing POTG as the dietary fat. The second group was fed on semisynthetic diet containing POTG supplemented with 72 ppm of tocotrienol; the third group was fed on semisynthetic diet containing POTG supplemented with 162 ppm of tocotrienol, and the fourth group was fed on semisynthetic diet containing POTG supplemented with 1000 ppm of tocotrienol.

Study 2. Effect of squalene, tocotrienols and tocopherols in serum and liver lipids. Four groups of hamsters were used. Group 1 was fed on a control semisynthetic diet which contained POTG as the dietary fat. Group 2 was fed on the control semisynthetic diet supplemented with 0.1% of squalene. Group 3 was fed on the control semisynthetic diet supplemented with 0.1% of squalene and 162 ppm of tocotrienol. Group 4 was fed on the control semisynthetic diet supplemented with 0.1% of squalene and 72 ppm of tocopherol.

Analysis of serum lipids
Serum total cholesterol, HDL-cholesterol and TG levels were determined by enzymatic methods using Sigma diagnostic kits (Sigma Chemical Co., St. Louis, USA). LDL-cholesterol level was determined from the supernatant of the HDL fraction according to that described by Pearson et al.

Analysis of liver lipids
Liver lipids were extracted with chloroform-methanol (2:1,v/v) as described by Folch et al. Liver lipids were fractionated into neutral and polar lipid fraction on a solid phase Florisil column. The neutral lipids were then separated into different lipid classes on HPLC using a 10 x 300 mm column and quantified with a pyrolytic evaporation light scattering detector.

Results
The hamsters grew well and appeared healthy on these semisynthetic diets and no significant difference in body weights was evident at the end of the feeding period. Supplementation of the POTG diet with different levels of tocotrienol and tocopherol had significant effect on the serum lipids. As shown in Figure 1, the serum total cholesterol (TC), LDL-C and HDL-C levels were somewhat elevated, whereas not significantly, when the hamsters were fed a diet supplemented with 72 ppm of tocopherol (POTG-T) as compared to the control, POTG group which received only tocopherol supplementation. When tocotrienols were supplemented at 162 ppm the serum TC, LDL-C and HDL-C levels were lower as compared to that of the control POTG group, but statistical difference was observed only between TC and HDL-C differences of the control (POTG) and tocotrienol-treated (POTG-T3L) groups. The level of tocotrienol supplementation was raised from 162 ppm to 1000 ppm in the diet, the serum TC, LDL-C and HDL-C levels were not further reduced but on the contrary somewhat raised as compared to the POTG-T3L group.

Figure 1. Effect of tocopherol and tocotrienol supplementation on the serum lipids level in the hamster.

Supplementation with either tocotrienols or tocopherol appeared to lower the serum TG level as compared with the control group but due to variation in TG values among hamsters groups there was no statistically significant effect. There also appeared to be no difference on the serum HDL-C levels.

Supplementation of the diets with tocopherols and tocotrienols produced no effect on the liver total lipids. Similarly tocotrienol supplementation alone, was found on the control semisynthetic diet whereas tocotrienol supplementation caused a slight reduction in liver TC when compared to the control (Figure 2). The slight elevation in liver TC in POTG and POTG-T3L groups as compared to the control was due to substantial increases in free cholesterol (FC) levels, the liver TG level was actually to be slightly elevated by tocopherol and tocotrienol supplementation (Figure 2). The other liver lipids such as diacylglycerols (DAG), monacylglycerols (MAG) and free fatty acids (FFA) were not much affected.

Figure 2. Effect of tocopherol and tocotrienol supplementation on liver lipid level in the hamster.
Lipidaemic effects of tocotrienols, tocopherols and squalene

J.T. Khor and D.Y. Chiang


Summary

Syrian hamsters were used to evaluate the ability of tocotrienols and tocopherols to reduce cholesterol levels in the liver and plasma. The tocopherol group was fed a diet containing 5% tocopherols and the tocotrienol group was fed a diet containing 12.5% tocopherols. The results showed that tocotrienols had a greater effect on reducing cholesterol levels than tocopherols.

Discussion

The antiproliferative effect of vitamin E, both tocotrienols (in vitro) and tocopherols, in biological systems is well established, but the cholesterol-lowering effect of these vitamins is still controversial. Previous studies in humans showed that tocotrienol supplementation had no effect on serum cholesterol levels or actually showed a slight lowering effect on serum cholesterol in some individuals. In animal models, Khor and Chiang reported that short-term (6 days) treatment of rats with tocopherols produced no effect on serum cholesterol levels, but Chen et al. reported that adding 100 ppm tocopherol to a semipurified diet containing vitamin E-stripped corn oil significantly increased serum total cholesterol and HDL-C levels in male Sprague-Dawley rats. Our recent study showed that hamsters fed on semipurified diets containing 72 ppm of tocopherols for 45 days had no significant increase in serum total cholesterol levels suggesting that tocopherols are probably neutral.

Figure 3. Effect of squalene, tocopherol, and tocotrienol supplementation on serum liver lipids in the hamster.

Squalene supplementation in the diet also produced significant changes in the liver lipid profiles. Squalene supplementation in the diet resulted in significant increase in liver total lipids (TL) as compared to the control group (Figure 4). The increase in liver TL in the squalene-supplemented group was due to significant increase in liver cholesterol content which was attributed to significant increase in cholesteryl esters; the free cholesterol content was not affected. There was a slight increase in liver TG content of squalene-supplemented group as compared to the control (Figure 5). The other liver lipids, namely DAG, MAG, and FFA, were not much affected by squalene or tocotrienol or tocopherol supplementation.

Figure 4. Effect of squalene, tocopherol, and tocotrienol supplementation on liver total lipids in the hamster.

Tocotrienols isolated from barley was shown to inhibit HMG CoA reductase activity in vitro. More recently Khor et al. reported that tocotrienols isolated from palm oil fatty acid distillate inhibited HMG CoA reductase activity in the guinea pig liver, whereas tocopherols were not effective in lowering cholesterol levels in the guinea pig. However, tocopherols were not effective in lowering cholesterol levels in the guinea pig. In the present study adding 0.1% squalene to the diet significantly lowered serum total cholesterol levels in the hamster. In our present study adding 0.1% squalene to the diet significantly lowered serum total cholesterol levels in the hamster. The differences in the above observation could be due to differences in the animal models and the level of squalene supplementation. In our study we lowered the serum cholesterol levels was associated with a significant increase in liver cholesterol esters (Figure 5). It appears that squalene may enhance the cholesterol esterase activity in the liver resulting in accumulation of cholesterol esters in the tissue. Further experiments are in progress to confirm this assumption.

Addition of tocotrienols to squalene-supplemented diet resulted in further reduction of serum cholesterol levels (Figure 3). This result confirms earlier observation that tocotrienols possessed hypocholesterolemic effect in the hamster. Unexpectedly addition of tocopherols to squalene supplemented also resulted in further reduction in serum cholesterol levels in the hamster (Figure 3).

Acknowledgement: This project was supported by research grants from the Palm Oil Research Institute of Malaysia and the University of Malaya.}

Lipidaemic effects of tocotrienols, tocopherols and squalene: studies in the hamster

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Syrian hamsters were used to evaluate the ability of tocotrienols and tocopherols to reduce cholesterol levels in the liver and plasma. The tocopherol group was fed a diet containing 5% tocopherols and the tocotrienol group was fed a diet containing 12.5% tocopherols. The results showed that tocotrienols had a greater effect on reducing cholesterol levels than tocopherols.

Discussion

The antiproliferative effect of vitamin E, both tocotrienols (in vitro) and tocopherols, in biological systems is well established, but the cholesterol-lowering effect of these vitamins is still controversial. Previous studies in humans showed that tocotrienol supplementation had no effect on serum cholesterol levels or actually showed a slight lowering effect on serum cholesterol in some individuals. In animal models, Khor and Chiang reported that short-term (6 days) treatment of male guinea pigs with tocopherols produced no effect on serum cholesterol levels, but Chen et al. reported that adding 100 ppm tocopherol to a semipurified diet containing vitamin E-stripped corn oil significantly increased serum total cholesterol and HDL-C levels in male Sprague-Dawley rats. Our recent study showed that hamsters fed on semipurified diets containing 72 ppm of tocopherols for 45 days had no significant increase in serum total cholesterol levels suggesting that tocopherols are probably neutral.

Figure 3. Effect of squalene, tocopherol, and tocotrienol supplementation on serum liver lipids in the hamster.

Squalene supplementation in the diet also produced significant changes in the liver lipid profiles. Squalene supplementation in the diet resulted in significant increase in liver total lipids (TL) as compared to the control group (Figure 4). The increase in liver TL in the squalene-supplemented group was due to significant increase in liver cholesterol content which was attributed to significant increase in cholesteryl esters; the free cholesterol content was not affected. There was a slight increase in liver TG content of squalene-supplemented group as compared to the control (Figure 5). The other liver lipids, namely DAG, MAG, and FFA, were not much affected by squalene or tocotrienol or tocopherol supplementation.

Figure 4. Effect of squalene, tocopherol, and tocotrienol supplementation on liver total lipids in the hamster.

Tocotrienols isolated from barley was shown to inhibit HMG CoA reductase activity in vitro. More recently Khor et al. reported that tocotrienols isolated from palm oil fatty acid distillate inhibited HMG CoA reductase activity in the guinea pig liver, whereas tocopherols were not effective in lowering cholesterol levels in the guinea pig. However, tocopherols were not effective in lowering cholesterol levels in the guinea pig. In the present study adding 0.1% squalene to the diet significantly lowered serum total cholesterol levels in the hamster. In our present study adding 0.1% squalene to the diet significantly lowered serum total cholesterol levels in the hamster. The differences in the above observation could be due to differences in the animal models and the level of squalene supplementation. In our study we lowered the serum cholesterol levels was associated with a significant increase in liver cholesterol esters (Figure 5). It appears that squalene may enhance the cholesterol esterase activity in the liver resulting in accumulation of cholesterol esters in the tissue. Further experiments are in progress to confirm this assumption.

Addition of tocotrienols to squalene-supplemented diet resulted in further reduction of serum cholesterol levels (Figure 3). This result confirms earlier observation that tocotrienols possessed hypocholesterolemic effect in the hamster. Unexpectedly addition of tocopherols to squalene supplemented also resulted in further reduction in serum cholesterol levels in the hamster (Figure 3).

Acknowledgement: This project was supported by research grants from the Palm Oil Research Institute of Malaysia and the University of Malaya. The authors wish to thank the palm oil industry for providing tocopherol and tocotrienol concentrates. The authors also wish to thank the Department of Microbiology, University of Malaya, for their assistance in the biochemical analysis of the blood samples.
Lipidemic effects of tocotrienols, tocopherols and squalene

Squalene supplementation in the diet also produced significant changes in the liver lipid profiles. Squalene supplementation in the liver resulted in significant increase in liver total lipids (TL) as compared to the control group (Figure 4). The increase in liver TL in the squalene-supplemented group was due to significant increase in liver cholesterol content which was attributed to squalene supplementation. The free cholesterol content was unaffected. There was a slight increase in liver TG content of squalene-supplemented group as compared to the control (Figure 5). The other liver lipids, namely DAG, MAG, and FFA, were not much affected by squalene or tocotrienol or tocopherol supplementation.

Figure 5. Effect of tocotrienol, tocopherol and tocotrienol supplementation on serum cholesterol levels in the hamster.

Tocotrienols isolated from barley was shown to inhibit HMG CoA reductase activity in hamsters. More recently, Khor et al. reported that tocotrienols isolated from palm oil fatty acid distillate inhibited HMG CoA reductase activity in hamsters. In the present study, tocotrienols supplemented in the diet resulted in significant decrease in serum cholesterol levels (Figure 3) which were not observed with tocopherol supplementation. The decrease in serum cholesterol levels was associated with significant decrease in liver cholesterol content (Figure 4) and the hamsters fed on semipurified diet containing vitamin E-stripped corn oil significantly increased serum total cholesterol and HDL-C levels in male Sprague-Dawley rats. Our results suggest that tocotrienols may have hypolipidemic action in the hamster model. The differences in the above observation could be due to differences in the animal models and the level of squalene supplementation. In our study the lowering of serum cholesterol levels was associated with a significant increase in liver cholesterol esters (Figure 5). It appears that tocotrienols may activate cholesterol esterase activity in the liver resulting in accumulation of cholesterol esters in the tissue. Further experiments are in progress to confirm this assumption. Additional tocotrienol supplementation diet resulted in further reduction of serum cholesterol levels (Figure 3). This result confirms earlier observation that tocotrienols possessed hypocholesteremic effect in the hamster.

Figure 6. Effect of tocotrienol, tocopherol and tocotrienol supplementation on serum total lipids in the hamster.

Discussion
The antioxidant effect of vitamin E, both tocotrienols (only in vitro) and tocopherol, in biological systems is well established but the cholesterol-lowering effect of these vitamin E is still controversial. Previous studies in humans showed that tocotrienol supplementation had no effect on serum cholesterol level or actually showed a slight lowering effect on serum cholesterol in some individuals. In animal models, Khor and Chiang reported that tocotrienol supplementation had no effect on serum cholesterol level in male guinea pigs with tocopherol supplementation in the diet, or in male Sprague-Dawley rats whereas Huang et al. reported that adding 1% of squalene to the diet significantly increased serum cholesterol levels in the rat. In our present study adding 0.1% squalene in the diet significantly lowered serum total cholesterol level in the hamster (Figure 3). The differences in the above observation could be due to differences in the animal models and the level of squalene supplementation. In our study the lowering of serum cholesterol levels was associated with a significant increase in liver cholesterol esters (Figure 5). It appears that tocotrienols may increase cholesterol esterase activity in the liver resulting in accumulation of cholesterol esters in the tissue. Further experiments are in progress to confirm this assumption.

In conclusion, our results show that tocotrienol possesses hypolipidemic effect while tocopherol may have a hypothermic effect in the hamster. Squalene at 0.1% supplementation shows hypolipidemic effect and tocotrienols and tocopherol may enhance the hypolipidemic action of squalene. The hypolipidemic action of squalene may be mediated by an accumulation of cholesterol esters in the liver.

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Lipidemic effects of tocotrienols, tocopherols and squalene: studies in the hamster

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生育三烯酚, 生育酚和薰烯的催脂血作用

摘要

去筋穆粥样硬化和心心血管疾病的研究, 增长的脂肪组织的反应存在着许多与人类相似的。从油性精制的含生三烯酚碎片 (TRF) 的催脂血作用在人类实验中仍未得到一致的效果。因此研究用为缺血非氧化物的精制油三烯酚甘油酯碎片 (POTE) 作著脂血吸食者。我们第一个食验结果显示, 加入162ppm生育三烯酚POTE (POTE - T3L) 膜中, 与POTE膜组比较可明显降低血脂总固醇 (Tc) (P<0.05); 但POTE - T3L膜的血清LDL - c, HDL - c和TG水平下降没有明显下降 (P>0.05), 增加补充生育三烯酚于膳食中 (POTE - T3H) 與原来低添三烯酚POTE (POTE - T3L) 比较似可增加血脂Tc, LDL - c和HDL - c水平。这种し的 作用是有趣的, 但未得到较好的解释。当生育酚加至72ppm于POTE膜中, 可观察到血清 Tc, LDL - c和HDL - c水平与原来POTE膜组比较均有程度的增加。这些结果指出了生育三烯酚和生育酚在脂质体内外也许有促进血液脂肪酸的作用, 情況下用生育酚和生育酚的催脂血作用的进一步实验是必要的。我們第二個食验结果顯示, 加入0.1%薰烯于膳食, 与POTE膜组比较, 可明显降低血脂Tc (P<0.05)。薰烯的加入似可降低LDL - c, HDL - c和Tc水平。但是POTE - SO组与POTE组比较没有统计学上的显著性 (P>0.05)。當加入生育三烯酚和生育酚于含有薰烯的POTE膜中, 与POTE膜组和POTE - SO组比较, 可进一步降低Tc和LDL - c水平 (P<0.01), 但HDL - c和Tc水平不受影响。这些结果指出了生育三烯酚和薰烯的催脂血作用。生育酚也已增高脂酸的作用。
Palm oil tocotrienols and plant flavonoids act synergistically with each other and with Tamoxifen in inhibiting proliferation and growth of estrogen receptor-negative MDA-MB-435 and -positive MCF-7 human breast cancer cells in culture

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Palm oil, unlike many other dietary oils, does not increase the yield of chemically-induced mammary tumors in rats fed at high levels in the diet. This difference appears to be due to the presence of tocotrienol fraction in the palm oil rich in tocotrienol, since palm oil stripped of this fraction does increase tumor yields. Experiments in our laboratory have shown that tocotrienols inhibit proliferation and growth of both MDA-MB-435 and MCF-7 cells in culture much more effectively than tocopherol. In addition, it was found that combinations of tocotrienols with Tamoxifen, a drug widely used for treatment of breast cancer, inhibit these cells more effectively than either tocotrienols or Tamoxifen alone. However, the mechanisms by which these effects occur have not been fully elucidated. In this study, we have now shown synergistic effects between tocotrienols and a number of other flavonoids from various plant sources, including citrus fruits, in the inhibition of both MDA-MB-435 and MCF-7 cells (IC50: 0.03-25 and 0.02-5 mg/ml, respectively). In the MCF-7 cells, 1:1 combinations of tocotrienols, flavonoids and Tamoxifen were even more effective, with the best combination being b-tocotrienol, baertepin and Tamoxifen (ICso. 0.005 µg/ml). These results suggest that diets containing palm oil may reduce the risk of breast cancer, particularly when eaten with other plant foods containing flavonoids, and may also enhance the effectiveness of Tamoxifen for treatment of breast cancer.

Introduction

Previous studies have shown that diets containing a high level of palm oil not only reduce the risk of breast cancer but also other chronic diseases such as cardiovascular disease, cancer and osteoporosis in rats.4,5 Evidence that this inhibition is related to the vitamin E fraction of palm oil, consisting mainly of tocotrienols, was provided by Neuhäusler et al.2 They showed that rats treated with the mammary carcinoma, 7,12-dimethylbenz(a)anthracene (DMBA), and fed vitamin E free palm oil developed more tumors than those fed palm oil containing vitamin E. Tocotrienols also caused a delay in the onset of subcutaneous lymphoma in B6F1 mice by 2-4 weeks and the life span of mice inoculated with transplanted tumor cells was increased by tocotrienols.5 Flavonoids are polyphenolic compounds that occur ubiquitously in plant foods and are important constituents of the human diet.6-10 They have also been investigated for their anticancer properties.11,12 Genistein, an isoflavone found in soybean, has also been extensively studied as a possible anti-cancer agent.12-14 Quercetin, another flavonoid found in fruits and vegetables, has also been investigated for its anticancer activity. It has been shown to have growth inhibitory activity in vitro in human breast cancer cells and to reduce the incidence of chemically-induced mammary tumors in rats.15-18 Previous studies in our laboratory have shown that both tocotrienols19 and citrus flavonoids20 are effective inhibitors of human breast cancer cell growth. We have also shown that rats treated with the mammary carcinoma DMBA and given quercetin by juice, developed fewer tumors than controls21 which may be due to the flavonoid, hesperitin, in orange juice.

A number of epidemiological studies have been concerned with relationships between diet and cancer and have provided evidence that consumption of fruits and vegetables protects against various types of cancer.22-24 Although this protective effect has generally been attributed to the antioxidant capacities of vitamin C and E-carotenoids in these foods, it may also be related to other constituents of vegetables and fruits, such as the flavonoids.

In our investigations, a non-steroidal estrogen antagonist, has been extensively used in the treatment of hormone-responsive breast cancer.25 It acts mainly by blocking the stimulatory action of estrogens in hormone-responsive breast cancer cells.26 Most breast cancers consist of hormone-independent as well as dependent cells27 and tumors invariably develop resistance to tamoxifen.28,29 We became interested in tocotrienols as a result of the observation that palm oil stripped of its vitamin E fraction promoted the growth of breast cancer cells in vivo as effectively as other fats30 and in flavonoids because of our observation that naringenin, a flavonoid in grapefruit, is a more effective inhibitor of proliferation and growth of human breast cancer cells in vitro than genistein.29 Since combinations of drugs are often more effective than single drugs in chemotherapy,28 we tested 1:1 combinations of tocotrienols, flavonoids and Tamoxifen to determine whether this is the case.31-32 Our results suggest that tocotrienol, flavonoid and Tamoxifen can act synergistically in the treatment of breast cancer and that such combinations may be effective even in the presence of tamoxifen resistance.