

## ANTIHYPERLIPIDEMIC EFFECT OF *DL*-ALPHA-TOCOPHEROL ACETATE IN RATS FED A HIGH-CHOLESTEROL DIET

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### ABSTRACT

*DL- $\alpha$ -tocopherol acetate (TA)* is a synthetic form of vitamin E, consisting of a mixture of eight diastereomers. The aim of the current study was to assess the influence of TA on plasma total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and triglycerides (TG) in rats fed a cholesterol-free standard diet (SD) and a 1% cholesterol-containing diet (ChD). TA was applied intraperitoneally for 30 days at doses of 25, 50 and 100 mg/kg. TA did not significantly influence plasma lipids of rats fed the SD. In rats fed the ChD the three doses of TA significantly prevented the dietary-induced increase of plasma TC and LDL-C. The HDL-cholesterol levels were not significantly influenced either by the ChD, or by the application of TA. The ChD induced a certain elevation of plasma TG while TA reduced their levels, the effect being significant at the highest TA dose. In conclusion, TA showed an antihyperlipidemic effect in rats with experimentally induced hyperlipidemia and could be valuable in reducing lipemia as a factor of cardiovascular risk.

**Keywords:** *dl- $\alpha$ -tocopherol acetate, high-cholesterol diet, plasma lipids, rats*

### INTRODUCTION

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defense system. Natural vitamin E is a mixture of *d*-tocopherols and *d*-tocotrienols (**6**-, **8**-, **11**-, **14**-tocopherol, and **6**-, **8**-, **11**-, **14**-tocotrienol), synthesized only by plants. The biological effectiveness *in vivo* of the forms of vitamin E has been established as **6** > **8** > **11** > **14** (2). In animal models of diet-induced hypercholesterolemia, where the animals are not deficient in vitamin E, **6**-tocopherol supplementation often decreases plasma cholesterol (9,13,18). This is not always the case however; in some studies either no change (7,12,16) or even an increase (14) in plasma cholesterol was observed. There is also a widely available synthetic form, *dl- $\alpha$ -tocopherol* (all-rac-**6**-tocopherol), prepared by coupling trimethylhydroquinone with isophytol. This consists of a mixture of eight diastereomers (RRS-SSR, RRR-SSS, RSR-SRS, RSS-SRR) in approximately equal proportions (17).

The aim of this study was to assess in rats fed a cholesterol-free and a 1% cholesterol-containing diet the influence of *dl- $\alpha$ -tocopherol acetate (TA)* on plasma total cho-

lesterol (TC), LDL-cholesterol (LDL-C), HDL cholesterol (HDL-C) and triglycerides (TG).

### MATERIALS AND METHODS

*Animals and Diets.* Male Wistar rats ( $n = 56$ ) with a mean weight of  $220 \pm 20$ g at the beginning of the experiment were used. The animals were housed in plastic cages in a well ventilated room maintained at 23-25°C and on a 12/12 light/dark cycle (light 7:00-19:00). They had unrestricted access to food and drinking water.

The rats were fed either a standard diet for laboratory animals or a 1% cholesterol-containing diet. The standard diet (SD) was prepared from wheat (30%), oats (35%), maize (15%), ground fish (6%), milk powder (6%), blood powder (2%), egg powder (1%), vitaminized cod-liver oil (1.5%), yeast powder (1.5%), chalk (1%), salt (0.5%), gelatine (0.5%). It contained by weight: 61.56% carbohydrates, 20% proteins, 3.8% fats, 0.88% lysine, 0.72% methionin/cystin, 0.82% calcium, 0.63% phosphorus, 0.39% chlorides, 11.2% moisture. The energetic value of the diet was 294.2 kcal/100 g.

The 1% cholesterol-containing diet (ChD) was prepared as 1 g cholesterol was added to 99 g ground SD. Distilled water was added to obtain a substance of the consistency of thick dough. This dough was hand-rolled into 2-3 cm long forms of roughly 1 cm diameter, and dried at room temperature.

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All procedures concerning animal treatment and experimentation were in accordance with the Guiding Principles in the Care and Use of Animals, approved by the Council of the American Physiological Society, with European Communities Council Directives 86/609/EEC.

**Chemicals.** Cholesterol was from Sigma Chemie (Germany) and *dl*-**6**-tocopherol acetate (TA) was from Merck (Germany).

**Experimental Procedure.** The experimental animals were randomly divided into eight groups of 7 rats. During the 30 days of the experiment the rats were treated intraperitoneally either with sterile sunflower oil (SO) in a volume of 2 ml/kg or with TA as a solution in SO in a vol-

**Statistical Analysis.** Results are presented as mean  $\pm$ SEM. The data were tested by one-way ANOVA, followed by Dunnett's multiple comparison post test. Two independent groups were compared by Student's *t*-test. All analyses were performed using GraphPad Prism statistical software. A level of  $p < 0.05$  was considered significant.

## RESULTS AND DISCUSSION

Plasma TC, LDL-C, HDL-C and TG of rats fed the SD and treated with TA did not differ significantly from those of the control group (Table 1).

Table 1. Plasma levels of total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and triglycerides (TG) in rats fed a standard diet (SD) or a 1% cholesterol-containing diet (ChD) treated with sunflower oil (SO) or with *dl*-**6**-tocopherol acetate (TA) at doses of 25, 50 and 100 mg/kg

Animal group	TC (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)
Control (SD/SO)	1.62 $\pm$ 0.12	0.38 $\pm$ 0.05	0.94 $\pm$ 0.10	0.66 $\pm$ 0.06
SD/TA25	1.41 $\pm$ 0.10	0.32 $\pm$ 0.04	0.87 $\pm$ 0.06	0.54 $\pm$ 0.08
SD/TA50	1.29 $\pm$ 0.09	0.27 $\pm$ 0.04	0.77 $\pm$ 0.09	0.47 $\pm$ 0.10
SD/TA100	1.37 $\pm$ 0.07	0.25 $\pm$ 0.04	0.90 $\pm$ 0.14	0.48 $\pm$ 0.07
ChD/SO	2.01 $\pm$ 0.10 <sup>#</sup>	0.81 $\pm$ 0.10 <sup>##</sup>	0.82 $\pm$ 0.16	0.81 $\pm$ 0.07
ChD/TA25	1.57 $\pm$ 0.12 <sup>*</sup>	0.54 $\pm$ 0.04 <sup>*</sup>	0.80 $\pm$ 0.13	0.86 $\pm$ 0.09
ChD/TA50	1.52 $\pm$ 0.12 <sup>*</sup>	0.41 $\pm$ 0.07 <sup>**</sup>	0.71 $\pm$ 0.09	0.62 $\pm$ 0.05
ChD/TA100	1.55 $\pm$ 0.15 <sup>*</sup>	0.52 $\pm$ 0.08 <sup>*</sup>	0.79 $\pm$ 0.13	0.52 $\pm$ 0.05 <sup>*</sup>

<sup>#</sup> $p < 0.05$ , <sup>##</sup> $p < 0.01$  vs Control (SD/SO); <sup>\*</sup> $p < 0.05$ , <sup>\*\*</sup> $p < 0.01$  vs ChD/SO

ume of 2 ml/kg. The animals of the control (SD/SO) group were fed the SD and received SO. The rats from animal groups named SD/TA<sub>25</sub>, SD/TA<sub>50</sub> and SD/TA<sub>100</sub> were fed the SD and were treated with TA at doses of 25, 50 or 100 mg/kg. The rats from groups named ChD/SO, ChD/TA<sub>25</sub>, ChD/TA<sub>50</sub> and ChD/TA<sub>100</sub> were fed the ChD and were treated respectively with SO and with TA at doses of 25, 50 or 100 mg/kg.

**Biochemical Assays.** After 30 days of feeding, the rats were fasted overnight. The next day the animals were anaesthetized with diethylether and blood was collected from the sublingual veins. It was centrifuged at 2000 x g for 10 min and plasma was obtained. The plasma TC, HDL-C and TG (mmol/l) were measured with an automatic analyzer (RA 1000 - Technicon - USA) using the standard test kits of Pointe Scientific Inc. (USA). TC was measured by the CHOD-PAP method, HDL-C – by the direct method without precipitation, TG – by the GPO-PAP method. LDL-C was calculated using the Friedwald formula (4):  

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{VLDL-C}; \text{VLDL-C was calculated as } 0.456 \times \text{TG concentration} .$$

There are data that feeding of rats for 30 days with a 1% cholesterol-containing diet is a suitable experimental model of hyperlipidemia (8): plasma TC and TG are increased and remain elevated after 18-21 days of feeding. The ChD in our experiment induced a significant elevation of plasma TC ( $p < 0.05$  vs control), of LDL-C ( $p < 0.01$  vs control) and did not induce significant changes in the concentrations of plasma HDL-C and TG (Table 1).

The three doses of TA applied to rats fed the ChD significantly hindered the elevation of plasma TC and LDL-C and their levels did not differ from the control values (Table 1). The application of TA to rats fed the ChD did not induce significant changes in the concentrations of plasma HDL-C in comparison either with the control level or with that of the ChD/SO group (Table 1). In rats fed the ChD the two doses of TA (50 mg/kg and 100 mg/kg) reduced plasma TG, the effect being significant ( $p < 0.05$  vs ChD/SO) at the dose of 100 mg/kg (Table 1).

The effect of TA on plasma lipids (TC, LDL-C, HDL-C and TG) in our experiment is similar to the effects of **6**-tocopherol acetate (15) and of vitamin E (6) in rats fed high-cholesterol diets.

The cholesterol-lowering effect of TA might be due to its effect of the LDL receptor, a cell surface protein which plays an important role in controlling blood cholesterol. Pal et al. (10) demonstrated a specific, concentration-dependent biphasic "up then down" effect of  $\alpha$ -tocopherol on the LDL receptor of highly differentiated hepatocytes (HepG2 cells). The biphasic nature of the LDL receptor response explains why in animals made hypercholesterolaemic by diet,  $\alpha$ -tocopherol supplementation can result in a decrease (9), an increase (14) or no change (7,16) in plasma cholesterol. The decrease occurs only in animals whose vitamin E level prior to supplementation is lower than optimal. If we suppose that the effect of the synthetic TA is similar to that of  $\alpha$ -tocopherol, the administration TA in our experiment might have resulted in an increase of LDL receptor activity, most prominent at the dose of 50 mg/kg.

Vitamin E influences the activity of the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase which is the rate-limiting enzyme in cholesterol synthesis. Choi et al. (3) and Park et al. (11) demonstrated in rats that this enzyme activity was significantly lowered with an increase in the dietary vitamin E. This might also be the effect of the synthetic TA.

TA might also influence other factors of cardiovascular risk. Oxidized LDL-cholesterol has been proposed as an atherogenic factor in heart disease, promoting cholesterol ester accumulation and foam cell formation (1). The natural vitamin E and to a smaller extent the synthetic *dl*- $\alpha$ -tocopherol are attached directly to the LDL lipoproteins and prevent their oxidative modification and even restore the oxidized cholesterol (5).

## CONCLUSION

Our study has shown that TA prevents the elevation of plasma lipid levels in rats fed a high-cholesterol diet. TA might have a beneficial effect in reducing the hyperlipidemia which is a risk factor for atherosclerosis.

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