Anti-Cancer Drugs 1995, **6**, pp. 771–774

α-Tocopherol ameliorates cyclophosphamide-induced hyperlipidemia in fibrosarcoma-bearing rats

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Cyclophosphamide, an alkylating agent, is currently being used for the treatment of various types of cancer, either alone or in combination with other cytostatic drugs. However, cyclophosphamide has a detrimental effect on lipid metabolism and causes hyperlipidemia in patients. Since α-tocopherol is known to reduce hyperlipidemia, we have investigated the effects of adding α-tocopherol to the cyclophosphamide treatment. Our study, carried out on fibrosarcoma-bearing rats, shows that α-tocopherol markedly reduces cyclophosphamide-induced hyperlipidemia and brings lipid metabolism down to values observed in untreated controls.

Key words: α-Tocopherol, cyclophosphamide, fibrosarcoma, lipids.

Introduction

Peroxidation of unsaturated membrane lipids by free radicals is known to contribute to the toxic side effects observed following administration of cytotoxic drugs.[1–5] Cyclophosphamide-induced lipid peroxidation has been well documented.[6] There are numerous reports indicating a direct relationship between lipid peroxidation and subsequent alteration in lipid metabolism. High doses of cyclophosphamide inhibit lipoprotein lipase activity,[7] thereby retarding plasma triglyceride hydrolysis. The administration of cyclophosphamide to rabbits has been reported to enhance the total cholesterol level with accumulation of very low density lipoprotein (VLDL) cholesterol.[8]

VLDL and low density lipoprotein (LDL), when oxidized, become toxic to cells.[9,10] Antioxidants such as vitamin E, a lipid-soluble free radical scavenger, inhibit both the oxidation and formation of cytotoxic LDL due to their free radical quenching activity.[11] That vitamin E regulates lipid metabolism has been well documented. Vitamin E is not reported to have any side effect when administered in therapeutic doses.[12]

One of the important objectives of chemotherapy in cancer treatment is to minimize the side effects of neoplastic agents. Since vitamin E is an antioxidant and a scavenger of free radicals, we have investigated whether it can be used to limit lipid abnormalities in fibrosarcoma-bearing rats treated with cyclophosphamide.

Materials and methods

Fibrosarcoma

Adult male albino rats derived from the Wistar strain, weighing 100–120 g, were used in the study. The animals were maintained on standard pellet diet and water provided *ad libitum*. Fibrosarcomas were induced in the rats with 20-methylcholanthrene.[13] The fibrosarcoma was maintained in Wistar rats by transplanting 0.5 ml of 10% tumor cell suspension in saline in the axillary region through a puncture in the inguinal region.

Treatment protocol

The rats were divided into five groups of six animals each. All animals were given food and water *ad libitum*. Group I (control) was not injected with tumor cells and was untreated. Groups II–V were given fibrosarcoma cells. Group II received no further treatment. Group III received cyclophosphamide (20 mg/kg body weight) orally in 6 ml of sunflower oil for 28 days using a stomach tube from the day of tumor transplantation. Group IV animals received α-tocopherol (400 mg/kg body weight) orally in 4 ml of sunflower oil for 28 days using a stomach tube from the day of tumor transplantation. Group V rats were given cyclophosphamide

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(20 mg/kg body weight) and α-tocopherol (400 mg/kg body weight) in sunflower oil for 28 days, orally, from the day of transplantation.

At the end of the experimental period, the animals were sacrificed by cervical dislocation, blood samples were collected, and the liver and kidney were excised. Lipid extract of kidney and liver, prepared by the method of Folch,[14] was used for the analysis.

Serum and lipid extracts of the tissues of control and experimental animals were analyzed for lipid content. Total cholesterol was estimated by the method of Parekh and Jung,[15] and free cholesterol was estimated by the method of Leffler and McDougald.[16] Triglycerides were estimated by the method of Rice, based on the method of Van Handel.[17] Free fatty acid content was estimated by the method of Horn and Menahan,[18] and phospholipid was estimated by the method of Rouser.[19]

Results

The levels of total cholesterol, free cholesterol, ester cholesterol, triglycerides, free fatty acids and phospholipids in plasma, liver and kidney of Groups I–V are depicted in Tables 1–3.

Levels of lipid components (total cholesterol, free cholesterol, ester cholesterol, triglycerides, free fatty acids and phospholipids) were increased significantly ($p < 0.001$) in Group II when compared with Group I. By contrast, ester cholesterol was not increased. A further increase in the lipid components were observed in cyclophosphamide-treated rats. By contrast, values for lipid components were decreased significantly in Group IV animals (α-tocopherol treated). In Group V animals, which were given the combination therapy (cyclophosphamide + α-tocopherol) the lipid component levels were diminished significantly ($p < 0.001$) compared with Group III animals (cyclophosphamide treated) and were nearly similar to the values (normal levels) of Group I controls. Conversely, the level of ester cholesterol was found to be decreased in Groups II and III when compared with controls. By contrast, Group IV animals showed increased ester cholesterol levels and in Group V the esterchолesterol was increased to near normal values.

Discussion

Cyclophosphamide induces significant increases in the levels of total and free cholesterol, triglycerides and free fatty acids in our rat fibrosarcoma model. Similar to our results, Radcliffe[20] has reported increased levels of total cholesterol, triglycerides and free fatty acids in serum of rats bearing methylcholanthrene-induced sarcoma.

The increase in total cholesterol observed in Group II rats may be the reason for the decrease in the rate of cholesterol esterification. The enzyme lecithin, cholesterol acyl transferase (LCAT) is responsible for the esterification of cholesterol. Speigel *et al.*[21] have reported a decrease in LCAT activity in plasma of leukemia and lymphoma patients. Due to the decrease in LCAT activity, the free cholesterol is not esterified to ester cholesterol. This may explain why the free cholesterol level is increased with a parallel decrease in ester cholesterol in our Group II fibrosarcoma rats. In addition, due to the increased catabolism of lipids, the ester cholesterol is hydrolyzed into free cholesterol, thereby causing an increase in free cholesterol and decrease in ester cholesterol levels.

The alterations in lipoprotein transport and metabolism play an important role in the context of

Table 1. Levels of lipid components in the plasma of control and experimental groups at the end of experimental period (mean ± SD)

Parameters (mg/dl)	Group I	Group II	Group III	Group IV	Group V
Total cholesterol	93.12 ± 7.08	121.46 ± 9.03a**	132.20 ± 8.25b*	103.42 ± 7.51	96.09 ± 7.16d**, e**
Free cholesterol	33.19 ± 2.23	72.39 ± 4.48a**	87.47 ± 6.38b**	47.31 ± 4.14c**	36.22 ± 2.92d**, e**
Ester cholesterol	59.93 ± 4.42	49.07 ± 3.64a**	44.73 ± 3.29b*	56.11 ± 3.06	59.8 ± 3.78d**, e**
Triglycerides	43.51 ± 3.83	58.72 ± 4.65a**	66.14 ± 5.14b*	49.56 ± 4.03	45.34 ± 4.02d**, e**
Free fatty acids	5.87 ± 1.14	13.19 ± 2.34a**	17.38 ± 2.70b*	9.40 ± 1.24	6.55 ± 1.68d**, e**
Phospholipids	115.72 ± 12.31	161.86 ± 16.28a**	176.07 ± 19.37	137.62 ± 15.81c*	121.81 ± 13.14d**, e**

Group I, untreated and tumor-free controls; Group II, untreated tumor-bearing rats; Group III, cyclophosphamide-treated tumor-bearing rats; Group IV, α-tocopherol-treated tumor-bearing rats; Group V, α-tocopherol + cyclophosphamide-treated tumor-bearing rats.
For statistical evaluation of significant variations: (a) Group II compared with Group I; (b) Group III compared with Group II; (c) Group IV compared with Group II; (d) Group V compared with Group II; (e) Group V compared with Group III. Statistical alterations are expressed as: * $p < 0.05$; ** $p < 0.001$.

Table 2. Levels of lipid components in the liver of control and experimental groups at the end of experimental period (mean ± SD)

Parameters (mg/g tissue)	Group I	Group II	Group III	Group IV	Group V
Total cholesterol	7.52 ± 0.92	12.42 ± 1.18a**	14.70 ± 1.46b*	9.83 ± 1.04	8.31 ± 1.11d**, e**
Free cholesterol	3.05 ± 0.28	8.81 ± 0.64a**	11.22 ± 0.75b**	5.86 ± 0.43c**	4.12 ± 0.36d**, e**
Ester cholesterol	4.46 ± 0.29	3.61 ± 0.26a**	3.48 ± 0.24	3.97 ± 0.30c**	4.19 ± 0.32d**, e**
Triglycerides	15.37 ± 1.87	21.84 ± 2.09a**	24.06 ± 2.24	18.32 ± 1.75c**	16.52 ± 1.90e**
Free fatty acids	2.04 ± 0.28	4.31 ± 0.37a**	4.90 ± 0.42b*	3.07 ± 0.31c**	2.38 ± 0.27d**, e**
Phospholipids	14.73 ± 2.91	26.83 ± 4.06a**	31.49 ± 4.65	20.89 ± 3.62c*	16.37 ± 3.07d**, e**

See Table 1 for legend.

Table 3. Levels of lipid components in the kidney of control and experimental groups at the end of experimental period (mean ± SD)

Parameters (mg/g tissue)	Group I	Group II	Group III	Group IV	Group V
Total cholesterol	5.82 ± 0.41	9.74 ± 1.28a**	12.09 ± 1.42b**	7.34 ± 1.17	6.37 ± 0.93d**, e**
Free cholesterol	3.08 ± 0.50	7.68 ± 0.82a**	10.22 ± 1.14b**	4.96 ± 0.72	3.84 ± 0.55d**, e**
Ester cholesterol	2.74 ± 0.32	2.06 ± 0.23a**	1.87 ± 0.20	2.38 ± 0.25c*	2.53 ± 0.29d*, e**
Triglycerides	10.83 ± 1.35	15.90 ± 1.62a**	18.01 ± 1.86	13.68 ± 1.71c*	11.26 ± 1.45**, e**
Free fatty acids	1.57 ± 0.29	2.83 ± 0.34a**	3.22 ± 0.28b*	2.36 ± 0.33	1.80 ± 0.27d**, e**
Phospholipids	11.84 ± 1.54	20.34 ± 2.08a**	24.52 ± 2.44	16.18 ± 1.87	13.11 ± 1.72d**, e**

See Table 1 for legend.

changes in plasma as well as tissue lipids. The increased levels of triglycerides observed in our Group II animals may be due to the increased synthesis of VLDL cholesterol. Alternatively, the increase in triglycerides and free fatty acids may also be due to decrease in lipoprotein lipase (LPL) activity. Nestel *et al.*[22] have reported a decrease in LPL activity in tumor-bearing animals; this inhibits the hydrolysis of chylomicrons (that yield triglycerides to adipose tissue), and thus provokes the increase in triglyceride and free fatty acids. The increase in phospholipids in Group II animals is due to the increased mobilization of lipids from lipid stores.

In cyclophosphamide administered rats (Group III), a more pronounced elevation was observed in these lipid components. Cyclophosphamide-induced hyperlipidemia has been well documented.[23] Loudet and coworkers[8] have reported that cyclophosphamide administration inhibits LCAT and LPL activity in rabbits. This may be the reason for a further increase in total cholesterol, free cholesterol and triglycerides observed in cyclophosphamide-treated animals (Group III). Loudet *et al.*[8] have also observed that cyclophosphamide administration shows an accumulation of VLDL cholesterol with a decrease in HDL cholesterol.

The lipid components were found to be reverted to near normal in Group IV α-tocopherol administered animals. Vitamin E supplementation has been shown to reduce the number of chemically induced tumors in animals.[24,25] Excess rates of lipid peroxidation may be at the root of the hyperlipidemia found in many cancer patients.[26] Vitamin E is a well accepted first line defense mechanism against lipid peroxidation. Komaratat *et al.*[27] have observed a decrease in total cholesterol with dose-response rate of vitamin E in control rabbits.

The animals which were given a combination therapy of cyclophosphamide and vitamin E showed an ameliorating response—lipid values being almost reverted to the near normal observed in Group I controls. Vitamin E has been proposed as an anticarcinogen because it suppresses the metabolism of lipids, causing a lower rate of turnover.

In conclusion, therefore, our results suggest that the addition of α-tocopherol reverts the abnormally increased levels of lipids due to cyclophosphamide in fibrosarcoma animals to normal levels. These results suggest that cyclophosphamide treatment is more effective when administered together with α-tocopherol, because the latter counteracts the cyclophosphamide-induced hyperlipidemia.

References

1. Plaa GL, Witschi H. Chemicals, drugs and lipid peroxidation. *Annu Rev Pharmacol Toxicol* 1976; **16**: 125–41.
2. Bus JS, Gibson JE. Lipid peroxidation and its role in toxicology. *Rev Biochem Toxicol* 1976; **1**: 125–49.
3. Cummings J, Anderson L, Willmott N, *et al.* The molecular pharmacology and doxorubicin *in vivo*. *Eur J Cancer* 1991; **27**: 532–5.
4. Patel JM. Metabolism and pulmonary toxicity of cyclophosphamide. *Pharmacol Ther* 1990; **47**: 137–46.
5. Tritton TR. Cell surface action of adriamycin. *Pharmacol Ther* 1990; **49**: 293–9.
6. Berrigan MJ, Struck RF, Gurtoo HL. Lipid peroxidation induced by cyclophosphamide. *Cancer Biochem Biophys* 1987; **9**: 265–70.
7. Lespine A, Azema C, Gavels M, *et al.* Lipoprotein lipase regulation in cyclophosphamide treated rabbit: dependence on nutritional status. *J Lipid Res* 1993; **34**: 23–36.
8. Loudet AM, Dousset N, Carton M, *et al.* Effects of an antimitotic agent (cyclophosphamide) on lipoproteins. *Biochem Pharmacol* 1984; **33**: 2961–5.
9. Steinbrecher UP, Zhang H, Lougheed M. Role of oxidatively modified LDL in atheroslcerosis. *Free Radical Biol Med* 1990; **9**: 155–68.
10. Quehenberger O, Koller E, Jurgens G, *et al.* Investigation of lipid peroxidation in human low density lipoproteins. *Free Radical Res Commun* 1987; **3**: 233–42.
11. Esterbauer H, Dieber-Rothernderm M, Striegl G, *et al.* Role of vitamin E in preventing the oxidation of low density lipoprotein. *Am J Clin Nutr* 1991; **53** (Suppl 3): 14–21.
12. Murray RK. Cancer, Oncogenes and growth factors. In: Murray RK, Granner DK, Mayes PA, eds. *Harper's biochemistry* 21st edn. Connecticut: Appleton and Lange 1988.
13. Mohana K, Purushothaman KK. Plumbagin: a study of its anticancer, antibacterial and antifungal properties. *Ind J Exp Biol* 1980; **18**: 876–7.
14. Folch J, Lees M, Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; **226**: 497.
15. Parekh AC, Jung DH. Cholesterol determination with ferric acetate–uranyl acetate and sulphuric acid–ferrous sulphate reagents. *Anal Chem* 1970; **42**: 1423–7.
16. Leffler HH, McDougald CH. A colorimetric method for estimation of cholesterol. *Am J Clin Pathol* 1963; **39**: 311–5.
17. Van Handel E. Modification of the micro-determining triglycerides. *Clin Chem* 1961; **7**: 249–51.
18. Horn WT, Menahan LA. A sensitive method for determination of free fatty acids in plasma. *Lipid Res* 1981; **22**: 377–81.
19. Rouser G, Fliesher S, Yamamoto A. Two dimensional thin layer chromatographic separation of polar lipids. Determination of phospholipids by phosphorus analysis of spots. *Lipids* 1970; **5**: 494–6.
20. Radcliffe JD. The effect of methylcholanthrene induced Sarcoma on lipid status of the Fisher rat. *Nutr Rep Int* 1989; **39**: 409–14.
21. Speigel RJ, Schaefer EJ, Hagrath IT, *et al.* Plasma Lipid alterations in leukemia and lymphoma. *J Med* 1982; **72**: 755–82.
22. Nestel PJ, Havel RJ, Benzman A. Relation between incorporation of triglycerides, fatty acids and heparin released lipoprotein lipase from adipose tissue slices. *J Clin Invest* 1963; **42**: 1313.
23. Lespine A, Dousset N, Perret B, *et al.* Accumulation of large VLDL in cyclophosphamide treated rabbits. Relationship with lipoprotein lipase deficiency. *Biochem Biophys Res Commun* 1988; **154**: 635–40.
24. Shklar G. Oral mucosal carcinogenesis in hamsters: inhibition by Vitamin E. *J Natl Cancer Inst* 1982; **68**: 791–7.
25. Cook MG, McNamara P. Effect of vitamin-E on dimethyl hydrazine induced colonic tumors in mice. *Cancer Res* 1980; **40**: 1329–31.
26. Bast A, Haenen GRMM, Doelman CTA. Oxidants and antioxidants. State of the art (Symposium on oxidants and antioxidants). *Am J Med* 1991; **91** (Suppl 3C); 2–22.
27. Komaratat P, Chypuk charoen N, Wilairat P. Effect of vitamin E on cholesterol plasma lipoprotein distribution and metabolism in rabbit. *Int J Vitamin Nutr Res* 2985; **55**: 267–71.

(Received 15 June 1995; received in revised form 10 June 1995; accepted 20 July 1995)