Hypolipidemic Effect of Ethyl all-cis-5,8,11,14,17-Icosapentaenoate (EPA-E) in Rats

Kiyoshi Mizuguchi1, Takashi Yano1, Masahiro Kojima1, Yasuo Tanaka1, Masaaki Ishibashi1, Atsuhiro Masada2, Masami Sato2, Masahiro Mizota1, Katsuhiko Fukutake1 and Yasushi Saito3

1Fuji Central Research Laboratory, Mochida Pharmaceutical Co., Ltd., 722 Jimba-aza-Uenohara, Gotemba, Shizuoka 412, Japan
2Toxicology Laboratory, Mochida Pharmaceutical Co., Ltd., 542 Gensuke, Fujieda, Shizuoka 426, Japan
3The Second Department of Internal Medicine, School of Medicine, Chiba University, 1-8-1 Inohana, Chiba 280, Japan

Received January 10, 1992 Accepted April 16, 1992

ABSTRACT—We examined the effect of ethyl all-cis-5,8,11,14,17-icosapentaenoate (EPA-E) with high purity on circulating lipids in rats under several experimental conditions. In normolipidemic rats, EPA-E decreased the lipids in a dose-dependent manner. Clofibrate (100 mg/kg/day) was more potent in lowering the lipids than EPA-E (1000 mg/kg/day). In high cholesterol diet-fed rats, EPA-E (300 mg/kg/day) decreased the total cholesterol. However, clofibrate (300 mg/kg/day) had little effect on the total cholesterol. In hypertriglycemic rats induced by corn oil, EPA-E (300 mg/kg/day) or clofibrate (100 mg/kg/day) reduced the rise of triglycerides. EPA-E (300 mg/kg/day), clinofibrate (100 mg/kg/day) or clofibrate (300 mg/kg/day) caused a significant reduction in the lipids induced by the injection of Triton WR-1339. Furthermore, EPA-E (300 mg/kg/day) or clinofibrate (100 mg/kg/day) decreased the elevation of lipids produced by feeding the rats a casein-rich diet. These results show that EPA-E possesses potent inhibitory activity on experimental hyperlipidemia induced either exogenously or endogenously.

Keywords: EPA-E (ethyl all-cis-5,8,11,14,17-icosapentaenoate), Hypolipidemic action, Clofibrate

Polyunsaturated fatty acids (PUFA) regulate various biological functions and are involved in a variety of diseases. Since several studies have revealed that 5,8,11,14,17-icosapentaenoic acid (EPA) is an active substance of PUFA (1, 2), considerable effort has been made to clarify the possible pharmacological function(s) of EPA. Administration of PUFA containing EPA has been reported to have a number of actions including suppression of platelet aggregability (3, 4), decrease in blood pressure (5, 6), and reduction in restenosis after coronary angioplasty (7). Furthermore, EPA has been also suggested to have a hypolipidemic effect (8–11). Recently, we have successfully prepared a highly purified ethyl ester derivative of EPA, ethyl all-cis-5,8,11,14,17-icosapentaenoate (EPA-E). In the present study, we designed experiments to determine if EPA-E has a hypolipidemic effect on serum or plasma lipids in rats. The results presented show unequivocally that EPA-E is capable of reducing circulating lipids in the animals not only with exogenously or endogenously induced hyperlipidemia, but also in those with normo-

MATERIALS AND METHODS

Animals and diets

Male Wistar rats (Japan SLC, Inc.) weighing 200–290 g were used. They were all maintained under the following environmental conditions: room temperature, 23 ± 2°C; relative humidity, 55 ± 5%; illumination, 12 hr from 7.00 am to 7.00 pm.

As a standard diet, a laboratory commercial chow pellet not containing fish products (basal chow, F1®; Funabashi Farm) was used. A semisynthetic diet (12) containing 20% casein without cholesterol purchased from Oriental Yeast was used as the casein-rich diet (CRD). F1® supplemented with both 1% cholesterol and 1% cholic acid (Funabashi Farm) was used as the high cholesterol-containing diet (HCD).

Drugs

EPA-E (Lot. 870224, purity: 90.7%; Lot. 870814,
purity: 89.2%; Mochida Pharmaceutical Co., Ltd.), clofibrate (Wako Pure Chemical Industries, Ltd.) and clinofibrate (extracted from LIPOCLIN Tab®, Sumitomo) were emulsified or suspended in 5% gum arabic solution with an ultrasonic cell homogenizer (5202, Ohtake Works Co., Ltd.) and orally administered via a stomach tube.

Experimental procedures

*Normolipidemic rats*: EPA-E at doses of 100–1000 mg/kg or clofibrate at the dose of 100 mg/kg was administered to rats daily for 7 days.

*HCD-induced hypercholesterolemic rats*: Rats were given HCD containing both 1% cholesterol and 1% cholic acid for 4 weeks. EPA-E at doses of 100–1000 mg/kg or clofibrate at a dose of 100 or 300 mg/kg was administered daily during the period of HCD feeding.

*Corn oil-induced hypertriglycemic rats*: The above-described rats given EPA-E or clofibrate for 7 days were employed. To induce hypertriglycemia, an emulsion of 50% (w/w) corn oil (Nacalai Tesque, Ltd.) at a dose of 10 ml/kg was orally administered to the rats.

*Triton WR-1339-induced hyperlipidemic rats*: EPA-E at doses of 100–1000 mg/kg, clinofibrate at 100 mg/kg or clofibrate at 300 mg/kg was administered to rats after an overnight fast. At 3 hr after the drug administration, 300 mg/kg of Triton WR-1339 (Ruger Chemical Co., Inc.) was injected into the tail vein.

*CRD-induced hyperlipidemic rats*: Rats were given CRD without cholesterol for 4 weeks. EPA-E at doses of 100–1000 mg/kg or clinofibrate at the dose of 100 mg/kg was administered daily during the period of CRD feeding.

Measurement of lipids

Serum or plasma was separated from the blood samples by centrifugation (1500 × g for 15 min). Serum lipoproteins were fractionated by density gradient ultracentrifugation (13). Measurement of total cholesterol (TC), triglycerides (TG) and phospholipids (PL) in the serum, plasma or lipoprotein fraction were all carried out by enzymatic methods with commercial kits (Wako Pure Chemical Industries, Ltd.).

Statistical analyses

Results were expressed as the mean ± S.E. Statistical analyses were performed by the Student's t-test. If P < 0.05, a value was considered to be significantly different.

RESULTS

*Normolipidemic rats*

EPA-E significantly lowered plasma TC in rats by 12% at 300 mg/kg and by 18% at 1000 mg/kg (Fig. 1). Clofibrate at 100 mg/kg caused a much greater reduction of TC to 51%. TG was reduced by EPA-E at 1000 mg/kg by 20% and by clofibrate at 100 mg/kg by 23%.

*HCD-induced hypercholesterolemic rats*

Serum TC in rats fed HCD increased to 135 ± 10 mg/dl from the basal level of 58 ± 3 mg/dl in standard-diet-fed animals (Fig. 2). EPA-E at 100, 300 and 1000 mg/kg significantly reduced the serum TC elevation. TG was also lowered by EPA-E in a dose-dependent manner. It is interesting to note that clofibrate at 100 and 300 mg/kg decreased TG but was without effect on TC.

**Fig. 1.** Effects of EPA-E and clofibrate on plasma lipids in normal rats. TC, total cholesterol; TG, triglycerides. Drugs were orally administered for 7 days. The vehicle solution was administered to the control animals in the same way. Blood was obtained from the inferior vena cava about 24 hr after the last administration. Each value represents the mean ± S.E. Significant differences from the control value are indicated: *P < 0.05 and **P < 0.01.
Corn oil-induced hypertriglyceremic rats

Plasma TG in rats after oral administration of corn oil became elevated, with a peak at 4 hr (Fig. 3). Such an increase of TG was significantly suppressed by EPA-E at 300 and 1000 mg/kg and by clofibrate at 100 mg/kg.

Triton WR-1339-induced hyperlipidemic rats

Both serum TC and TG in rats were markedly increased 12 hr after the injection of Triton WR-1339 (Table 1). EPA-E at 300 and 1000 mg/kg, clinofibrate at 100 mg/kg and clofibrate at 300 mg/kg caused significant reductions of both TC and TG.

**Fig. 2.** Effects of EPA-E and clofibrate on serum lipids in rats fed the high cholesterol diet (HCD). TC, total cholesterol; TG, triglycerides. Animals except for the untreated ones were fed HCD containing both 1% cholesterol and 1% cholic acid for 4 weeks. Drugs or the vehicle solution were orally administered during this period. Blood was collected from the abdominal aorta under pentobarbital anesthesia about 24 hr after the final administration. Each value represents the mean ± S.E. Significant differences from the control value are indicated: *$P < 0.05$ and **$P < 0.01$.

**Table 1.** Effects of EPA-E, clofibrate and clinofibrate on serum lipids in rats injected with Triton WR-1339

| Group         | Dose (mg/kg) | N | TC (mg/dl) | TG (mg/dl) |
|---------------|--------------|---|------------|------------|
| Untreated     | —            | 6 | 41 ± 4**   | 95 ± 20**  |
| Control       | —            | 8 | 323 ± 7    | 3108 ± 69  |
| EPA-E 100     | 100          | 8 | 321 ± 8    | 3004 ± 86  |
| EPA-E 300     | 100          | 8 | 299 ± 3**  | 2749 ± 82**|
| EPA-E 1000    | 1000         | 8 | 293 ± 5**  | 2670 ± 118**|
| Clinofibrate  | 100          | 8 | 237 ± 9**  | 2337 ± 88**|
| Clofibrate 300| 300          | 8 | 244 ± 8**  | 1739 ± 54**|

TC, total cholesterol; TG, triglycerides. Animals except for the untreated ones were intravenously injected with 300 mg/kg of Triton WR-1339. After 12 hr, blood was collected from the ophthalmic venous plexus with a glass capillary tube. Drugs or the vehicle solution were orally administered 3 hr before the injection. Each value represents mean ± S.E. Significant differences from the control value are indicated: *$P < 0.05$ and **$P < 0.01$.

**Fig. 3.** Effects of EPA-E and clofibrate on plasma triglycerides (TG) after oral administration of corn oil in rats. Drugs or the vehicle solution were orally administered for 7 days. An emulsified corn oil (50%, w/w) at 10 ml/kg was given orally about 24 hr after the final administration of the drugs. Blood was taken from the inferior vena cava at 2, 4, 6 and 8 hr after the corn oil ingestion. Each point represents the mean ± S.E. of 8 animals. Significant differences from the control value are indicated: *$P < 0.05$ and **$P < 0.01$. 
CRD-induced hyperlipidemic rats

Serum TC and TG in rats gradually increased until 3 to 4 weeks after beginning CRD (Fig. 4). At 4 weeks, TCs in the lipoprotein fractions of $d < 1.006$ and $1.063 < d < 1.125$ were significantly increased as compared with the corresponding values obtained from the standard-diet-fed rats (Fig. 5). However, such increases of TC and TG in rats given EPA-E at doses of 300 mg/kg or more were significantly suppressed. Clinofibrate at 30 mg/kg decreased both TC and TG. TC in the fractions of $d < 1.006$ and $1.063 < d < 1.125$ and TG in those of $d < 1.006$ and $1.125 < d$ were markedly reduced by EPA-E at 1000 mg/kg.

DISCUSSION

We have demonstrated here that EPA-E, with daily administration, is capable of lowering plasma lipids in normolipidemic rats. Moreover, the hypolipidemic effect of EPA-E was shown in exogenously and endogenously induced hyperlipidemic animals.

Experimental animals being fed HCD have been frequently employed as a suitable model for inducing hypercholesterolemia exogenously. EPA-E considerably reduced the increment of TC in this model. As a clue to the mechanism for the action of EPA-E, it is interesting to note that exogenous HCD-induced augmentation of TC can be prevented by agents possessing

![Graph showing effects of EPA-E and clinofibrate on serum lipids in rats fed the casein-rich diet (CRD). TC, total cholesterol; TG, triglycerides. Animals except for the untreated ones were fed semisynthetic CRD containing 20% casein without cholesterol for 4 weeks. Drugs or the vehicle solution were orally administered during this period. Blood was taken from the ophthalmic venous plexus every week. About 24 hr after the final administration, blood was collected from the abdominal aorta under pentobarbital anesthesia. Each value represents the mean ± S.E. of 9 to 10 animals. Significant differences from the control value are indicated: *P < 0.05 and **P < 0.01.](image1)

![Graph showing effects of EPA-E on serum lipids of lipoprotein fractions in rats fed the casein-rich diet (CRD). TC, total cholesterol; TG, triglycerides; PL, phospholipids; d, density; ND, non-detectable (< 10 mg/dl). Serum was obtained from the animals of Fig. 4. The serum lipoprotein fractions were separated by ultracentrifugation. Each value represents the mean ± S.E. of 9 to 10 animals. Significant differences from the control value are indicated: *P < 0.05 and **P < 0.01.](image2)
an inhibitory effect on intestinal cholesterol absorption (14, 15). Recently, Rustan et al. have reported that EPA possessed the ability to inhibit acyl-CoA cholesterol acyltransferase (ACAT) (16), which is the most important enzyme in the regulation of intestinal cholesterol absorption. Therefore, one of the primary actions of EPA-E might be at the step of cholesterol absorption, which would result in a fall of serum TC in HCD-fed animals.

Pretreatment with EPA-E significantly attenuated the elevation of plasma TG in rats induced by oral administration of corn oil. From this result, it can be deduced that another inhibitory action of EPA-E exists at the level of intestinal absorption of fatty acids and/or enzymatic degradation of TG via lipases. However, it is not yet clear how EPA or EPA-E influences these steps in lipid metabolism. Effects of EPA-E on intestinal lipid absorption and lipoprotein lipase activity are under investigation in our laboratory.

In connection with endogenously induced animal models of hyperlipidemia, various reports have become available based on the following methods: injection of Triton WR-1339 into rats (17) and oral administration of a semisynthetic diet containing casein with no cholesterol to rats (18) and rabbits (19). It is well-established that rats injected with Triton WR-1339 show an increase in hepatic cholesterol synthesis (20); hence drugs with an inhibitory action on 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase are effective (21). Furthermore, it has been reported that dietary n-3 polyunsaturated fatty acids, which are rich in EPA, decrease the HMG-CoA reductase activity of hepatic microsomes in rats (22) and rabbits (23). We also demonstrated in the present study that EPA-E diminished the increased output of TC induced by Triton WR-1339 injection to rats. It is therefore suggested that the other hypolipidemic effects of EPA-E might be due to the suppression of endogenous cholesterol biosynthesis in the liver.

As shown in Fig. 5, EPA-E also reduced serum lipids in CRD-fed rats, in which the content of TC, TG and PL in the fraction of d < 1.006 was significantly decreased by EPA-E. These results strongly suggest that EPA-E causes the level of very low density lipoprotein (VLDL) to fall. The reasons why EPA-E reduces TC and PL in the fraction of 1.063 < d < 1.125 (HDL2) and TG and PL in 1.125 < d (HDL3) is still not clear. However, the reduction of TC and PL in HDL2 might be attributed to the decrease of VLDL, since the free cholesterol, PL and apolipoprotein C of HDL2 are transferred from that of TG-rich lipoproteins as VLDL (24).

Clofibrate and/or clinofibrate significantly lowered the lipids in normolipidemic rats, in Triton WR-1339-injected rats and in CRD-fed rats, and also attenuated the elevation of TG in corn oil-loaded rats. In these animal models, the potency of the lipid lowering effect of clofibrate was greater than that of EPA-E. However, we demonstrated here that clofibrate is without any effect on TC and TG in HCD-fed rats. There have been several reports to support our results that clofibrate at the same dose as used here fails to exert any effect on the lipids on serum TC in HCD-fed rats (25, 26).

On the other hand, it has been demonstrated that EPA-E is effective in lowering the lipids in all conditions tested, under normolipidemic and hyperlipidemic conditions induced either exogenously or endogenously. We also observed that EPA-E possesses abilities to decrease the lipids in normolipidemic hamsters and HCD-fed rabbits (data not shown). Recently, Abbey et al. reported that highly purified EPA-E (purity, 75%) improved the increment of plasma TC induced by an atherogenic diet in marmosets (27). These findings indicate that EPA-E appears to be a promising drug for the treatment of human hyperlipidemia and related diseases. The precise mechanism of the hypolipidemic action of EPA-E is now under investigation in our laboratory, but we can suggest at least three possibilities for the mode of action of EPA-E as follows: (1) inhibitory effect on intestinal lipid absorption, (2) inhibitory effect on lipid biosynthesis or secretion in the liver, and (3) stimulatory effect on lipid catabolism in the blood.

Acknowledgment

The authors wish to thank Dr. Kazutoshi Yanagibashi for helpful advice for preparing this manuscript.

REFERENCES

1 Kromhout, D., Bosschieter, E.B. and de Lezenne Coulinder, C.: The inverse relation between fish consumption and 20-year mortality from coronary heart diseases. N. Engl. J. Med. 312, 1205–1209 (1985)
2 Hirai, A., Hamazaki, T., Terano, T., Nishikawa, T., Tamura, Y., Kumagai, A. and Sajiki, J.: Eicosapentaenoic acid and platelet function in Japanese. Lancet ii, 1132 (1980)
3 Siess, W., Roth, P., Schere, B., Kurzmann, I., Bohling, B. and Weber, P.C.: Platelet-membrane fatty acids, platelet aggregation and thromboxane formation during a mackerel diet. Lancet i, 441–444 (1980)
4 Goodnight, S.H., Harris, W.S. and Connor, W.E.: The effect of dietary o-3 fatty acids on platelet composition and function in man. Blood 58, 880–885 (1981)
5 Norris, P.G., Jones, C.J.H. and Weston, M.J.: Effect of dietary supplementation with fish oil on systolic blood pressure in mild essential hypertension. Br. Med. J. 293, 104–105 (1986)
6 Knapp, H.R. and FitzGerald, G.A.: The antihypertensive effects of fish oil. N. Engl. J. Med. 320, 1037–1043 (1989)

7 Dehmer, G.J., Popma, J.J., van del Berg, E.K., Eichhorn, E.J., Prewitt, J.B., Cambell, W.B., Jennings, L., Willerson, J.T. and Schmitz, J.M.: Reduction in the rate of early restenosis after coronary angioplasty by a diet supplemented with n-3 fatty acids. N. Engl. J. Med. 319, 734–740 (1988)

8 Harris, W.S., Connor, W.E. and McMurry, M.P.: The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats; salmon oil vs. vegetable oils. Metabolism 32, 179–184 (1983)

9 Von Lossonczy, T.O., Ruiter, A., Bronseest-Schoute, H.C., van Gent, C.M. and Hermus, R.J.J.: The effect of a fish diet on serum lipids in healthy human subjects. Am. J. Clin. Nutr. 31, 1340–1346 (1978)

10 Phillipson, B.E., Rothrock, D.W., Connor, W.S., Harris, W.S. and Illingworth, D.R.: Reduction of plasma lipids, lipoproteins and apoproteins by dietary fish oils in patients with hypertriglycemia. N. Engl. J. Med. 312, 1210–1216 (1985)

11 Balasubramaniam, S., On the effects of dietary n-3 fatty acids (Maxepa) on plasma lipids and lipoproteins in patients with hyperlipidemia. Atherosclerosis 54, 75–88 (1985)

12 Yagasaki, K., Okada, K., Takagi, K. and Inukura, T.: Effect of 4-(4'-chlorobenzyloxy) benzyl nicotinate (KCD-232) on cholesterol metabolism in rats fed an amino acid imbalance diet. Agric. Biol. Chem. 48, 1417–1423 (1984)

13 Hata, Y., Shigematsu, H., Tsushima, M., Oikawa, T., Yamamoto, M., Yamaguchi, Y., Hirose, N. and Ueno, T.: Serum VLDL, LDL- and HDL-cholesterol levels in healthy man. —Serum lipoproteins fractionated with a use of Beckman Lp-42 Ti Rotor— Domyakukoka 9, 769–778 (1981) (Abs. in English)

14 Balasubramaniam, S., Simons, L.A., Chang, S., Roach, P.D. and Nestel, P.J.: On the mechanism by which an ACAT inhibitor (CL277082) influences plasma lipoproteins in rat. Atherosclerosis 82, 1–5 (1990)

15 Heider, J.G., Pickens, C.E. and Kelly, L.A.: Role of acyl CoA cholesterol acyltransferase in cholesterol absorption and its inhibition by 57–118 in the rabbits. J. Lipid Res. 24, 1127–1134 (1983)

16 Rustan, A.C., Nossen, J.O., Osmundsen, H. and Drevon, C.A.: Eicosapentaenoic acid inhibit cholesterol esterification in cultured parenchymal cells and isolated microsomes from liver. J. Biol. Chem. 263, 8162–8132 (1988)

17 Schurr, P.E., Schults, J.R. and Parkinson, T.M.: Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs. Lipids 7, 68–74 (1972)

18 Hevia, P., Kari, F.W., Ulman, E.A. and Visek, W.J.: Serum and liver lipids in growing rats fed casein with L-lysine. J. Nutr. 110, 1224–1230 (1980)

19 Terpstra, A.H.M., Harkes, L.H. and van Veen, F.H.: The effect of different proportions of casein in semipurified diets on the concentration of serum cholesterol and the lipoprotein composition in rabbits. Lipids 16, 114–119 (1981)

20 Kuroda, M., Tanzawa, K., Tsujita, Y. and Endo, A.: Mechanism for elevation of hepatic cholesterol synthesis and serum cholesterol levels in Triton WR-1339 induced hyperlipidemia. Biochim. Biophys. Acta 489, 119–125 (1977)

21 Endo, A., Tsujita, Y., Kuroda, M. and Tanzawa, K.: Effects of ML-236B on cholesterol metabolism in mice and rats. Lack of hypocholesterolemic activity in normal animals. Biochim. Biophys. Acta 575, 266–276 (1979)

22 Choi, Y.S., Goto, S., Ikeda, I. and Sugano, M.: Effect of n-3 polyunsaturated fatty acids on cholesterol synthesis and degradation in rats of different ages. Lipids 24, 45–50 (1989)

23 Tall, A.R. and Small, D.M.: Plasma high-density lipoproteins. N. Engl. J. Med. 229, 1232–1236 (1978)

24 Wada, S., Koizumi, M., Neichi, T., Yamazaki, T., Onoda, F., Nakakimura, H. and Ando, K.: Anti-atherosclerotic activity of AZ 1355, ethyl-10,11-dihydro-4-methoxydibenz [b,f] [1,4] oxazepine-8-carboxylate. Domyakukoka 9, 659–669 (1981) (Abs. in English)

25 Seri, N., Matsuo, T., Taniguchi, T., Amemiya, K., Kudo, M., Saito, G. and Kato, T.: Hypolipidemic effects of S-methylmethionine (Vitamin U) using various experimental procedures. Arzneimittelforschung 30, 1694–1703 (1980)

26 Abbey, M., Clifton, P.M., McMurchie, E.J., McIntosh, G.H. and Nestel, P.J.: Effect of a high fat/cholesterol diet with or without eicosapentaenoic acid on plasma lipids, lipoproteins and lipid transfer protein activity in the marmoset. Atherosclerosis 81, 163–174 (1990)