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EXPERIMENTAL STUDY

Omega-3 fatty acids may be harmful to thickness of aortic intima-media

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Abstract: There are several studies confirming an association between nicotine exposure and increase in aortic intima-media thickness (aIMT) as a pre-atherosclerotic lesion. The ω-3 FAs are on the other hand reported to have an anti-atherogenic effect. We aimed to evaluate histopathologically the effect of nicotine exposure during pregnancy and lactation period on fetal growth and aIMT at postnatal 45 days of age in rat pups living in the same conditions and to determine the protective effect of ω-3 FAs.

Pregnant rats were assigned into four groups. In nicotine (N) group; pregnant rats received nicotine subcutaneously and extra-virgin olive oil by gavage during pregnancy from 1 to 21 days of gestation and lactation. In nicotine+ ω-3 FAs (N+O) group; nicotine was administered subcutaneously and ω-3 FAs by gavage, in omega-3(O) group; ω-3 FAs were administered by gavage and saline subcutaneously, in control(C) group; saline was administered subcutaneously and extra-virgin olive oil by gavage for the same period.

The aIMT was found to be greatest in N+O group, which indicated a significant difference compared to the control group ($p < 0.05$). No statistically significant difference was found among other groups.

Although the majority of studies on ω-3 FAs suggest a beneficial effect, our study showed that exposure to ω-3 FAs increased the aIMT *(Tab. 2, Fig. 3, Ref. 25)*. Text in PDF *www.elis.sk.*

Key words: omega-3 fatty acid, aortic intima media, atherosclerosis, newborn.

Introduction

Cigarette smoking is firmly established as a risk factor for coronary heart disease, peripheral vascular disease, and stroke. One possible explanation for this relation is that smoking increases the formation of atherosclerosis. Indeed, chronic cigarette smoking has been associated with an increased risk for atherosclerotic diseases of coronary, aortic, abdominal, and peripheral arteries and, more recently, of the extra-cranial carotid arteries (1–4). Increased aortic intima–media thickness (aIMT) has been shown to be one of the earliest signs of atheroma formation (5, 6). In our previous studies, we gave nicotine to dams during pregnancy and lactation, and demonstrated that it had negative effects on aIMT of juvenile rats (7).

Most early epidemiologic studies noted very low cardiovascular mortality in populations with high fish consumption. The apparent benefit of dietary fish is explained by the intake of very long chain, highly polyunsaturated omega ω-3 fatty acids. Dietary omega ω-3 fatty acid is correlated with thinner IMT (8, 9).

The aim of this study is to show that ω-3 FAs can prevent harmful effects of nicotine on the aIMT in the offspring of rats exposed to nicotine during pregnancy and lactation.

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Materials and methods

The study was approved by the Institutional Ethics Committee. The experiments are performed in accordance to the Council Directive of the European Communities.

Animals and feeding

Female adult white Sprague–Dawley virgin rats weighing 180–225 g (8- to 12-wk old) were purchased from the Medical Science Research Center of Erciyes University. The study was performed in September, 2011. The rats were acclimatized to caged laboratory conditions and allowed to feed with standard pellets during the study. Rats were housed in stainless steel cages at room temperature in a humidity-controlled room with a 12-h light/dark reversed schedule (lights on between 07:00 and 19:00 hours). The animals were fed up with stock diet (rat food; Aytekinler, Ankara, Turkey; protein, 24 %; cellulose, 7 %; ash, 8 %;metabolic energy, 2.65 kcal/g; calcium, 1 %; and phosphorus, 0.9 %). The food intake of animals in various groups was not measured. Pregnant rats were obtained by letting the virgin females to mate overnight with a sexually experienced male. We checked the plug sign every day, and pregnancy was confirmed by the presence of a vaginal plug of semen in the breeding cage in the following morning.

Fish oil preparation

A standardized fish oil formulation (GNC, USA) was used. Each 0.5-mL capsule contained 250 mg of DHA and 100 mg of

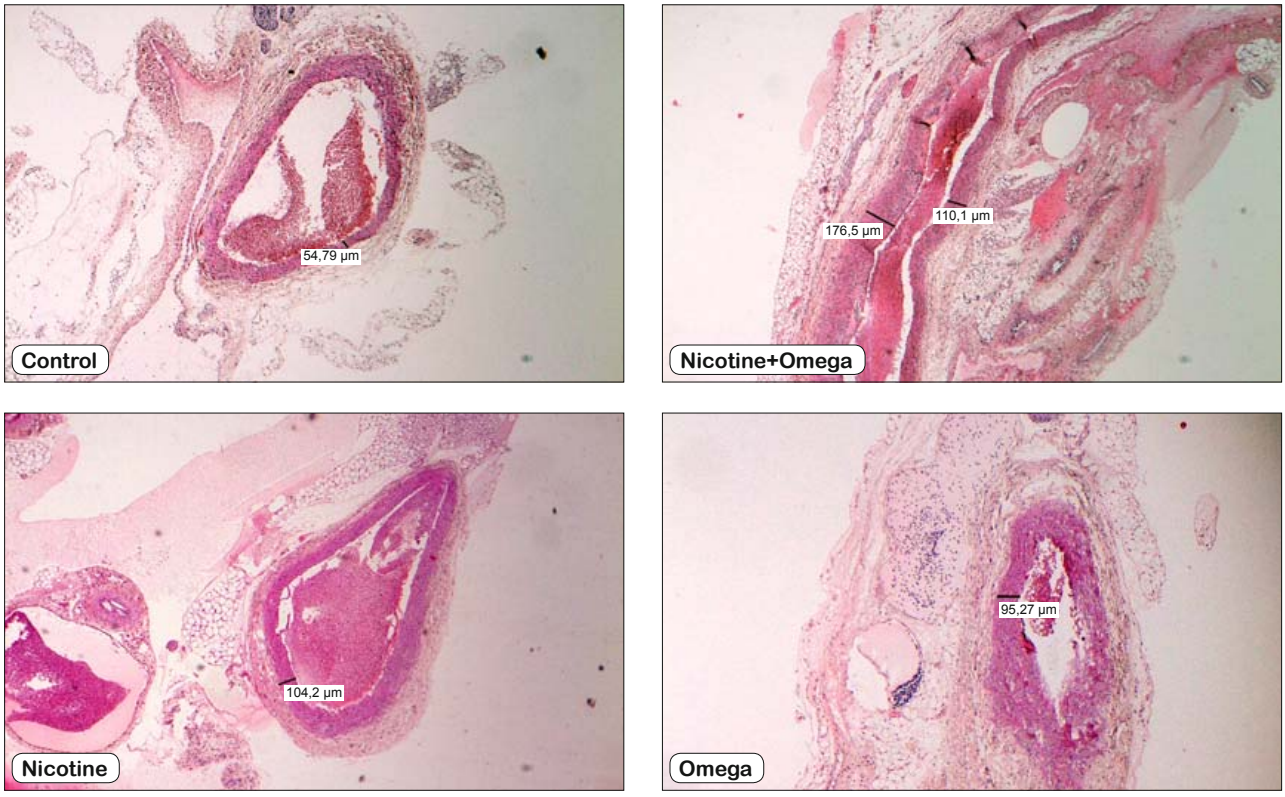

Fig. 1. Rat pups, abdominal aortic intima–media thickness (aIMT). aIMT was greatest in group N+O. aIMT was smallest in group C. (hematoxylin and eosin x40). (C – Control, O – Omega-3, N – Nikotine, N+O – Nicotine+Omega-3).

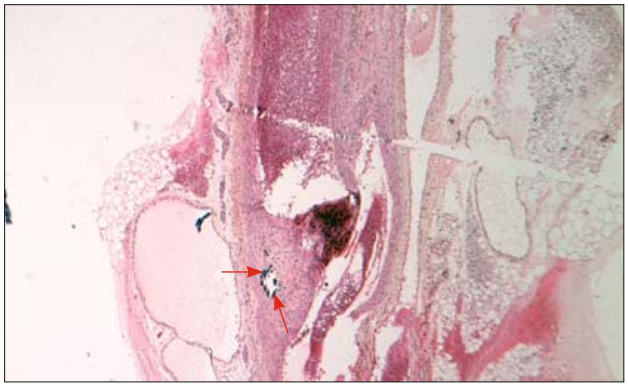

Fig. 2. Rat pups, group N+O: Calcified focal area (The arrows) (hematoxylin and eosin x40).

EPA without vitamin E. The content of two capsules was diluted with 1 mL of extra-virgin olive oil, the final DHA concentration of which was 250 mg/mL (10). In ω-3 FA treatment group, a constant volume of 0.15 ml per 100 g body weight was given daily by gavage. Under these ω-3 FA conditions, DHA and EPA concentrations were 375 mg/kg and 140 mg/kg, respectively. The ω-3 FA doses used in this study were modified from doses that had been previously used in rat studies. Long-term DHA treatment given at a dose of 360 mg/kg/day was found to substantially modify the membrane fatty acids without increasing the susceptibility to oxidative stress in mature rats (11).

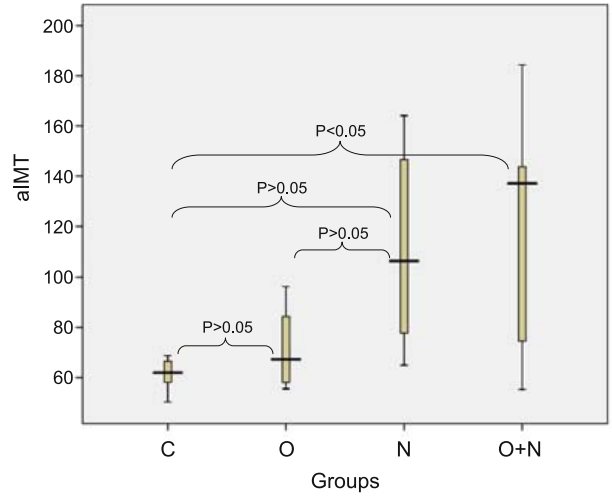

Fig. 3. aIMT in pups at postnatal 45 days (C – Control, O – Omega 3 fatty acids, N – Nicotine, O+N – Omega 3 fatty acids + Nicotine). aIMT was greatest in group O+N. It was seen that there was a significant difference between group O+N and C ($p < 0.05$). No statistically significant difference was found among other groups.

Study groups

After confirming pregnancy with the vaginal smear method, pregnant rats (dams) were randomly assigned into four equal groups: one control (n = 6) and three experimental groups. In

nicotine (N) group (n = 6), pregnant rats received 2 mg/kg/day subcutaneous (S.C) nicotine [N5260 (−) nicotine tartarate, Sigma Chemical Co., St. Louis, MO] and extra-virgin olive oil by gavage during pregnancy from 1 to 21 days of gestation and lactation (until postnatal day 21). In nicotine+omega-3 (N+O) group (n = 6), pregnant rats received 2 mg/kg/day nicotine S.C and 0.15 ml per 100 g body weight ω-3 FAs by gavage for the same period. In omega 3 fatty acid (O) group (n = 6), pregnant rats received 0.15 ml per 100 g body weight ω-3 FAs by gavage for the same period. In control (C) dams, saline solution was subcutaneously injected and extra-virgin olive oil given by gavage in same amounts daily during pregnancy and lactation for the same period. Nicotine and ω-3 FAs were only administered (s.c. and gavage), to mothers, which implies that it reached the fetuses and the neonates only via the placenta and mother's milk. The clinical status of the dams was monitored daily during nicotine treatment. All dams were allowed normal delivery on the day 20 or 21 of gestation, and body weight was recorded in all pups at birth and for 45 days.

Examination methodology

During lactation period (21 days), the pups were not isolated from mothers and continued to live under optimal environmental conditions. At the end of the mid-adolescent period (45 days), ketamine (1.2 mg/kg, i.p.) was applied to all pups, and the abdominal aorta was removed. All pups died due to hypovolemia. Abdominal aortas were prepared as distal abdominal aortas by cutting them from below to the level of the renal arteries and above the iliac bifurcation. The specimens of distal abdominal aortas obtained by dissection were fixed in 10 % formaldehyde, embedded in paraffin, and sectioned at a thickness of 6 μm. The mounted sections were stained with hematoxylin and eosin (Fig. 1). Aortic intima–media thickness was measured via Leica DMD 108 (Leica Microsystems GmbH, Wetzlar, Germany). Each sample was measured as "micrometer (μm)" from five different locations of the vessel wall, blindly. Arithmetic averages of these five measurements were included in statistical calculations. The samples were evaluated by two different expert pathologists who were blinded to the subjects and each other's examination findings.

Statistical analyses

One-Way ANOVA test (descriptives, Anova, Post hoc, Tukey HSD) was used to compare group averages. Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (SPSS Inc., version 15, Chicago, IL, USA). A p value of less than 0.05 was considered significant.

Results

There was no significant difference in baseline body weight of dams. On days 0 and 45, no significant difference was found in body weight of pups (Tab. 1).

The aIMTs (mean ± standard deviation) were measured as follows: 61.25 ± 6.63μm in group C, 71.45 ± 15.81μm in group O, 111.03 ± 38.99 μm in group N and 122.02 ± 48.16 μm in group N+O (Tab. 2, Fig. 1). Contrary to the initial assumption, aIMT was greatest in group N+O. It was seen that there was a significant difference between group N+O and C ($p < 0.05$). No statistically significant difference was found among other groups. When group N was compared to group C, it was seen that there was a marked difference between groups, which did not reach statistical significance ($p = 0.063$) (Fig. 3).

Discussion

Atherosclerosis is the main underlying problem in cardiovascular diseases such as heart disease, stroke and other forms of blood vessel disease such as peripheral vascular disease and abdominal aortic aneurysms (1–4). It has been shown that increased aIMT is one of the earliest findings of atheroma (5, 6). In a study by Tell et al, it was shown that there is a strong relationship between smoking and atherosclerotic diseases in elder people. Authors showed that wall thickness in both, internal and common carotid arteries as well as the degree of narrowing of the arterial lumen (stenosis) showed a wider range of expression of atherosclerotic disease (4). In our previous study, we administered nicotine (3 and 6 mg/kg/day) to the female rats during gestation and lactation and demonstrated that nicotine administration significantly increased aIMT in pups (7). Another of our studies showed that neonates whose mothers smoked have significantly increased aIMT (12). In the present study, we observed that there was a marked difference between group C and N; however, this difference did not reach statistical significance ($p = 0.063$). We attributed this finding to the lower nicotine doses used, namely 2 mg/kg/day in the present study.

Cardiovascular disease is the leading cause of death worldwide. The primary pathophysiological event is atherosclerosis (13). The ω-3 FAs have cardioprotective effects. Clinical and epidemiological studies showed that ω-3 FAs decrease the risk for coronary heart disease (8). The ω-3 FAs also have an anti-atherogenic effect (9). In a study, in which carotid intima-media thickness was compared between Japanese and White Americans, Sekikawa et al. found that Japanese had lower carotid intima-media thickness and 2-fold higher blood ω-3 FAs levels than white Americans. Authors attributed this finding to higher fish consumption in Japanese population (14). Skilton et al showed that the inverse relationship between arterial wall thickness and fetal growth might be prevented by adding ω-3 FAs to the diet in children (15).

We achieved unexpected results in the present study, which we planned by considering these effects. When we compared group O to C, we observed that aIMTs were greater rather than being smaller; however, it did not reach statistical significance ($p > 0.05$). When we compared group N+O to C, despite expecting better results in group N+O, the results we obtained in the latter group were the worst (Group C: 61.25 ± 6.63 μm vs. Group N+O: 122.02 ± 48.16 μm; $p < 0.05$). In addition, we observed calcified focal areas over intima-media of abdominal aorta (Fig. 2). No such areas were observed in other groups. Qeustions arise as to whether these focal calcification areas may

be precursors of atheroma plaques as well as to what causes these effects.

It was shown that Eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) has positive effects on cardiovascular risk factors. When EPA+DHA are given at a dose of 4 g/day, it provides a decrease in triacylgliserol by 25–30 % and increase in low-density lipoprotein-cholesterol (LDL-C) by 5–10 % and high-density lipoprotein-cholesterol (HDL-C) by 1–3 % (16, 17).

A number of randomized, controlled studies confirmed that LDL-cholesterol plays a pivotal role in the pathogenesis of atherosclerotic cardiovascular diseases. In the primary and secondary protection studies, it was shown that decreasing LDL-cholesterol by statin therapy resulted in decreased cardiovascular morbidity and mortality (18). In some studies, it was shown that ω-3 FAs may enhance sensitivity of LDL-C to oxidation (19, 20).

In other words, arterial wall may also be harmed similar to various organs and tissues, when oxidative stress is increased. In our study, increased aIMTs might have resulted from oxidative stress due to increased LDL-cholesterol. In addition, lipid metabolism resulted in formation of free radicals and lipid peroxidation. Multivitamin supplementation is recommended in total parenteral nutrition because of protective and antioxidant properties against free radicals (21). It could be possible that excessive ω-3 FAs given to dams caused tissue injury by excessive production of free radicals; in turn, this might have increased aIMT and resulted in calcified necrosis areas at aIMTs by enhancing the effect of nicotine in Group N+O.

There are studies suggesting that ω-3 FAs supplementation is harmful rather than beneficial. In a study with a 10-year follow-up, Oomen et al showed that dietary intake of α-linoleic acid had no effect on coronary artery disease (22). Moreover, Rice et al used ω-3 FAs supplementation in patients with acute pulmonary injury. They suggested that such supplementation was not useful, on the contrary it was even harmful (23).

Dietary supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFAs) may limit oxidative stress by increasing antioxidant capacity, but n-3 PUFAs are also highly susceptible to lipid peroxidation; hence n-3 PUFA supplementation is potentially harmful (24, 25).

May additional ω-3 FAs use be harmful during gestation and lactation? Despite the expected outcome that ω-3 FAs would decrease the effect of nicotine, the outcome contradicted outcomes reported in literature. Our results suggest that these finding should be addressed by further studies.

Our study has been conducted based on a single methodology while other methods could have been applied to enrich the outcome of the study. Nevertheless, this was not possible due to the lack of necessary budget.

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