Iranian Journal of Veterinary Research, Shiraz University, Vol. 11, No. 4, Ser. No. 33, 2010

Correlation between lipid-lowering and bone-protective effects of eicosapentaenoic acid in rats with steroid-induced bone loss

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(Received 26 Oct 2009; revised version 29 May 2010; accepted 13 Jun 2010)

Summary

This study was conducted to investigate the correlation between lipid-lowering and bone-protective effects of eicosapentaenoic acid on steroid-induced bone changes in rats. Twenty-one male 10-week-old Wistar rats were divided into 3 groups (n = 7 each) and treated with 0.9% NaCl SC (group 1) or methylprednisolone 7 mg/kg SC once a week (group 2) or methylprednisolone 7 mg/kg SC once a week + eicosapentaenoic acid 160 mg/kg, PO daily (group 3), for 6 weeks. At the end of the experiment, serum total cholesterol and triglycerides concentrations were determined using enzymatic colorimetric assays and bone histomorphometric analyses were performed on cancellous bone of femoral epiphysis and metaphysis using a photomicroscope and a digital camera. Histomorphometric parameters demonstrated a significant bone loss due to methylprednisolone administration, which was partly restored by eicosapentaenoic acid. A strong inverse correlation was observed between the serum total cholesterol concentration and epiphyseal trabecular width and metaphyseal trabecular osteoid width (R = -0.82, P = 0.04 and R = -0.86, P = 0.01, respectively). Serum triglycerides concentration was also strongly and inversely correlated with the above mentioned parameters (R = -0.88, P = 0.02 for epiphyseal trabecular width and R = -0.81, P = 0.02 for metaphyseal trabecular osteoid width). The correlation between the lipid-lowering and bone-protective effects of eicosapentaenoic acid may be helpful in the clarification of its effects on bone and lipid metabolism.

Key words: Eicosapentaenoic acid, Methylprednisolone, Lipid, Bone

Introduction

Atherosclerosis and osteoporosis are two almost ubiquitous metabolic diseases that underlie the most common causes of death and disability, especially in industrial societies. Eicosapentaenoic acid (EPA) is a dietary n-3 long chain polyunsaturated fatty acid found mainly in marine oils. It is well-known for several biological effects, including benefits on lipoprotein metabolism (Bézard *et al*., 1994) and atherosclerosis (Mori and Beilin, 2001; Leaf *et al*., 2008). Although best known for their cardio-protective role, long chain polyunsaturated fatty acids and their metabolites also regulate bone metabolism and consequently have a potential application in the prevention and/or treatment of osteoporosis (Poulsen *et al*., 2007). In animal models, it has been shown that n-3 polyunsaturated fatty acids deficiency causes severe osteoporosis and when deficient animals are replenished with n-3 polyunsaturated fatty acids, the process of bone degradation is reversed (Reinwald *et al*., 2004). Dietary n-3 fatty acids have decreased osteoclasto-genesis and loss of bone mass in ovariectomized mice (Sun *et al*., 2003) and an EPA-enriched diet has prevented loss of bone weight and strength in ovariectomized rats fed with a low calcium diet (Sakaguchi *et al*., 1994). Moreover, it has been demonstrated that EPA, especially at a dose of 160 mg/kg, exerts beneficial effects on steroid-induced bone loss in rats (Shomali *et*

al., 2009).

Considering the dual effects of EPA on osteoporosis and atherosclerosis, the investigation of putative relationships between these effects seems quite intriguing. The purpose of the present study was to clarify a possible correlation between the effects of EPA on serum total cholesterol and triglycerides concentrations and bone histomorphometric parameters in steroid-induced osteopenic rats.

Materials and Methods

Animals

Twenty-one male Wistar rats, 10 weeks of age, with a mean body weight of 300 g, were purchased from Razi Vaccine and Serum Research Institute (Karaj, Iran). After one week of adaptation, they were randomly divided into 3 experimental groups of equal number, and treated for 6 weeks as follows:

Group 1: 0.9% NaCl, once a week subcutaneouse (SC) administration (control group) (The volume of injection was chosen according to the volume of methyl-prednisolone injected in an animal with the same weight).

Group 2: methylprednisolone (Manufactured for Merck Generiques by Vianex, Greece, Merck® 500 mg) 7 mg/kg once a week SC administration (MP-group).

Group 3: methylprednisolone 7 mg/kg once a week SC + EPA (S.L.A. Pharma AG., Liestal, Switzerland, 99%) 160 mg/kg daily by oral gavage administration (EPA-group).

Dose, dosage interval and duration of administration of MP were borrowed from the studies performed by Wimalawansa *et al*. (1997) and Wimalawansa and Simmons (1998). The dosage regimen for the treatment with EPA was chosen according to the study performed by Shomali *et al*. (2009).

During the experimental period, the animals were maintained on a 12 h light-dark cycle at 20 ± 2°C. They were allowed to have free access to tap water and a pelleted standard rat chow diet (Razi Vaccine and Serum Research Institute, Karaj, Iran).

Animals were treated ethically in compliance with the local regulations of the University of Tehran, Faculty of Veterinary Medicine, according to research authorization number 7506006/6/3.

Sampling of blood and bones

On day 43, all rats were anesthetized with chloroform and blood samples were obtained by cardiac puncture. Sera were harvested within one h after sampling and the serum samples stored in -20°C until use. All the animals were euthanized by deepening the chloroform anesthesia and their left femoral bones were dissected for histomorphometric study.

Preparation of specimens for histomorphometric study

The left femoral bones were fixed in 4% formaldehyde solution (Sigma-Aldrich®, Taufkirchen, Germany) and decalcified using formic acid-sodium citrate (Sigma-Aldrich®, Taufkirchen, Germany) method (Kiernan, 1999). The samples were sectioned along the median plane in the distal epiphysis and metaphysis and 5 µm longitudinal sections were stained using Masson's trichrome method (Kiernan, 1999).

Histomorphometric parameters were determined by using a digital photomicroscope connected to a personal computer with Ziess Axio Vision LE software. Parameters measured in cancellous bone included; epiphyseal trabecular width, metaphyseal trabecular width, epiphyseal bone area/tissue area and metaphyseal trabecular osteoid width.

The region of cancellous bone marked for the measurements was the central zone of cancellous tissue, 2-3 mms below (epiphysis) or above (secondary spongiosa of metaphysis) the margins of the growth plate in the distal epiphysis and metaphysis of the femoral bone. All widths were reported as the mean value of 10 measurements for each parameter in each sample.

The parameters' nomenclature is in compliance with the American Society for Bone and Mineral Research (ASBMR) histomorphometry nomenclature (Parfitt *et al*., 1987).

Determination of serum parameters

Serum total cholesterol and triglycerides concentrations were determined using enzymatic colorimetric assay with cholesterol esterase and cholesterol oxidase and glycerol phosphate oxidase, respectively. Kits were prepared by Pars Azmun Co., Ltd., Tehran, Iran.

Statistical analysis

Data comparison among groups was performed by one-way ANOVA followed by Tukey's multiple comparison test. It should be mentioned that skewness was observed in the two groups and after using logarithmic scale, normality test was passed. Correlations were analyzed using the Pearson's product-moment correlation test. Statistical analysis was performed by using Sigma Stat 2 Statistical package. A significance level of $P<0.05$ was set for all analyses.

Results

Histomorphometric and lipid parameters

Epiphyseal trabecular width, epiphyseal bone area/tissue area and metaphyseal trabecular osteoid width significantly decreased in the MP-group compared to the control ($P<0.05$). EPA treatment significantly restored epiphyseal bone area/tissue area and metaphyseal trabecular osteoid width ($P<0.05$). Although no significant differences in the serum total cholesterol and triglycerides concentration were observed among the three groups ($P>0.05$), the serum levels of both the total cholesterol and triglycerides showed a decrease of about 16% in the EPA-group compared to the control group. Data for serum and histomorphometric parameters is summarized in Table 1.

Significant inverse correlations were noted between the serum total cholesterol concentration and the epiphyseal trabecular width, as well as the metaphyseal trabecular osteoid width in the EPA-group ($R = -0.82$, $P = 0.04$ and $R = -0.86$, $P = 0.01$, respectively). The correlations between the serum triglycerides concentrations and both of the above mentioned histomorphometric parameters were also significant ($R = -0.88$, $P = 0.02$ for epiphyseal trabecular width and $R = -0.81$, $P = 0.02$ for metaphyseal trabecular osteoid width) (Table 2).

Discussion

The epidemiological evidence links osteoporosis with cardiovascular diseases independently from age (Boukhris and Becker, 1972; Frye *et al*., 1992); the low bone mineral density has been closely associated with atherosclerosis in men (Yamaguchi *et al*., 2002). It has been reported that 3-hydroxy-3-methyl-glutaryl-Coenzyme A (HMG-Co A) reductase inhibitors (statins) can increase bone mineral density in postmenopausal women (Edwards *et al*., 2000) and stimulate bone formation *in vitro* and *in vivo* (Mundy *et al*., 1999). In the rabbit, lipid-lowering agents from statin (lovastatin) and non-statin (bezafibrate) categories alleviated steroid-induced osteoporosis with the same degree (Wang *et al*., 1995).

Nowadays, the positive effects of lipid-lowering agents on osteoporosis can be clarified (McFarlane *et al*., 2004), but the relationship between these two effects has

Table 1: Histomorphometric parameters and serum total cholesterol and triglycerides concentrations (n = 7 in each group). Data presented as mean ± SEM

Variables	Groups		
	Control group	MP-group	EPA-group
Epiphyseal trabecular width (μm)	63.02 ± 4.27^{a}	37.8 ± 3.81^{b}	49.89 ± 4.44^{ab}
Epiphyseal bone area/tissue area (%)	37.8 ± 1.10^{a}	22.87 ± 0.67^{b}	35.14 ± 1.17^{a}
Metaphyseal trabecular width (μm)	37.92 ± 2.75^{a}	33.97 ± 2.28^{a}	40.87 ± 2.15^{a}
Metaphyseal trabecular osteoid width (μm)	2.23 ± 0.10^{a}	1.60 ± 0.04^{b}	2.40 ± 0.17^{a}
Serum total cholesterol concentration (mg/dl)	57.83 ± 9.61^{a}	53 ± 7.46^{a}	48.57 ± 5.50^{a}
Serum triglycerides concentration (mg/dl)	73 ± 10.51^{a}	74.83 ± 11.75^{a}	61.29 ± 5.17^{a}

Different letters display significant differences in each row ($P<0.05$)

Table 2: Results of correlation analysis for histomorphometric and serum lipid parameters in EPA group (n = 7)

	Variables	R	P-values
Serum total cholesterol concentration (mg/dl)	Epiphyseal trabecular width (μm)	-0.82*	0.04
	Epiphyseal bone area/tissue area (%)	-0.23	0.61
	Metaphyseal trabecular width (μm)	-0.30	0.51
	Metaphyseal trabecular osteoid width (μm)	-0.86*	0.01
Serum triglycerides concentration (mg/dl)	Epiphyseal trabecular width (μm)	-0.88*	0.02
	Epiphyseal bone area/tissue area (%)	-0.30	0.50
	Metaphyseal trabecular width (μm)	-0.54	0.20
	Metaphyseal trabecular osteoid width (μm)	-0.81*	0.02

*A significance level of $P<0.05$ was set for all comparisons (data of epiphyseal trabecular width in one animal was missing)

not been addressed yet. The present study is conducted to investigate this correlation by using EPA, a well-known agent with hypolipidemic and anti-atherosclerotic effects (Angerer and Von Schacky, 2000; Hooper *et al*., 2001) as well as anti-osteoporotic properties (Shomali *et al*., 2009).

The main characteristic consequence of steroid-induced osteopenia in rats is thinning of the trabecular bone with unchanged connectivity, which is due to accelerated bone resorption and depressed bone formation (Nitta *et al*., 1999). The reduction of trabecular thickness is the key histological feature of steroid-induced bone loss (Manolagas and Weinstein, 1999). In the present study, epiphyseal trabecular width, epiphyseal bone area/tissue area and metaphyseal trabecular osteoid width significantly decreased in the MP-group compared to the control ($P<0.05$), as a result of decreased bone formation and increased bone resorption. EPA significantly restored the epiphyseal bone area/tissue area and metaphyseal trabecular osteoid width.

The absence of a significant lipid lowering effect of EPA in the present study might be due to the relatively short term of the experiment or the low dose of EPA administered and, with regard to the poor number of animals included in the present study, the findings should be interpreted cautiously.

The correlation between high serum cholesterol and osteoporosis has been previously described (Broulik and Kapitola, 1993). Yamaguchi *et al*. (2002) reported an inverse relationship between bone mineral density and the LDL-cholesterol level in postmenopausal women.

In the present study, both serum total cholesterol and triglycerides concentrations were strongly and inversely correlated with epiphyseal trabecular width as well as metaphyseal trabecular osteoid width, which are two important histomorphometric parameters of the bone remodeling process.

Parhami (2003) hypothesized that bioactive products of lipids accumulated in the bone and the lipoprotein oxidation could affect the bone formation and resorption processes, leading to osteoporosis. The lipid accumulation in the bone can occur with steroid administration (Warman and Boskey, 1983). Putting all these findings together, it seems that the lipid lowering effect may consequently result in an improvement of the bone condition.

On the other hand, it is well established that the mevalonate pathway plays a pivotal role in osteoclast formation, apoptosis and function (Roelofs *et al*., 2006). Farnesyl diphosphate synthase and HMG-Co A reductase are two key enzymes of this metabolic pathway. EPA administration significantly reduces the mRNA levels of these enzymes in hepatoma cells (Le Jossic-Corcos *et al*., 2005).

In conclusion, the correlation observed between lipid-lowering and bone-protective effects of eicosapentaenoic acid, in rats with steroid-induced bone loss, may be helpful in the clarification of its dual effects on bone and lipid metabolism.

Acknowledgement

Thanks are due to J. Slagel (S.L.A Pharma AG., Liestal, Switzerland) for the generous donation of eicosapentaenoic acid.

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