ORIGINAL ARTICLE

Isotretinoin increased carotid intima-media thickness in acne patients

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Abstract

Background: Isotretinoin (Iso) in acne treatment may cause dyslipidemia and increase in liver enzymes. Moreover, its effect on lipid and glucose metabolism may induce atherosclerotic complications. The aim of this study was to evaluate carotid intima-media thickness (CIMT), osteopontin (OPN), lipid, high sensitive C-reaktive protein (hs-CRP) levels, and insulin resistance (HOMA-IR) in acne patients before and after Iso treatment.

Materials: Twenty-one acne patients were treated with Iso (0.5-0.8 mg/kg) for four months. Blood tests for lipid profile, fasting glucose, liver enzymes, OPN, HOMA-IR, hs-CRP and CIMT measurements were performed before and after Iso treatment. Serum levels of OPN and, hs-CRP were measured by ELISA and particle-enhanced turbidimetric immunoassay respectively.

Results: Iso treatment significantly increased lipid levels, CIMT (0.60-0.74 mm; $p < 0.001$); whereas it non-significantly increased HOMA-IR (0.91-1.87; $p = 0.70$), OPN (4.32-5.44 ng/ml; $p = 0.27$), and hs-CRP (0.08-0.09 mg/dl; $p = 0.88$) levels. There was no correlation between OPN and CIMT ($p = 0.77$).

Conclusion: Isotretinoin treatment for four months significantly increased CIMT in acne patients. Hippokratia 2016, 20(1): 14-18

Keywords: Isotretinoin, osteopontin, carotid intima-media thickness, homeostasis model assessment of insulin resistance

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Introduction

Isotretinoin (Iso) is an active metabolite of retinoic acid and is the drug of choice for the treatment of acne. Dyslipidemia and increase in liver enzymes are the most frequently seen side effects of Iso treatment[1,2]. Also conflicting results are reported regarding the effect of Iso on insulin resistance although the alterations in lipid profile were reminiscent of the metabolic syndrome[1,2]. Long-term use of Iso may be a risk factor for atherosclerosis. It is also reported that retinoid X receptor (RXR) stimulation inhibits the proliferation in atherosclerosis and has been proposed to prevent artery occlusion[3].

Osteopontin (OPN) is a matrix-associated protein that is secreted out of the cell[4]. OPN has been suggested to have an important role on macrophage uptake, insulin resistance, and the regulation of inflammation in vascular and fat tissue[5,6]. OPN is involved in cellular and humoral actions of the inflammation, and these may cause tissue calcification and matrix restructuring[4]. Elevated OPN levels are found in the aorta of hyperglycemic diabetics, atherosclerotic lesions, fatty liver, end-stage renal disease and osteoporosis that are associated with insulin resistance and type 2 Diabetes Mellitus[7].

Increase in carotid intima-media thickness (CIMT) is associated with atherosclerotic risk factors. CIMT is proposed to demonstrate the subclinical atherosclerosis[3]. In the recent years, CIMT measurement is used to estimate the risk of myocardial infarction, stroke, and sudden cardiac death[8]. It is reported in the literature that, in spite of changes in lipid profile, retinoid reduced foam cell formation and stabilized atherosclerotic plaques[3]. RXR and peroxisome proliferator-activated receptor gamma (PPAR-γ) are heterodimer receptors. When one of them binds its ligand, they act together. So the activation of RXR and PPAR-γ resulted in depletion of foam cell formation by cholesterol efflux from the macrophages that were exposed to oxidized lipoproteins[9]. According to these results a decrease in CIMT is expected with the use of retinoids.

In the literature, there are not enough studies about the effect of Iso treatment on CIMT and OPN levels. The aim of this study was to evaluate the effect of Iso on OPN,

high sensitive C-reactive protein (hs-CRP) levels, insulin resistance (HOMA-IR), lipid levels and CIMT in acne patients before and after Iso treatment.

Materials and Methods

This prospective study included 21 patients with an age range of 18-34 years who presented to our dermatology clinic with acne. Patients with any of the following were excluded from the study: smoking, hypertension, using vitamin A supplements, previous therapy with oral retinoid, sensitivity or allergy to parabens, any thyroid or pituitary disease, pregnancy, coronary artery disease, diabetes mellitus, chronic renal failure, rheumatic disease, hypolipidemic treatment, cancer, recent antibiotic or anti-inflammatory drug administration, recent infection, and history of psychiatric disorders. Iso therapy was prescribed at a dose of 0.5-0.8 mg/kg for four months. Body mass index (BMI) measurements and physical examinations were performed before and after the treatment.

The study was approved by the Medical Ethical Committee of Sifa University (decision number/date: 09-16/18.04.2012) and was conducted from April 2012 to December 2012 according to the principles of the Declaration of Helsinki. Written informed consent was obtained by all patients prior to inclusion in the study.

Biochemical parameters

Serum concentrations of glucose, triglyceride, total cholesterol, high-density lipoprotein (HDL), and serum glutamic oxaloacetic transaminase (SGOT) were determined by enzymatic procedures, while serum insulin was measured by chemiluminescence. Serum low-density lipoprotein (LDL) levels were estimated using the Friedwald formula. Serum levels of OPN were measured by enzyme-linked immunosorbent assays (ELISAs) (Human Osteopontin Platinum ELISA BMS2066/BMS2066TEN eBioscience Kit, San Diego, CA, USA) (normal range 2.30-75.24 ng/ml). hs-CRP levels were measured by particle-enhanced turbidimetric immunoassay (normal range 0.03-2.76 mg/dl) (Cobas Integra C-Reactive Protein Latex, Roche Diagnostics, Indianapolis, USA).

Insulin resistance was calculated using the homeostasis model assessment (HOMA) according to the following formula[10]:

HOMA-IR = [fasting plasma insulin (μU/ml)] x [(fasting plasma glucose (mmol/l)]/22.5

After a full night of fasting, blood samples were collected from patients the day before initiation and the day after completion of the treatment. Blood samples were centrifuged in gel tubes (2500 g for five minutes) and, consecutively serum was examined and analyzed in the laboratory. Hematological parameters were also measured (results not included herein) which detected no abnormality.

Measurements of CIMT

All ultrasonography (US) measurements were performed by the same experienced radiologist, using a US scanner (Acuson Sequoia 512; Siemens AG Medical Solutions, Erlangen, Germany) and a high-frequency 15L8-MHz linear-array transducer according to a standard scanning protocol.

With the patients in the supine posture the common carotid artery (CCA), carotid bifurcation, and proximal portion of internal carotid artery were evaluated. The CIMT was identified at the far wall of the CCA using the semi-automated edge detection software. The region of interest (about two centimeters in length and one centimeter away from bifurcation) was placed perpendicular to the vessel wall. The lumen-intima and the media-adventitia interface at the far wall of the vessel was digitally calculated with the software and the mean CIMT is reported[11].

Statistical analyses

Statistical analyses were performed using the Rstudio software via R language (version 0.98.501, Wirtschaftsuniversität, Vienna, Austria). The Kolmogorov-Smirnov test was used to check data for normality. All numerical variables with normal distribution are reported as mean ± standard deviation and median (IQR). For comparison between the pre and post-treatment data, the paired sample t-test was used for homogenous data. The non-homogeneous data was analyzed using the Wilcoxon Signed Rank test. A p value of less than 0.05 was considered to be statistically significant.

Results

Twenty-one acne patients (6 male and 15 female) with mean age 23.0 ± 4.1 years, were treated with Iso for four months. There was no drop out during the study. There was no significant difference in the BMI of the patients before and after the treatment (Table 1).

No significant differences were seen in fasting glucose, insulin, SGOT levels before and after Iso treatment. However, the HOMA-IR levels were non-significantly increased. Iso treatment increased total cholesterol and LDL-cholesterol levels while the HDL-cholesterol levels were decreased significantly. After Iso treatment, OPN and hs-CRP levels were non-significantly increased while CIMT measurements were significantly increased (Table 1). There was no correlation between the OPN and CIMT measurements (p =0.77; Pearson correlation test).

Discussion

It is reported in the literature that treatment with Iso for three months or longer induced alterations in lipid profile[12]. Reduced removal rate of triglycerides from the plasma and induced expression of Apo E gene may be the cause of the increase in triglyceride levels[13]. A significant decrease in HDL-cholesterol levels was explained as down regulation of the expression of apolipoprotein A-1 mRNA in rat hepatocytes that it is the major component of HDL-cholesterol[14]. Iso treatment, as expected, significantly increased total-cholesterol, LDL-cholesterol, triglyceride and decreased HDL-cholesterol levels in our study.

Table 1: Pre- and post-treatment values of blood tests for lipid profile, liver enzymes, fasting glucose and insulin, insulin resistance, C-reactive protein, and measurements for carotid intima-media thickness in 21 acne patients treated with ısotretinoin for four months.

Variables	Pre treatment		Post treatment		p
	Mean ± SD	Median (IR)	Mean ± SD	Median (IR)	
BMI (kg/m2)	23.7 ± 3.2	23.5 (4.7)	24.2 ± 2.9	24.1 (4.1)	p =0.25
Fasting glucose (mg/dl)	87.6 ± 9.7	89.0 (44.0)	88.1 ± 7.0	88.0 (11.0)	p =0.28
Fasting insulin (μU/ml)	10.8 ± 8.6	8.2 (6)	10.1 ± 3.9	9.9 (5.6)	p =0.77
HOMA-IR	2.2 ± 0.9	2.1 (1.2)	2.3 ± 1.9	1.7 (1.5)	p =0.70
SGOT (U/l)	14.1 ± 7.8	12.0 (4.0)	14.9 ± 5.7	13.0 (10.0)	p =0.81
Total-Cholesterol (mg/dl)	163.6 ± 28.6	167.0 (39.0)	187.5 ± 33.5	188.0 (53.5)	p <0.001
Triglyceride (mg/dl)	71.0 ± 27.4	68.0 (38.0)	99.6 ± 37.6	96.0 (52.0)	p <0.001
LDL-Cholesterol (mg/dl)	90.1 ± 26.9	87.0 (37.4)	121.8 ± 37.3	126.0 (60.0)	p <0.001
HDL-Cholesterol (mg/dl)	60.8 ± 13.1	58.0 (18.0)	55.2 ± 11.6	55.0 (23.0)	p <0.001
Osteopontin (ng/ml)	3.7 ± 1.6	3.5 (1.2)	4.9 ± 3.8	3.3 (13.8)	p =0.27
hs-CRP (mg/dl)	0.19 ± 0.39	0.08 (0.12)	0.20 ± 0.34	0.09 (0.12)	p =0.88
CIMT (mm)	0.64 ± 0.13	0.60 (0.15)	0.79 ± 0.17	0.74 (0.24)	p <0.001

SD: standard deviation, IR: interquartile, BMI: body mass index, HOMA-IR: homeostasis model assessment-insulin resistance, SGOT: serum glutamic oxaloacetic transaminase, LDL: low-density lipoprotein, HDL: high-density lipoprotein, hs-CRP: high sensitive C-reactive protein, CIMT: carotid intima media thickness.

The relation between insulin resistance and Iso treatment is not clear so far. It was reported that both oxidative and non-oxidative total glucose disposal rate was significantly decreased in 11 male patients treated with Iso for five months. Thus, insulin sensitivity was decreased[15]. Ertugrul et al treated acne vulgaris patients with Iso for three months and concluded that it did not affect insulin sensitivity[16]. In a recent study, it is reported that six months of Iso treatment of acne patients did not affect the insulin sensitivity[17]. In our study, Iso treatment was given for four months and at the end of the study HOMA-IR was non-significantly increased.

The effect of Iso on lipid and glucose metabolism is probably mediated by the retinoic acid-related orphan receptors α/β and γ (ROR α/β and γ). These receptors play a regulatory role in lipid/glucose metabolism. The studies revealed that the RORα-deficient mice are thin, have no hepatosteatosis, insulin resistance and glucose intolerance. The RORγ-deficiency causes an increase in insulin sensitivity in mice[18]. All-trans retinoic acid and the synthetic retinoids functions as antagonist for RORβ and RORγ, but not for RORα[19]. So retinoid with RORα agonist effect may cause obesity, hepatosteatosis and lead to an increase in insulin resistance while with RORγ antagonist effect cause a decrease in insulin resistance[18]. In another mechanism, Iso binds to the serum retinol binding protein (RBP) in the circulation and is transported into cells by being stimulated by retinoic acid 6 (STRA6). The molecular mechanism of RBP was reported after the discovery of STRA6. Besides STRA6, there is a vitamin A transporter that also functions as a surface signal receptor. After the binding of RBP-ROH (retinol binding protein-retinol) to STRA6, it induces the phosphorylation of tyrosine residue in the receptor's C-terminal. This activates the janus kinase (JAK)-signal transducer and activator of transcription (STAT) cascade. As adipocyte cells expressing STRA6, RBP-ROH induce STAT target genes including SOCS3 (suppressor of cytokine signaling 3), suppress insulin signaling and PPARγ (peroxisome proliferator-activated receptor gamma); and this may cause an increase in insulin resistance[20]. In our study four months of Iso treatment increased HOMA-IR non-significantly.

OPN is a glycoprotein and acts as a multifunctional proinflammatory cytokine. It is secreted by activated T cells, NK cells, dendritic cells and monocytes/macrophages. OPN plays an important role in physiologic and pathologic events including angiogenesis, apoptosis and inflammation[21,22]. OPN levels are increased in chronic inflammation, and it may have a function in the pathogenesis of atherosclerosis[23]. OPN levels are reported to be correlated with the intima-media thickness in hypertension patients[24]. In our study, the OPN levels were not correlated with CIMT. Iso is known to have effects on the lipid/glucose metabolism as discussed before. It may also have effects on some mediators that affect atherosclerosis. Therefore, we aimed to investigate the effect of Iso on OPN. In the literature, there are no studies about the effect of Iso on OPN. Krskova et al reported that short-term application of 13-cis retinoic acid increased PPAR-γ mRNA in adipose tissue of rat[25]. Earlier studies demonstrated that in the short term application of 13-cis retinoic acid reduces insulin sensitivity, but it is considered as an adaptation of the adipocytes in the beginning by increasing the PPAR-γ as well as the other mediators like adiponectin[26]. In another study Wang et al reported that induced myofibroblasts and macrophages were found in the aortic valves of rabbits that were fed with higher cholesterol diets. In the aortic valves, mRNA of OPN levels was also found increased[27]. These conclude that Iso-induced dyslipidemia may increase OPN levels but in our study, the OPN levels were non-significantly increased.

CIMT is now recognized as an indicator of atherosclerosis[8]. At the end of our study Iso treatment led to significantly increased LDL-cholesterol, triglyceride and decreased HDL-cholesterol levels and this may result in an increase of CIMT. Herdeg et al reported that all-trans-

retinoic acid reduced the restenosis of rabbit carotid artery after balloon-dilation[28]. RXR and PPAR-γ activation resulted in depletion of foam cell formation by cholesterol efflux from the macrophages that were exposed to oxidized lipoproteins[9].

hs-CRP is accepted as a risk factor for atherosclerosis, myocardial infarction, and stroke[29]. hs-CRP activates many processes involved in inflammation[29]. In the study of Heliövaara et al three months of 13-cis retinoic acid treatment non-significantly increased CRP but this parameter returned to base levels after cessation of therapy[30]. In this study, many acute-phase proteins like ceruloplasmin, A1GP, α-1 antitrypsin, C3, SAA, sE-selectin, fibrinogen, haptoglobin, and an inflammation marker (erythrocyte sedimentation rate) were tested but not all were affected by 13-cis retinoic acid. These conflicting results may be attributed to the sensitivity of these parameters to the inflammatory stimulus that is induced by 13-cis retinoic acid[30]. It was observed that although IL-6 levels did not change initially by the 13-cis retinoic acid treatment, the CRP levels were increased but not significantly. Later in the treatment, both IL-6 and CRP levels significantly decreased[30]. In our study, hs-CRP levels non-significantly increased.

Limitations of the current study are the limited study period, small patient number, and absence of untreated control group. Significant results could be obtained if the study period was prolonged and the number of patients increased. Another limitation is the fact that the reversible laboratory findings and CIMT measurements after discontinuation of Iso treatment were not studied.

In conclusion Iso treatment for four months significantly increased CIMT, while it non-significantly increased HOMA-IR, OPN and hs-CRP levels.

Conflict of interest

Authors declare no conflict of interest.

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