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Short communication

Influence of ezetimibe monotherapy on ischemia-modified albumin levels in hypercholesterolemic patients

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Abstract:
Ischemia-modified albumin (IMA) is considered to be a novel biochemical marker for ischemic and atherosclerotic conditions. This study aimed to investigate the influence of ezetimibe monotherapy on circulating IMA levels in hypercholesterolemic patients. A total of 31 patients (mean age 65.7 years) received 10 mg of ezetimibe daily during a 12-week treatment period. The levels of low-density lipoprotein cholesterol and IMA were significantly reduced after ezetimibe treatment. The adjusted regression analyses revealed that the changes in the IMA levels were not significantly correlated with those of the other atherosclerotic risk markers, such as body mass index, blood pressure, glucose and lipid panels. The significant reduction of the IMA levels following ezetimibe treatment, which was independent of the reduction of low-density lipoprotein cholesterol levels, suggests that ezetimibe may improve the oxidative stress burden in hypercholesterolemic patients.

Key words:
IMA, oxidative stress, reactive oxygen species, intestinal cholesterol transport inhibitor, atherosclerosis

Abbreviations: BMI – body mass index, HDL-C – high-density lipoprotein cholesterol, IMA – ischemia-modified albumin, LDL-C – low-density lipoprotein cholesterol, MBP – mean blood pressure, TG – triglycerides

Introduction

Atherosclerosis, a chronic pathology involving medium and large arteries, is a serious health issue that stems from complex interactions between genetic and environmental risk factors, including hypercholesterolemia [16]. The atherosclerotic process is usually accompanied by oxidative stress, which results in the damage of biomolecules by reactive oxygen species [16]. The capacity of albumin to bind various ligands, including toxic molecules, acts as a buffering factor to maintain health [11]. The N-terminal region (the aspartyl-alanyl-histidyl-lysine sequence) of albumin is a specific binding site for transitional metals (i.e., cobalt, copper and nickel), and albumin is converted to

ischemia-modified albumin (IMA) in the circulation by a reduction of the metal binding capacity of the albumin N-terminus under certain pathological conditions, such as localized oxidative stress produced in the environment of an atheroma plaque [11]. Thus, IMA is currently considered to be a novel biochemical marker of atherosclerosis-induced ischemia *in vivo* [11]. Interestingly, higher IMA levels have not only been reported in patients with acute coronary syndromes, but they have also been measured in patients without an acute coronary episode who have atherosclerotic risk factors, such as hypercholesterolemia [6]. In addition, although very few reports exist, IMA levels have been shown to predict cardiac outcomes or mortality in patients with myocardial infarction or renal failure [12, 15].

Ezetimibe, a new-concept agent, is a selective inhibitor of intestinal cholesterol transport that suppresses the absorption of dietary and biliary cholesterol *via* the Niemann-Pick C1-like 1 enterocyte receptor, thereby reducing circulating concentrations of low-density lipoprotein cholesterol (LDL-C) [3, 9]. Although little is known about whether ezetimibe reduces oxidative stress, recent studies using oxidative stress-related markers of lipids, lipoproteins and proteins (i.e., oxidized low-density lipoproteins, protein carbonyl and 8-isoprostane) have indicated that this agent may improve oxidative stress environments [13, 14]. Thus, exploring a putative association between ezetimibe treatment and IMA levels is of interest. The aim of the present study was to investigate the influence of ezetimibe treatment on the circulating levels of IMA and other atherosclerotic risk factors examined in daily practice in patients with hypercholesterolemia.

Subjects and Methods

The study included 31 hypercholesterolemic patients with serum LDL-C concentrations ≤ 3.64 mmol/l (male/female = 13/18; mean age = 65.7 ± 5.6 years) who received 10 mg of ezetimibe daily during a 12-week treatment period. These patients had no history of cardiovascular, thyroid, kidney or liver diseases, and they did not receive any other lipid-lowering agents. Although 15 patients were receiving antihypertensive agents and 13 patients were current smokers, the prescriptions were unaltered and smoking was continued during the treatment period. The study was approved by the institutional ethics committee, and each subject gave informed consent.

After an overnight fast in both the pre- and post-treatment phases of this study, standard atherosclerotic risk markers were measured, such as body mass index (BMI), mean blood pressure (MBP, as calculated according to the following equation: diastolic blood pressure plus [systolic blood pressure minus diastolic blood pressure]/3) and plasma glucose and serum lipid levels (LDL-C, triglycerides [TG] and high-density lipoprotein cholesterol [HDL-C]). The glucose and lipid levels were measured by standard enzymatic methods. In addition, serum IMA levels were measured by the decrease in cobalt 2^{+} binding, according to previously published methods [2, 6]. Briefly, we added 100 µl of the whole serum sample to 25 µl of a solution of 1 g/l cobalt chloride. After agitation and a 10-min incubation at 25°C, dithiothreitol (25 µl of a 1.5 g/l solution) was added. The absorbance of the mixture was read at 470 nm, and IMA was expressed as the delta absorbance in arbitrary units between the individual control (without dithiothreitol) and reaction samples. The intra-assay CV was 5.0% at a mean absorbance of 0.51 units.

The data are expressed as the mean ± standard deviation or the median plus the interquartile range. A paired *t*-test was used to compare the pre- and post-treatment levels of the respective markers. Simple (Pearson's correlation) and multiple linear regression analyses, controlled for age, gender, smoking and all of the measured markers, were used to determine the correlations between the changes in the respective marker levels (Δ: post-treatment data minus pre-treatment data). The TG values were log-transformed because of the skewed distribution. $P < 0.05$ was considered significant.

Results

During the treatment period, the levels of IMA and LDL-C were significantly reduced, and there were small changes in the other atherosclerotic risk markers (Tab. 1).

A simple correlation analysis revealed that the ΔIMA levels were not significantly correlated with those of the other atherosclerotic risk markers

Tab. 1. Comparison of the measured markers pre- and post-treatment

Markers	Pre-treatment levels	Post-treatment levels	p-value
Body mass index, kg/m^2	25.5 ± 3.4	25.4 ± 3.2	0.688
Mean blood pressure, mmHg	94.1 ± 11.4	92.5 ± 11.7	0.341
LDL cholesterol, mmol/l	3.98 ± 0.58	3.18 ± 0.56	< 0.0001**
HDL cholesterol, mmol/l	1.40 ± 0.38	1.39 ± 0.38	0.616
Triglycerides, mmol/l	1.49 (1.23–1.97)	1.32 (1.16–1.91)	0.087
Fasting plasma glucose, mmol/l	6.3 ± 0.6	6.3 ± 1.7	0.995
IMA, units	0.57 ± 0.52	0.55 ± 0.62	0.036*

LDL: low-density lipoprotein, HDL: high-density lipoprotein, IMA: ischemia-modified albumin. The data are shown as the mean ± standard deviation or the median (interquartile range). A paired-*t* test was used to measure the changes in the respective markers. Significance level: * $p < 0.05$, ** $p < 0.01$

(r-coefficient (p-value)): ΔBMI 0.001 (0.998), ΔMBP 0.266 (0.147), ΔLDL-C –0.104 (0.577), ΔHDL-C –0.106 (0.572), ΔTG –0.100 (0.592) and Δglucose 0.147 (0.429). A multiple linear regression analysis also failed to show significant correlations between the ΔIMA levels and those of the other markers (β-coefficient (p-value)): ΔBMI 0.152 (0.484), ΔMBP 0.213 (0.299), ΔLDL-C –0.062 (0.786), ΔHDL-C –0.178 (0.449), ΔTG –0.219 (0.332) and Δglucose –0.039 (0.877).

Discussion

The present study demonstrated, for the first time, a significant reduction of the IMA levels of hypercholesterolemic patients during ezetimibe treatment. Taking into account the fact that increased IMA levels are a surrogate marker for atherosclerosis/ischemia-mediated damage of biomolecules *in vivo* [6, 11, 12, 15], the present finding suggesting that ezetimibe may reduce this oxidative pathway in an atherosclerosis-prone population is notable. In addition, because intervention studies on ezetimibe 'mono' therapy are limited, and the IMA-ezetimibe association had not been previously examined, the present data are novel and deserve further exploration.

Our finding may be in agreement with recent studies examining other oxidative stress-related markers that suggest that ezetimibe decreases the impact of oxidative pathways [13, 14]. Although the precise mechanisms of this phenomenon remain unclear, there are some possible explanations. Remnant lipoproteins are associated with oxidative conditions [4, 8, 10], and ezetimibe reduces the generation of remnant lipoproteins by suppressing intestinal lipid transport and/or hepatic production of remnant lipoproteins [3, 5, 7, 9]. Furthermore, oxidized low-density lipoproteins are associated with oxidative stress conditions [10], and ezetimibe can reduce the levels of oxidized low-density lipoproteins [13, 14]. The modulation of oxidative environments by ezetimibe may be a causative link in the observed reduction of IMA levels; however, this requires further exploration. A role for high-density lipoproteins in the antioxidant effect of ezetimibe has also been proposed [1]. In the present study, ezetimibe treatment significantly reduced the LDL-C levels; however, there was only a weak correlation between the change in the IMA concentration and the standard atherosclerotic risk markers, including LDL-C and HDL-C. Because ezetimibe can have pleiotropic effects, further investigations are required to ascertain which biological mechanisms are affected by the present findings.

The effects of ezetimibe in clinical scenarios of cardiovascular disease remain to be sufficiently established, as compared to other lipid-lowering agents, such as statins. Similar studies observing IMA changes under statin treatment may provide relevant information; therefore, future research in this area is required. In addition, there are some limitations to this study. Even though this was a prospective study, a randomized-controlled design was not employed, and there were no control subjects who did not receive ezetimibe. The number of patients enrolled in

this study was relatively small, and the treatment period was relatively short. Unfortunately, we did not measure the patients' total serum albumin concentrations; however, our patients did not have any nutritional deficiencies or thyroid, kidney, liver or renal diseases. Therefore, large shifts in albumin concentrations were not expected, and in previous studies, barring the aforementioned conditions, IMA has been shown to be a biomarker of ischemia irrespective of the total albumin concentrations [6, 11, 15].

Conclusions

In summary, there was a significant reduction of circulating IMA levels in hypercholesterolemic patients after ezetimibe treatment. While the present study provides insight into daily lipid management in hypercholesterolemic patients, more studies are necessary to confirm the clinical relevance of the present findings and to verify the mechanisms underlying the relationship between ezetimibe and circulating IMA levels.

Conflict of interest:
The authors have no further conflicts of interest to disclose.

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References:

1. Bays HE, Ose L, Fraser N, Tribble DL, Quinto K, Reyes R, Johnson-Levonas AO et al.: A multicenter, randomized, double-blind, placebo-controlled, factorial design study to evaluate the lipid-altering efficacy and safety profile of the ezetimibe/simvastatin tablet compared with ezetimibe and simvastatin monotherapy in patients with primary hypercholesterolemia. Clin Ther, 2004, 26, 1758–1773.
2. Bhagavan NV, Lai EM, Rios PA, Yang J, Ortega-Lopez AM, Shinoda H, Honda SA et al.: Evaluation of human serum albumin cobalt binding assay for the assessment of myocardial ischemia and myocardial infarction. Clin Chem, 2003, 49, 581–585.
3. Betters JL, Yu L: NPC1L1 and cholesterol transport. FEBS Lett, 2010, 584, 2740–2747.
4. Cohn JS: Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. Can J Cardiol, 1998, 14, B18–27.
5. Deushi M, Nomura M, Kawakami A, Haraguchi M, Ito M, Okazaki M, Ishii H, Yoshida M: Ezetimibe improves liver steatosis and insulin resistance in obese rat model of metabolic syndrome. FEBS Lett, 2007, 581, 5664–5670.
6. Duarte MM, Rocha JB, Moresco RN, Duarte T, Da Cruz IB, Loro VL, Schetinger MR: Association between ischemia-modified albumin, lipids and inflammation biomarkers in patients with hypercholesterolemia. Clin Biochem, 2009, 42, 666–671.
7. Fukuda M, Nakamura T, Kataoka K, Nako H, Tokutomi Y, Dong YF, Yasuda O et al.: Ezetimibe ameliorates cardiovascular complications and hepatic steatosis in obese and type 2 diabetic db/db mice. J Pharmacol Exp Ther, 2010, 335, 70–75.
8. Jagla A, Schrezenmeir J: Postprandial triglycerides and endothelial function. Exp Clin Endocrinol Diabetes, 2001, 109, S533–547.
9. Lioudaki E, Ganotakis ES, Mikhailidis DP: Ezetimibe; more than a low density lipoprotein cholesterol lowering drug? An update after 4 years. Curr Vasc Pharmacol, 2011, 9, 62–86.
10. Nakajima K, Nakano T, Tanaka A: The oxidative modification hypothesis of atherosclerosis: the comparison of atherogenic effects on oxidized LDL and remnant lipoproteins in plasma. Clin Chim Acta, 2006, 367, 36–47.
11. Sbarouni E, Georgiadou P, Voudris V: Ischemia modified albumin changes - review and clinical implications. Clin Chem Lab Med, 2011, 49, 177–184.
12. Sharma R, Gaze DC, Pellerin D, Mehta RL, Gregson H, Streather CP, Collinson PO, Brecker SJ: Ischemia-modified albumin predicts mortality in ESRD. Am J Kidney Dis, 2006, 47, 493–502.
13. Turfaner N, Uzun H, Balci H, Ercan MA, Karter YH, Caner M, Sipahioglu F, Genc H:. Ezetimibe therapy and its influence on oxidative stress and fibrinolytic activity. South Med J, 2010, 103, 428–433.
14. Ueda S, Miyake I, Takata K, Shimizu N, Shirotani K, Ishida S, Yonemitsu K et al.: Ezetimibe, an inhibitor of intestinal cholesterol absorption, decreases serum level of malondialdehyde-modified low-density lipoprotein in patients with hypercholesterolemia. Int J Cardiol, 2011, 146, 420–421.
15. Van Belle E, Dallongeville J, Vicaut E, Degrandsart A, Baulac C, Montalescot G: Ischemia-modified albumin levels predict long-term outcome in patients with acute myocardial infarction. The French Nationwide OPERA study. Am Heart J, 2010, 159, 570–576.
16. Victor VM, Rocha M, Solá E, Bañuls C, Garcia-Malpartida K, Hernández-Mijares A: Oxidative stress, endothelial dysfunction and atherosclerosis. Curr Pharm Des, 2009, 15, 2988–3002.

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