

Serologic APE1/Ref-1: New Biomarker for Vascular Inflammation in Atherosclerosis[†]

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Abstract: Apurinic/aprimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) is a secreted multifunctional protein. Since the concept of APE1/Ref-1 secretion was established in 2013, several studies have demonstrated the usefulness of APE1/Ref-1 as a serological biomarker. However, the role of APE1/Ref-1 as a new biomarker in atherosclerotic vascular inflammation is unclear. Herein, we investigated the role of APE1/Ref-1 in atherosclerotic apolipoprotein E (ApoE^{-/-}) mice fed with a Western-type diet as an animal model of vascular inflammation. We found that serologic APE1/Ref-1 was strongly correlated with vascular inflammation. Neutrophil/lymphocyte ratio, endothelial cell/macrophage activation, and atherosclerotic plaque formation, reflected by atherosclerotic inflammation, were increased in the ApoE^{-/-} mice fed with a Western-type diet. Correlation analysis showed a high correlation between plasma APE1/Ref-1 levels and neutrophil/lymphocyte ratio, a marker of systemic inflammation. We conclude that APE1/Ref-1 expression is upregulated in aortic endothelial cells/macrophages of atherosclerotic mice, and that plasma APE1/Ref-1 levels could predict atherosclerotic inflammation, it is a useful biomarker for vascular inflammation in atherosclerosis.

Keywords: APE1/Ref-1; atherosclerosis; ApoE knockout mouse; atorvastatin; VCAM-1; galectin-3; neutrophil/lymphocyte ratio

1. Introduction

Arteriosclerosis is a chronic vascular inflammatory disease that is increasing with changes in diet, characterized by inflammation within the arterial walls. Apurinic/aprimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) is a secreted multifunctional protein involved in DNA base-excision repair and in redox regulation of several functional proteins. Since the concept of APE1/Ref-1 secretion was established in 2013 [1], several studies have demonstrated the usefulness of APE1/Ref-1 as a serological biomarker [2]. However, the role of APE1/Ref-1 in atherosclerotic vascular inflammation is unclear. Here, we investigated the role of APE1/Ref-1 in atherosclerotic apolipoprotein E (ApoE^{-/-}) mice fed with a Western-type diet as an animal model of vascular inflammation.

2. Methods

In this study, we used 8-week-old male apolipoprotein E-knockout mice (ApoE^{-/-}; Jackson Laboratory, Bar Harbor, ME, USA) and age- and sex-matched C57BL/6J mice (DooYeol Biotech, Seoul, Korea.). In

addition to C57BL/6J wild-type (WT) control group, ApoE^{-/-} mice were randomly subdivided into three groups: the normal diet (ND) group, the Western-type diet group (WD), and the atorvastatin-treated ApoE^{-/-} mice fed with a Western-type diet (WD + statin). Mice were sacrificed at 20 weeks after commencement of the diet. Plasma levels of APE1/Ref-1 were determined using an APE1/Ref-1 sandwich enzyme-linked immunosorbent assay kit (MediRedox, Daejeon, Korea). Immunohistochemistry and immunofluorescence for VCAM-1, galectin-3, APE1/Ref-1, CD31, smooth muscle protein 22 alpha were performed in aortic tissues. Values are expressed as mean \pm standard error of the mean. Data were analyzed using one-way ANOVA.

3. Results and Discussion

The percentage of neutrophils in ApoE^{-/-} mice fed with a Western-type diet (WD) was significantly increased, while lymphocyte percentages were decreased, leukocyte/lymphocyte ratio is used as a marker for inflammation and is associated with atherosclerosis. WD groups showed the increased NLR, suggesting the systemic inflammation. In ApoE^{-/-} mice fed with a Western-type diet (WD), APE1/Ref-1 expression was markedly increased in whole aortic wall, especially in the innermost layer of endothelial lining, in a fatty streak of plaque, in the smooth muscle layer. Additionally, the expression of VCAM-1 and galectin-3 were significantly increased in those mice. These results suggest that APE1/Ref-1 expression was increased in endothelial cell- and/or macrophage-activated aortic tissues of atherosclerotic mice. The signal for APE1/Ref-1 was merged with that for galectin-3, thereby indicating the co-localization of APE1/Ref-1 in macrophages. Also, it was mainly merged with that for CD31, which is a specific marker for endothelial cells in the aortas of WD mice, this suggests that APE1/Ref-1 expression was upregulated, and it may have been derived from macrophages and endothelial cells in atherosclerotic plaques. Plasma APE1/Ref-1 level in mice was evaluated with sandwich ELISA assay as described in the experimental section. As shown in Figure 1, the levels of plasma APE1/Ref-1 in ApoE^{-/-} mice fed with a Western-type diet (WD) were significantly increased compared with those of the ND group (11.36 ± 2.17 ng/mL for WD vs. 2.74 ± 0.82 ng/mL as for ND). This suggests that plasma levels of APE1/Ref-1 were increased under hypercholesterolemic conditions accompanied by inflammation. Based on the ROC curve, the cut-off value for plasma APE1/Ref-1 level for diagnosis of atherosclerosis in ApoE^{-/-} mice fed with a Western-type diet (WD) as compared with wild-type control mice (WT) was set at 4.90 ng/mL, with an area under the ROC curve of 1.0, a sensitivity of 100%, and a specificity of 91%. In correlation analysis with hematologic parameter with plasma APE1/Ref-1, plasma levels of APE1/Ref-1 were correlated mainly with the neutrophil/lymphocyte ratio, which is used as a marker of systemic inflammation.

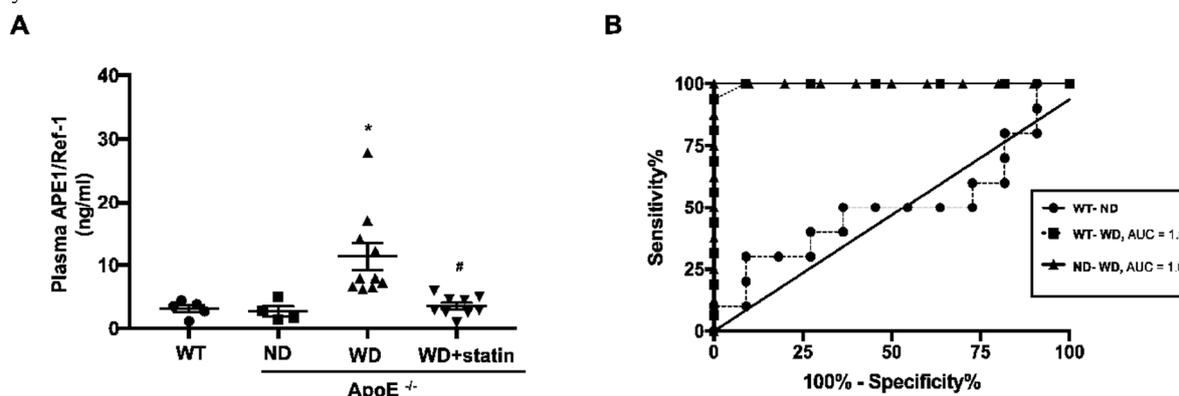


Figure 1. Plasma APE1/Ref-1 level was significantly elevated in ApoE^{-/-} mice fed with a Western-type diet. (A) Quantitative analysis of plasma APE1/Ref-1 levels in each experimental group (B) Receiver operating curves of plasma APE1/Ref-1 levels for the diagnosis of atherosclerosis in ApoE^{-/-} mice fed with a Western-type diet (WD) compared with those of wildtype control mice (WT). (Cited in Biomedicines 2020 [3]).

4. Conclusions

In our present study, we confirmed that serologic APE1/Ref-1 was increased in the plasma of ApoE^{-/-} mice, and that its levels were decreased by treatment with atorvastatin. These results strongly suggest that APE1/Ref-1 could be used as a new serologic biomarker to detect the progression of atherosclerosis. This suggests that the plasma level of APE1/Ref-1 could be a reliable serologic biomarker for the evaluation of atherosclerosis.

References

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