Role of Adiponectin in Atherosclerosis

Evy Sulistyoningrum*, Rina Susilowati**
* Pascasarjana Ilmu kedokteran dasar dan biomedis, Universitas Gadjah Mada Yogyakarta
** Bagian Histologi dan Biologi Sel, Universitas Gadjah Mada, Yogyakarta

Introduction

Adipose tissue has traditionally been considered as an energy storage organ but recently, it is also considered as a hormonally active system in the control of metabolism. Adipose tissue secretes a large number of factors with diverse functions. These factors include free fatty acids (FFA) and protein, which act in autocrine, paracrine, or endocrine manners to control various metabolic functions.[1,2] The terms 'adipocytokines' have been used to refer to a number of adipocyte-derived biologically active molecules which may influence the function as well as the structural integrity of other tissues. Some examples of these substances are leptin, TNF-α, resistin and interleukin-6.[1,2,3] Leptin is an adipocyte-derived peptide hormone with central and peripheral effects on energy balance.[2] Another product of adipose tissue is Tumor Necrosis Factor-α (TNF-α), which is a pro-inflammatory cytokine that has been implicated in the pathogenesis of insulin resistance through increasing release of FFA by adipocytes, reduction in adiponectin synthesis and impairment of insulin signaling. On the other hand, IL-6 is a pleiotropic circulating cytokine with effect from inflammation to host defense to tissue injury. IL-6 also has glucoregulatory effect and increases circulating FFA.[1,3]

Adiponectin is a recently discovered adipocytokine, and its plasma level is negatively-correlated with body mass index. Furthermore, the plasma level of adiponectin is also decreased in many obesity-related diseases like diabetes, insulin resistance and coronary artery diseases. The physiological role for adiponectin and its role in many pathological conditions have not fully established. Adiponectin increases tissue oxidation which leads to decreasing FFA level and tissue trigliserides, and furthermore increases insulin sensitivity. Data from animal studies, adiponectin has anti-diabetic activity[4] and anti-atherogenic property in certain species.[4,5,6] In populational studies, plasma level of adiponectin can predict incidence of metabolic syndrome in youth,[7] also, higher level of adiponectin were associated with lower risk for coronary heart disease.[8] This brief article will review the current understanding about the structure, function, and effects of adiponectin on atherosclerosis and provide insight into its potential therapeutic and prognostic relevances.

The Discovery and Molecular Structure of Adiponectin

Adiponectin is known by multiple names which are Acrp30, AdipoQ, apM1 and gelatin-binding protein 28 (GBP 28). This adipose tissue-specific protein is abundant in plasma, accounting for up to 0.05% of total serum protein.[1] Scherer *et al.* (1995)[9] described a novel 30-kDa secretory protein that is made exclusively in adipose tissues, which is structurally similar to complement factor C1q. Therefore, adiponectin is called Acrp30 (adipocyte complement-related protein of 30 kDa). Northern blot analyses showed that Acrp30 contained H" 244 amino acids, which can be distinguished in four domains: amino-terminal signal sequence, a variable region, a collagenous domain and a carboxy-terminal globular domain homologous with globular domain of the subunits of complement factor C1q.

Plasma concentration of adiponectin is 5-30 µg/ml.[1,2] Adiponectin can be found in several forms. Adiponectin monomer can exist as the full length or globular fragment, but most of adiponectin exist as full length structure, only a small amount of globular adiponectin in the plasma.[10,11] Monomeric-full length adiponectin has not been observed in the circulation and appears to be confined to the adipocyte.[10] Monomeric structure of adiponectin and its domains are shown in Figure 1.
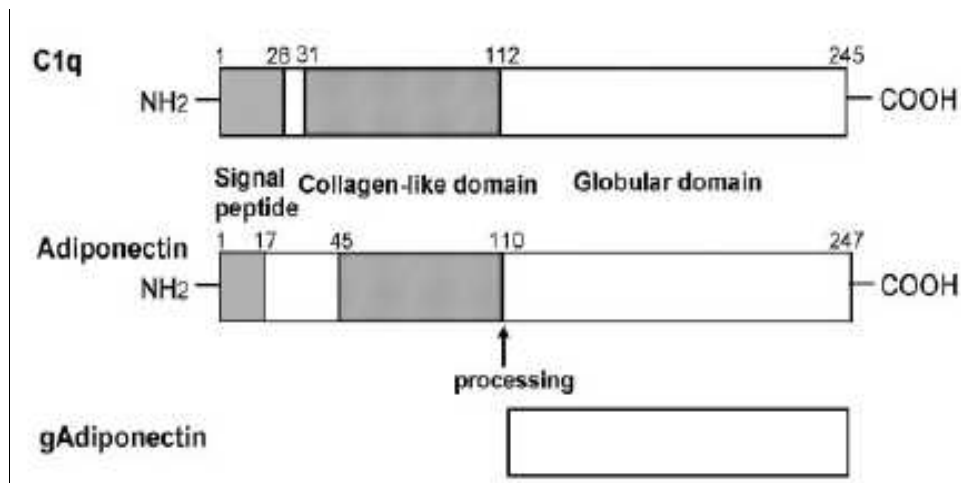
Figure 1. Structure and Domains of Adiponectin[11]

Three monomers of adiponectin associate at the globular domains form the trimeric structure of adiponectin. The trimeric structure of adiponectin is a basic building block of adiponectin. Four to six trimers associate through their collagenous domains via the disulfide bonds to form higher order structures, or multimers. Thus, from SDS PAGE analyses on serum adiponectin found that adiponectin can be distinguished by its molecular weight into low molecular weight, medium molecular weight and high molecular weight.[10,12] The polimerization of adiponectin monomers depends on disulfide bond formation by Cys at codon 39. Change in adiponectin's structure can change its potent bioactivity.[13] Model of adiponectin polymerization is shown in Figure 2.
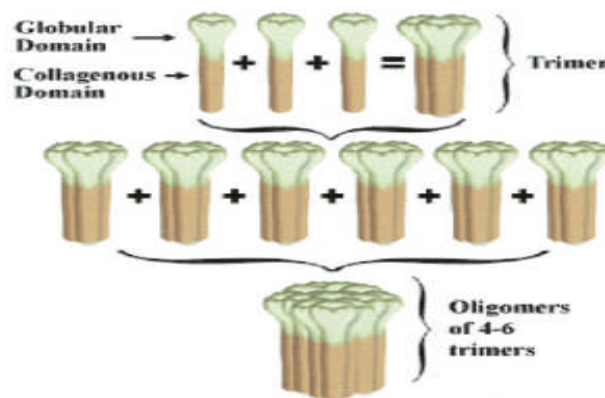

Figure 2. Models for Adiponectin Polymerization[10]

Anti-atherogenic Actions of Adiponectin

Atherosclerosis and ASCVD is one of the complications of metabolic syndrome. Atherosclerosis now has been regarded as an inflammatory disease. Atherosclerotic cellular changes consist of basically the following 3 cellular phenomena: monocyte adhesion to endothelial cells by the upregulated-expression of adhesion molecules, oxidized Low Density Lipoprotein (LDL) uptake of macrophages through scavenger receptors, and proliferation of migrated smooth muscle cells by the action of Platelet-Derived Growth Factors (PDGF) or Heparin-Binding Epidermal Growth Factor (EGF)-like Growth Factors (HB-EGF). Adiponectin has potential

inhibitory activities of these atherogenic cellular phenomena.[14]

The initial atherosclerotic lesion consists of monocytes/macrophages and T lymphocytes. At this stage, adhesion molecules on arterial endothelial cell surfaces are responsible for the accumulation of these haemocytes. Vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and intercellular adhesion molecule-1(ICAM-1) have been detected in human atherosclerotic lesions. The expression of adhesion molecules plays an important role in the regulation of inflammatory reactions. These expressions of adhesion molecules are up-regulated in the inflammatory states. The change in the endothelium will induce increasing endothelial permeability to lipoprotein and other plasma constituents and will lead to further processeses.[15]

Ouchi *et al.* (1999)[16] in their in vitro study with the human aortic endothelial cells (HAECs) showed that physiological concentration of adiponectin inhibits TNF-α–induced monocyte adhesion and expression of adhesion molecules. In this study, TNF-α treatment increased the surface expression of VCAM-1, E-selectin, and ICAM-1 on HAECs. Dose-dependent suppression of TNF-α-induced surface expression of VCAM-1, E-selectin, and ICAM-1 was observed when HAECs were pre-treated with adiponectin. The suppressive effects of adiponectin on cell-surface expression of these adhesion molecules were accompanied by changes in steady-state mRNA levels analyzed by Northern blot. Later on, other research reports that adiponectin action in inhibiting TNF-α-induced nuclear factor-κB activation (NF-κB) is performed through the inhibition of IκB phosphorylation.[17] Therefore, from these studies, we can conclude that adiponectin acts as an endogenous regulator of endothelial cells in response to inflammatory stimuli.

Another *in vitro* study of adiponectin's anti-atherogenic property is held in 2001, that demonstrated physiological concentrations of adiponectin (3 - 30 µg/mL) had significant inhibitory effects on human monocyte-derived macrophage. Adiponectin suppressed intracellular lipid accumulation as well as class-A Macrophage Scavenger Receptor (MSR) expression. Adiponectin also suppressed class-A MSR ligand binding and uptake activities of the cells, thus inhibit macrophage to foam cell transformation. The generation of lipid-laden foam cells is considered a key step in the pathogenesis of atherosclerosis. Class-A and class-B MSRs (CD36) play a pivotal role in foam cell formation by mediating the uptake of modified LDL. Therefore, these observations suggest that adiponectin may act as a negative endocrine modulator for foam cell formation through the inhibition of class A MSR expression. In this study, adiponectin specifically decreased the protein levels of class A MSR without altering those of CD36 because expression of these 2 proteins was differentially regulated by the nuclear receptor pathways. These observations suggest that adiponectin may modulate the macrophage-to-foam cell transformation.[18]

In cultured human aortic smooth muscle cells (HASMC), adiponectin attenuated DNA synthesis induced by several growth factor including PDGF and HB-EGF, thus inhibits the cells' proliferation. Adiponectin also reduce the growth factor-induced migration of HASMC. This inhibition was shown to be attributable to the inhibition of signal transduction trough the extracellular-related signal kinase (ERK) pathway.[19] Vascular smooth muscle cells' proliferation and migration is one of the key mechanisms of atherosclerosis. These smooth muscle cells migrate from tunica media to tunica intima and together with other cells like foam cell and platelets they will form fatty streaks. Furthermore, the fatty streaks progress to intermediate and advanced lesions, they tend to form a fibrous cap that walls off the lesion from the lumen. The fibrous cap covers a mixture of leukocytes, lipid, and debris, which may form a necrotic core. Rupture of the fibrous cap or ulceration of the fibrous plaque can rapidly lead to thrombosis and usually occurs at sites of thinning of the fibrous cap that covers the advanced lesion. Thinning of the fibrous cap is apparently due to the continuing influx and activation of macrophages, which release metalloproteinases and other proteolytic enzymes at these sites. These enzymes cause degradation of the matrix, which can lead to hemorrhage from the vasa vasorum or from the lumen of the artery and can result in thrombus formation and occlusion of the artery.[15]

Okamoto *et al.* (2002)[5] in their experimental study show that adiponectin can reduce athero-sclerotic lesion *in vivo*. In this study they use ApolipoproteinE knock-out (ApoE$^{-/-}$) mice as a mouse model for atherosclerosis. ApoE$^{-/-}$ is the best characterized model of atherosclerosis model,[20] these mice can spontaneously develop hyper-lipidemia and all phases of atherogenic lesion throughout arterial tree in both high fat and normal diet.[20,21] In ApoE$^{-/-}$ mice treated with recombinant adenovirus expressing human adiponectin, atherosclerotic lesion area and the diameter of lipid droplet are lower than in ApoE$^{-/-}$ mice treated

recombinant adenovirus expressing â-galactosidase. Adiponectin treated mice also expressing low mRNA level of VCAM-1, Class-A MSR, and TNF-á, but expressing normal CD36 mRNA level.[5]

Ouedraogo *et al.* (2007)[22] reports adiponectin protects the vasculature *in vivo* via increased endothelial Nitrit Oxide (eNO) bioavailability with suppression of leukocyte-endothelium interactions. Loss of adiponectin induces a primary state of endothelial dysfunction with increased leukocyte-endothelium adhesiveness. Using intravital microscopy to measure leukocyte-endothelium interactions in adiponectin-deficient (Ad^{−/−}) mice, they found that adiponectin deficiency was associated with a 2-fold increase in leukocyte rolling and a 5-fold increase in leukocyte adhesion in the microcirculation. Adiponectin deficiency also drastically reduced levels of eNO in the vascular wall. Immunohistochemical analyses also demonstrated increased expression of VCAM-1 and E-selectin in their vascular wall. Systemic administration of the recombinant globular adiponectin domain (gAd) to

Ad^{−/−} mice restoring physiologic levels of eNO, decreased surface expression of VCAM-1 and E-selectin, thus attenuated leukocyte-endothelium interactions.

Other study reports local adiponectin treatment significantly reduced the size of atherosclerotic plaque counted with ultrasonography. Adenovirus expressing adiponectin gene is locally transferred at the abdominal aorta of high-fat dieted-rabbits. Adiponectin also significantly suppressed the mRNA expression of VCAM-1 and ICAM-1, suggest that local adiponectin treatment suppresses the development of atherosclerosis *in vivo.*[6] Immunohistochemical staining on aortas taken from human subjects underwent an operation for abdominal aortic aneurysms showed that adiponectin was detected around macrophages in the human injured aorta where a thrombus was attached. Thus, adiponectin rapidly accumulate in the vascular wall when the endothelial barrier is injured.[18] The role of adiponectin in atherosclerosis can be shown in figure 3.
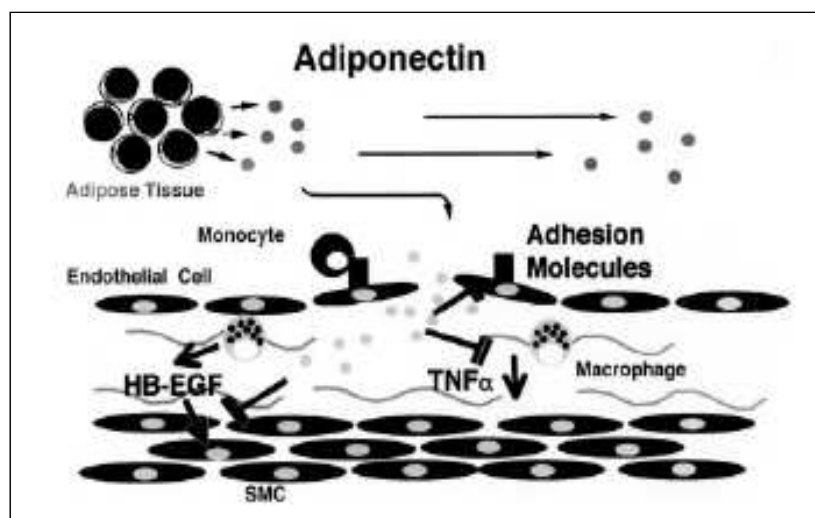

Figure. 3. Molecular mechanism of anti-atherogenic property of adiponectin [14]

Conclusion and Perspective

From this review, we can conclude that adiponectin has many biologic activity related to atherosclerosis, from the molecular to experimental level of researches. Therefore it can be speculated that adiponectin might play a role in the therapeutic strategy against atherosclerotic disease. Further investigations are required to determine the potential therapeutic benefits of adiponectin.

REFERENCES

1. Pittass AG, Joseph N A, and Greenberg AS. Adipocytokines and Insulin Resistance, J. Clin. Endocrinol. Metab. 2004; 89 (2): 447-52.
2. Diez JJ and Iglesias P. The Role of the Novel Adipocyte-derived Hormone Adiponectin in Human Disease. European Journal of Endocrinology. 2003; 148: 293-300.
3. Fantuzzi G and Mazzone T. Adipose Tissue and Atherosclerosis: Exploring the Connection, Arterioscler. Thromb. Vasc. Biol. 2007; 27: 996-1000.

4. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, et al. Globular Adiponectin Protected ob/ob Mice from Diabetes and ApoE-deficient Mice from Atherosclerosis. J. Biol. Chem. 2003; 278 (4): 2461-68.

5. Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, et al. Adiponectin Reduces Athero-sclerosis in Apolipoprotein E-Deficient Mice. Circulation. 2002; 106: 2767-70

6. Li CJ, Sun HW, Zhu1 FL, Chen L, Rong Y Y, Zhang Y, et al. Local Adiponectin Treatment Reduces Athero-sclerotic Plaque Size in Rabbits. Journal of Endo-crinology, 2007; 193: 137–45.

7. Shaibi GQ, Cruz ML, Weigensberg MJ, Toledo-Coral, C M, Lane C J, Kelly L A et al. Adiponectin Indepen-dently Predicts Metabolic Syndrome in Overweight Latino Youth. J. Clin. Endocrinol. Metab. 2007; 92: 1809-13.

8. Frystyk J, Berne C, Berglund L, Jensevik K, Flybjerg A, and Zethelius B. Serum Adiponectin Is a Predictor of Coronary Heart Disease: A populational-based-10 Year Follow-up Study in Elderly Men. J. Clin. Endocrinol. Metab. 2007; 92: 571-76.

9. Scherer PE, Williams S, Fogliano M, Baldini G, and Lodish HF. A Novel Serum Protein Similar to C1q, Produced Exclusively in Adipocytes. J. Biol. Chem.1995; 270 (45): 26746-49.

10. Chandran M, Phillips S A, Ciaraldi T, and Henry R. Adiponectin: More Than Just Another Fat Cell Hormone? Diabetes Care. 2003; 26 (8): 2442-50.

11. Kadowaki T and Yamauchi T. Adiponectin and Adiponectin Receptor, Endocrine Reviews, 2005; 26: 439-51.

12. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita, S. et al. Impaired Multimerization of Human Adipo-nectin Mutants Associated with Diabetes Molecular Structure and Multimer Formation of Adiponectin. J. Biol. Chem. 2003; 278 (41): 40352-63.

13. Pajvani U B, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, et al. Structure-Function Studies of the Adipocyte-secreted Hormone Acrp30/Adiponectin. J. Biol. Chem. 2003; 278 (11): 9073-85.

14. Matsuzawa Y, Funahashi T, Kihara S, and Shimomura I. Adiponectin and Metabolic Syndrome. Arterioscler. Thromb. Vasc. Biol. 2004; 24: 29-33.

15. Ross R. Atherosclerosis-An Inflammatory Disease. NEJM. 1999; 340 (2): 115-26.

16. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y et al. Novel Modulator for Endothelial Adhesion Molecules: Adipocyte-Derived Plasma Protein Adiponectin. Circulation. 1999; 100: 2473-76.

17. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A., Okamoto, et al. Adipocyte-Derived Plasma Protein, Adiponectin, Suppresses Lipid Accumulation and Class A Scavenger Receptor Expression in Human Monocyte-Derived Macrophages. Circulation, 2001; 103: 1057-63.

18. Ouchi N, Kihara S, Arita Y, OkamotoY, Maeda K, Kuriyama et al. Adiponectin, an Adipocyte-derived Plasma Protein, Inhibits Endothelial NF-κB Signal-ing through A cAMP-dependent Pathway. Circulation. 2000; 102: 1296–1301.

19. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto et al. Adipocyte-derived Plasma Protein Adiponectin Acts as A Platelet-derived Growth Factor-BB-binding Protein and Regulates Growth Factor-induced Common Postreceptor Signal in Vascular Smooth Muscle Cell. Circulation. 2002; 105: 2893-98.

20. Madamanchi A. Mouse Models in Cardiology Research. MJM. 2004; 8: 34-39.

21. Nakashima Y, Plump AS, Raines EW, Breslow JL, and Ross R. ApoE-Deficient Mice Develop Lesions of All Phases of Atherosclerosis Throughout the Arterial Tree. Arterioscler. Thromb. 1994; 14: 133-40.

22. Ouedraogo R, Gong Y, Berzins B, Wu X, Mahadev K, Hough K et al. Adiponectin Deficiency Increases Leukocyte-Endothelium Interactions via Up-regulat-ion of Endothelial Cell Adhesion Molecules in Vivo. J. Clin. Inves. 2007; 117: 1718–26.