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CONTENTS

RESEARCH

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LITERATURE REVIEW

THE ROLE OF PLATELETS SCD40L TO ATHEROGENESIS

(Peran sCD40L Trombosit terhadap Aterogenesis)

Liong Boy Kurniawan

ABSTRAK

Peran CD40 dan CD40L terhadap maturasi dan diferensiasi sel limfosit telah diteliti sebelumnya. CD40L diekspresikan oleh berbagai sel lainnya seperti: makrofag, sel dendritik, neutrofil dan endotelial. Trombosit juga dapat mengekspresikan CD40L dan dapat dilepaskan dalam bentuk terlarut yaitu sCD40L. Telaahan ini bertujuan untuk mengetahui peran sCD4-0L yang dihasilkan oleh trombosit pada aterogenesis lewat penjelasan. sCD40L dapat menyebabkan gangguan fungsi endotel, pelepasan ROS, peningkatan aktivitas ICAM, VCAM dan MMP, aktivasi trombosit dan destabilisasi plak melalui interaksi dengan berbagai molekul lain seperti OxLDL. Aterogenesis dapat dipicu melalui interaksi sCD40L. Berbagai penemuan di bidang Farmakologi dan segi lain perlu dikaji untuk menghambat sCD40L dalam aterogenesis. Penelitian lebih lanjut dan mendalam masih diperlukan untuk membuktikan peran sCD40L sebagai petanda peramal kejadian aterogenesis.

***Kata kunci**: sCD40L, trombosit, aterogenesis*

ABSTRACT

The role of CD40 and CD40L in the maturation and differentiation of B lymphocyte cells has been previously described. CD40L is also expressed by other cells such as macrophages, dendrite cells, neutrophils and endothelial cells. Platelets are also proven to express CD40L and can release it into a soluble form, known as sCD40L. This review was aimed to the role of sCD40L produced by platelets in the atherogenesis process. Explain through the interaction with other molecules such as OxLDL, sCD40L may cause endothelial dysfunction, ROS releasing, increased activity of ICAM, VCAM and MMP, platelets activation and plaque destabilization. Atherogenesis can be triggered by sCD40L interaction. Findings in the pharmacology and other aspects need to be studied to inhibit sCD40L in the atherogenesis process. Further in depth research is required to prove the role of sCD40L as a predictor of atherogenesis.

Key words: sCD40L, platelets, atherogenesis

INTRODUCTION

Cluster of Differentiation (CD) 40 is a molecule that was originally discovered to play a role of the humoral immune system. This molecule was first identified in 1985 and is expressed at all stages of cell maturation and differentiation of B lymphocytes. On the other hand, CD40L ligand is mainly expressed in activated T $CD4^{+}$.1 CD40 is also expressed on a number of other cells such as epithelial cells, fibroblasts, smooth muscle cells and platelets. CD40 expression is induced by proinflammatory stimuli such as Interleukin (IL) 1, 3, 4, Tumor Necrosis Factor Alpha (TNF-α) and interferon-γ.2،3 Oxidized Low Density Lipoprotein (oxLDL) also induces CD40L gene expression.4

CD40 is a type I transmembrane protein receptor and is included in the tumor necrosis factor super family. This molecule is encoded in chromosome 20 (q12-q13.2).5 The gene that encodes CD40L lies in the X chromosome in position Xq26.3-Xq27.1 with the length of deoxynucleotide acid (DNA) at 12-13kb and has five exons.6 This molecule is a type 2 transmembrane and included in the same superfamily as CD40.7 Another name of CD40L is CD154 which has a size of 39 kD and can be transformed into a soluble form with a size of 18 kD.8 Soluble CD40L (sCD40L) still has the ability to bind the CD40 receptor and is believed to be biologically active.9 The interaction between CD40 and CD40L is originally known as a co-

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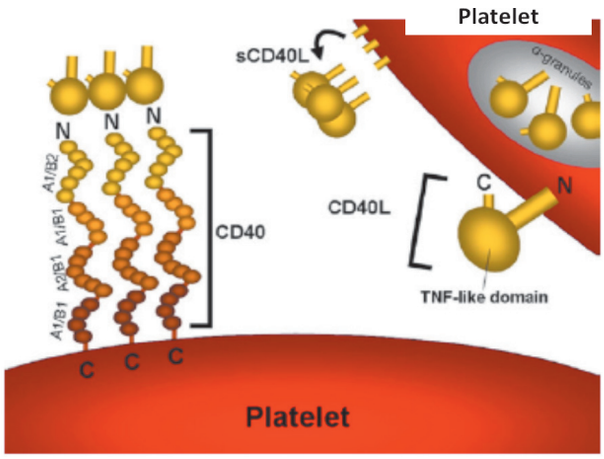

Figure 1. The structure of CD40, CD40L and sCD40L receptors, modification13

stimulator molecule which acts as antigen presenting cells and activated T CD4+. CD40 and CD40L are both expressed on the majority of immune cells (lymphocytes, monocytes, dendritic cells, neutrophils and mast) as well as non-immune cells (endothelial cells, vascular smooth muscle) and epithelium. Platelets are also proven to be the main source of CD40L and sCD40L.[2,3]

The platelets express CD40L after being stimulated by a number of platelet activators such as thrombin and thrombin receptor agonists such as collagen. CD40L expression on the surface of platelets depends on intracellular calcium and activation of protein kinase.10 Increased sCD40Llevel is also found in platelets of diabetic patients after stimulation with thrombin or peptide, thrombin activator. Insulin resistance induces the release of sCD40L from platelets and increases CD40L expression on murine megakaryocytes.11 CD40/CD40L the expression of platelets is partly regulated by nitric oxide. This study shows that the inhibition of nitric oxide synthase in human causes a reduction of phosphorylation platelets and induces platelet activation that increases CD40 expression excessively.12The structure of CD40, CD40 L and sCD40L receptors can be seen in Figure 1.

DISCUSSION

Numerous studies have demonstrated the role of sCD40L in cardiovascular incidence. A prospective study in a group of middle-aged females with the risk of cardiovascular for 4 years found that females with a level of sCD40L over the percentile of 95 (> 3.71 ng/mL) had a 3.3 times risk of suffering from cardiovascular disease in the future.[14] Another study comparing the expression of CD40L/CD154 and sCD40L/CD154 level in patients with acute myocardial infarction, unstable angina and stable angina found that the expression of CD40L/CD154 on platelets and sCD40L/CD154 increased in patients with unstable angina and myocardial infarction.[15] Similar findings were also reported previously in which the levels of membrane-bound sCD40L and CD40L were higher in patients with angina, especially unstable.[16] The results of the research involving 880 patients with atrial fibrillation connecting sCD40 to stroke found that sCD40L level was not related to the risk and course of the stroke with the group.[17] This probably reflects the role of platelet activation that is limited to thrombogenesis due to atrial fibrillation. Similar findings were also reported previously in which the levels of membrane-bound sCD40L and CD40L were higher in patients with angina, especially unstable one.[16] The results of the research involving 880 patients with atrial fibrillation that related sCD40 to stroke found that sCD40L level was not related to the risks and course of stroke in the group.[17] This probably indicated the role of platelet activation that was limited to thrombogenesis due to atrial fibrillation.

In a particular research involving micro particles of human atherosclerotic plaques that expressed CD40L and stimulated endothelial cell proliferation after ligation of CD40 and triggered angiogenesis in vivo.[18] Stimulation of platelets with recombination of sCD40L triggered the expression of M.RNA platelet aggregation and conjugation of platelets and leukocytes. The platelets isolated from CD40L deficient mice showed a decrease in the release of nitric oxide compared to the one from wild-type mice.[19] A report said that the level of sCD40L in blood components was higher than in fresh blood plasma. The highest levels of sCD40L were found in the sample of platelet concentrates from apheresis, followed by platelet concentrates from whole blood and the one from packed red blood cells.[20]

Patients with Diabetes Mellitus (DM) type 1 and 2 had higher levels of sCD40L than the comparison. The high level of SCD40L was associated with in vitro adhesion molecules, the release of Monocyte Chemo-Attractant Protein-1 (MCP-1), impaired endothelial cell migration as well as increased O2- resulted from monocytes.[21] sCD40L level and soluble P-selectin were used as an index of platelet activation, IL-6 served as proinflammatory cytokines and tissue factor which acted as the coagulation initiator which overall was higher in patients with DM type 2 than the comparison. Intervention in diabetic group lowered sCD40L level significantly.[22] The administration of thiazolidinedione for 12 weeks lowered sCD40L level up to 29% in patients with DM.[23] In the randomized, double blind study involving 110 patients with familial hypercholesterolemia treated with atorvastatin 80 mg/

day or simvastatin 40 mg/day for two (2) years showed a decrease in sCD40L level up to 40%.[24]

The pivotal role of CD40/CD40L in the development of atherosclerotic plaque was shown in LDL receptor-deficient mice fed a high cholesterol diet. The formation and progress of the atherosclerotic lesion were suddenly reduced by interrupting the signaling of CD40 in the mice using a neutralizing anti-CD40L antibody.[25] Initial trigger of the expression of CD40/CD40L in atheroma was still unclear but several studies showed that oxidized LDL likely played a role in this process. Oxidized LDL induced the expression of CD40 and CD40L in endothelial cells, smooth muscle and macrophage in human.[4] The signal of CD40/CD40L in endothelial cells stimulated the results of Reactive Oxygen Species (ROS), inhibited the results of endothelial Nitric Oxide (NO) and caused disruption to the function.[26]

The CD40 ligation to endothelial cells and smooth muscle induced the expression of adhesion molecules such as E-selectin, Vascular Cell adhesion Molecule1 (VCAM-1), intercellular adhesion Molecule1 (ICAM-1), which increased the recruitment of monocytes and lymphocytes to the lesion.[27] Leukocytes recruitment was then accelerated by the secretion of MCP-1, IL-1, IL-6 and TNF-α induced by CD40L.[2,3] CD40L in activated platelets could trigger inflammatory reactions expressing endothelial cells, which induced secretion of chemokines, the expressed adhesion molecule, increased recruitment and extravazation of leukocytes to the site of lesion.[28] A variety of cells expressing CD40 and or CD40L are shown in Table 1.

The platelets which expressed CD40 in a resting and activated state, or when CD40L was activated. sCD40L released by platelets during thrombosis had three (3) roles of process namely: inflammation through the product and release of proinflammatory cytokines from vascular cells and metalloproteinases matrix derived from cells, thrombosis by stabilizing the thrombus rich in platelets as well as restenosis by inhibiting reendothelisation injured blood vessels, resulting in the activation and proliferation of smooth muscle cells (see Figure 2).[29]

CD40L is not expressed in resting platelets, but will quickly be expressed once the platelets are activated. Expressed CD40L on the surface of platelets generally will be released after a period of several minutes to several hours to form soluble in trimeric form called sCD40L. Some of the compounds that can activate platelets include adenosine diphosphate, thrombin and collagen. The translocation of CD40L takes place along with the release of granule α contents including Platelet Derived Growth Factor (PDGF), Transforming Growth Factor Beta (TGF-β), Platelet Factor 4 (PF4) and thrombospondin (TSP). Antagonist GPIIb/IIIa

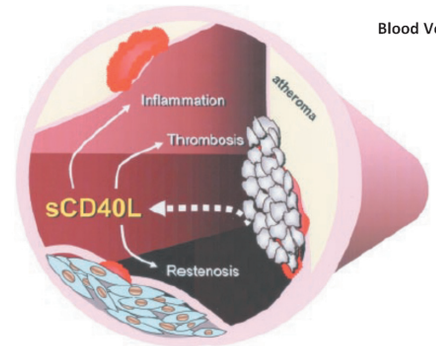

Figure 2. Three roles of sCD40L after being released from platelets during the modification of thrombosis[29]

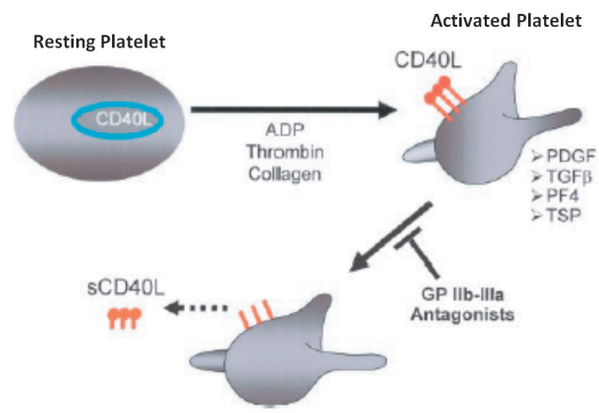

Figure 3. Process of releasing sCD40L in platelets stimulation, modification[29]

Table 1. A variety of cells that express CD40 and CD40L[9]

Type of expressing cell	CD40		CD40L		Form of CD40L
	Resting	Activated	Resting	Activated	
CD4+ T cells	(-)	(+++)	(-)	(+++)	membrane, soluble
B cells	(+++)	(+++)	(++)	(+++)	Membrane
Macrophages	(+)	(+++)	(+)	(+++)	Membrane
Platelets	(++)	(+++)	(-)	(+++)	Membrane, soluble
Dendritic cells	(-)	(+++)	(+)	(+)	Membrane
Neutrophils	(+)	(+++)	(+)	(+)	Membrane
Endothelial cells	(+)	(+++)	(+)	(+)	Membrane

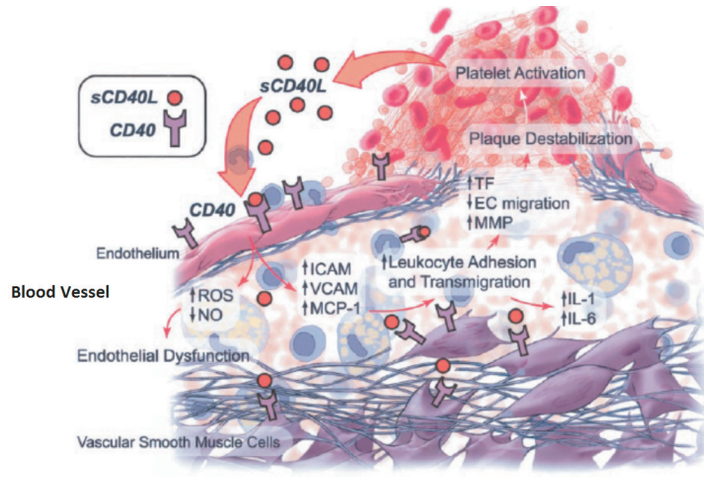

Figure 4. CD40/CD40L and the modification inflammatory[31]

inhibits hydrolysis and the release of sCD40L from platelets (Figure 3).

Some reports showed that more than 95% of CD40L circulating was derived from platelets.[29] Vascular endothelial cells, smooth muscle and macrophages in human increased the expression of matrix metalloproteinase (MMP) by ligation of CD40 including MMP-1, MMP-8 and MMP-13.[2,3] CD40 also made the plaque vulnerable by inducing the expression of tissue factor both in endothelial cells and smooth muscle. The increased level of TF triggered thrombogenic power in lesion rupturing through the extrinsic pathway of blood coagulation.[30] The process related to the role of sCD40L on the incidence of atherosclerosis is shown in Figure 4.

CONCLUSION

CD40L is expressed primarily on activated platelets cells other than playing a role in cueing T cells. The interaction with CD40 molecules plays a great role in atherosclerosis. CD40L expressed on platelets will experience the release and will form compounds soluble in the blood plasma called sCD40L. sCD40L is proven to play a great role in inflammation and atherogenity through interaction with various factors such as: oxLDL. Further process will lead to: impaired endothelial function, release of ROS, increased activity of ICAM, VCAM and MMP, as well as platelets and plaque destabilization

The studies on drugs that reduce the expression and release of sCD40L on platelets and inhibit the function are required given the important role of sCD40L in atherogenesis. Further studies on various other factors, in addition to drugs that can suppress the amount of sCD40L which will ultimately reduce the risk of atherosclerosis are also required. Further and in depth research on the role of sCD40L as a marker for atherogenesis also needs to be applied in the laboratory medicine.

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