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Regular Article

Oxidized low-density lipoprotein-induced CD147 expression and its inhibition by high-density lipoprotein on platelets *in vitro* ☆

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ABSTRACT

Introduction: Matrix metalloproteinases (MMPs) are believed to progressively degrade the collagenous components of the protective fibrous cap, leading to atherosclerotic plaque rupture or destabilization. Oxidized low-density lipoprotein (ox-LDL) enhances the release of CD147, known as the extracellular MMP inducer, from coronary smooth muscle cells. However, whether ox-LDL can induce platelet CD147 expression is unknown. Therefore, we investigated the influence of ox-LDL and high-density lipoprotein (HDL) on CD147 expression on human platelets.

Materials and Methods: Washed platelets were incubated with ox-LDL (or native LDL) and HDL or anti-LOX-1 monoclonal antibody prior to incubation with ox-LDL. In parallel, buffer (PBS) was added to washed platelets as a control. The expression levels of CD147, CD62P, CD63 and Annexin V were assessed by flow cytometry, and soluble CD147 from the platelets was assessed by an enzyme-linked immunosorbent assay. Laser scanning microscopy (LSM) and transmission electron microscopy (TEM) were used to visualize the morphological changes and granule release, respectively, from the platelets.

Results: Platelets treated with ox-LDL exhibited a significant increase in the expression of CD147 (or Annexin V), followed by increases in CD62P and CD63, compared with the control group. In contrast, HDL or anti-LOX-1 monoclonal antibody decreased these effects. The expression of soluble CD147 increased as the concentration of ox-LDL used to treat the platelets increased. After exposure to ox-LDL, morphological changes and granule release in the platelets were visualized by LSM and TEM. Additionally, the TEM revealed that HDL inhibits alpha-granule release.

Conclusions: In platelets, ox-LDL stimulates the release of CD147 via binding to LOX-1, whereas HDL inhibits this effect. This finding could provide new insights concerning the influence of ox-LDL and HDL on plaque stability by the up-regulation of CD147 on platelets.

Introduction

Most acute coronary syndromes (ACS), such as unstable angina, myocardial infarction, and sudden death, are triggered by plaque rupture and the subsequent thrombus [1–3]. CD147, an extracellular matrix metalloproteinase (MMP) inducer (EMMPRIN), can up-regulate MMPs, and the up-regulation of MMPs leads to atherosclerotic plaque rupture by degrading the extracellular matrix (ECM), which is the main component of fibrous caps [4–6]. CD147 was first identified as a surface protein on tumor cells [7] and was found to be expressed constitutively on monocytes, granulocytes, and lymphocytes [8].

CD147, as a novel receptor, was recently reported to be localized in the open canalicular system (OCS) of platelets and α granules, and CD147 activates platelets and stimulates MMP-9 synthesis in monocytes [9]. Platelet CD147 expression is up-regulated after washed platelets are exposed to various stimuli (e.g., thrombin, ADP, and collagen) *in vitro* [9]. Importantly, in vivo studies have shown that platelet CD147 expression is significantly greater in patients with coronary artery disease (CAD) compared with that in a control population and demonstrates a stronger association with age [10]. Furthermore, CD147 is able to enhance platelet-monocyte interactions in vivo and to promote monocyte recruitment to the arterial wall [11].

Ox-LDL is thought to be involved in the initiation of atherosclerotic lesions, mainly by leading to foam cell formation and vascular endothelial damage [12]. However, more importantly, a growing body of evidence suggests that elevated levels of circulating oxidized LDL serve as a sensitive marker for CAD [13], independently associate with the carotid intima-media thickness [14], display a significant positive correlation with the severity of acute coronary syndromes [15], and even serve as a

Abbreviations: Ox-LDL, oxidized low-density lipoprotein; HDL, high-density lipoprotein; MMPs, matrix metalloproteinases; EMMPRIN, extracellular matrix metalloproteinase inducer; ECM, extracellular matrix; ACS, acute coronary syndromes; CAD, coronary artery disease; PRP, platelet-rich plasma; OCS, open canalicular system.

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strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men [16]. Because ox-LDL is present in the circulation [17,18], it can make contact with platelets. Thus, research on the ox-LDL interaction with platelets is necessary. Previous studies have shown that mildly oxidized LDL enhances platelet aggregation via the activation of phospholipase A2 [19,20], whereas heavily oxidized LDL becomes a platelet aggregation inhibitor [21]. Although some studies reported that a low concentration of ox-LDL inhibited ADP-induced platelet aggregation [22,25], ox-LDL is generally thought to be bound to several receptors on platelets, including SRA [23], CD36 [24], LOX-1 [21], platelet activating factor (PAF) receptor [26], and SR-PSOX/CXCL16 [27], thereby leading to platelet activation. LOX-1 serves as a dominant receptor involved in ox-LDL binding to activated platelets. However, whether blocking LOX-1 affects the expression of CD147 in platelets is still unclear. Ox-LDL has been shown to increase CD62P expression in platelets [22,24]. Moreover, ox-LDL has been reported to enhance serotonin release from platelet-dense granules [28] and to induce CD147 expression on coronary artery smooth muscle cells [29]. However, whether ox-LDL stimulates platelet CD147 expression is unknown. In addition, HDL is capable of inhibiting platelet activation [30]. Therefore, we examined whether HDL has an inhibitory effect on platelet CD147 expression. In this study, we evaluated the effects of ox-LDL, HDL and anti-LOX-1 monoclonal antibody (mAb) in the expression of CD147 in platelets *in vitro*.

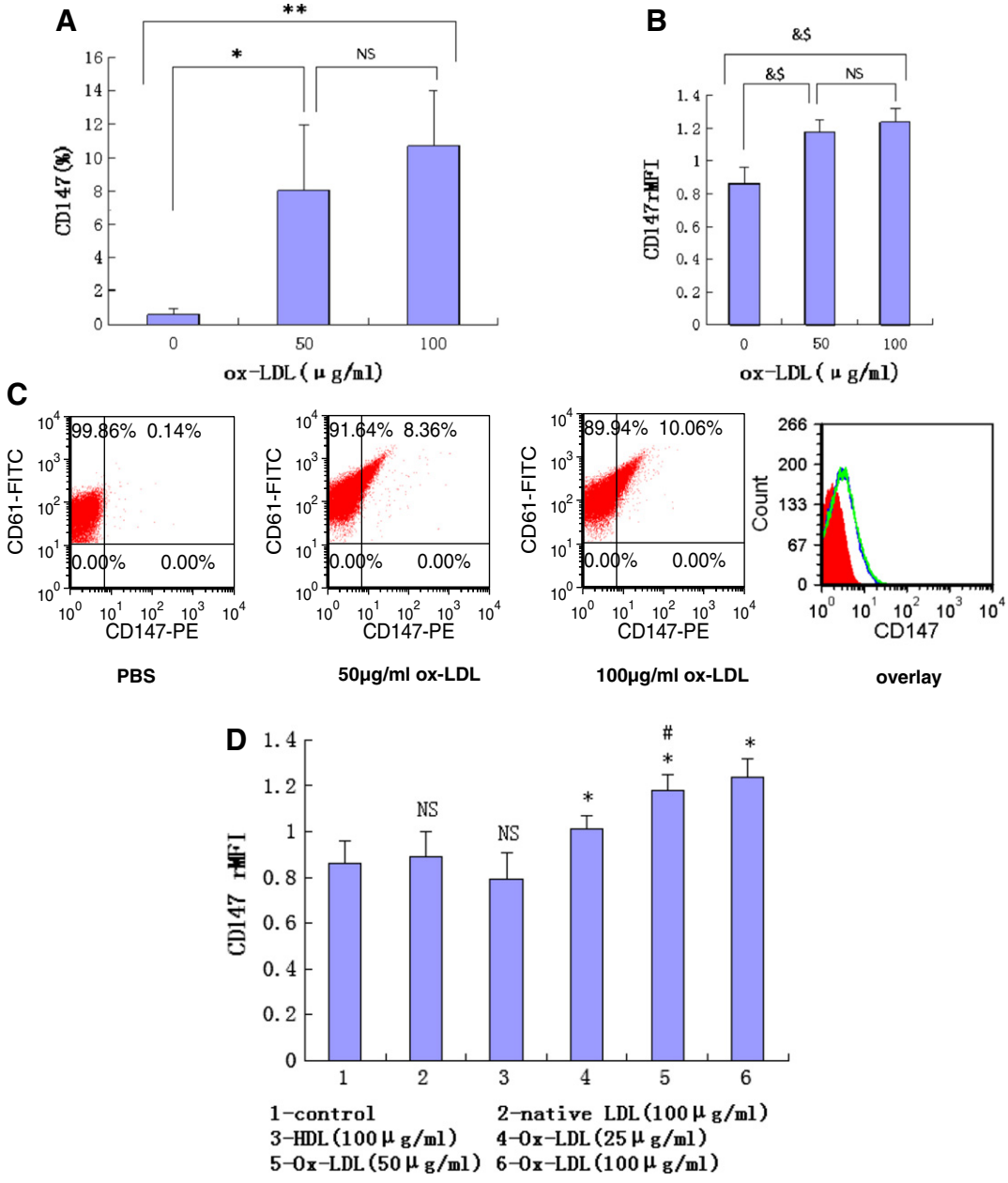

Fig. 1. Ox-LDL induces platelet CD147 expression. Flow cytometry was used to detect the expression of CD147 on platelets. A, Percentages of CD147-positive platelets treated with ox-LDL (0, 50 or 100 μg/ml); B, Change in the platelet CD147 relative mean fluorescence intensity (rMFI = monoclonal antibody/corresponding isotype control) following ox-LDL treatment. C, Representative of 5 experiments; the overlain histograms on the right show the expression levels of each platelet marker in PBS-treated and ox-LDL-treated platelets (PBS-treated, red; 50 μg/ml ox-LDL, blue; 100 μg/ml ox-LDL, green) $^{*}P = 0.032$, $^{**}P = 0.006$, $^{\&\$}P < 0.001$, NS = not significant. In D, NS vs. Control, $^{*}P < 0.01$ vs. control, $^{\#}P < 0.01$ vs. Ox-LDL (25 μg/ml). The data represent the means ± SD of 5 independent experiments.