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The combined effect of sidestream smoke and dynamic shear stress on endothelial cell inflammatory responses

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ABSTRACT

Both cigarette smoke and altered shear stress are risk factors for cardiovascular disease. Sidestream smoke is the major component of secondhand smoke. An *in vitro* model was developed to investigate the combined effect of sidestream smoke and physiologically relevant dynamic shear stress on endothelial cell inflammatory responses. Human coronary artery endothelial cells were exposed to sidestream smoke and dynamic shear stress (at normal or low level) simultaneously, and endothelial cell surface ICAM-1 and thrombomodulin expression was measured using a solid phase ELISA approach. Endothelial cell associated complement activation was assessed by cell surface C1q, C4d and iC3b deposition. The expression of complement inhibitors (C1 inhibitor and CR1) and C1q receptors (gC1qR and cC1qR) was also measured. The results demonstrated that sidestream smoke enhanced endothelial cell surface ICAM-1 expression and caused cell activation. While under normal pulsatile shear stress, endothelial cell surface ICAM-1 expression reduced to baseline level as thrombomodulin expression increased. Physiological dynamic shear stress also induced a significant increase in endothelial cell associated complement activation, through enhanced gC1qR and cC1qR expression. Subsequently, CR1 expression increased as well. Overall, physiological dynamic shear stress reduced endothelial cell activation by enhancing thrombomodulin expression. Physiological flow also enhanced the expression of endothelial cell surface C1q receptors, gC1qR and cC1qR, to promote C1q activation and initiate the classical pathway complement activation, which could be a potential protective mechanism to clear injured or damaged cells.

Introduction

Cigarette smoking is a risk factor for many cardiovascular diseases, including atherosclerosis and thrombosis [1]. Smoking not only affects smokers, but also nonsmokers, through secondhand smoking. Sidestream smoke, i.e., smoke coming from the smoldering end of a cigarette, is the major component of secondhand smoke (approximately 85%) [2]. Sidestream smoke has a higher concentration of carcinogens and is considered more toxic compared to mainstream smoke [3,4]. It has been reported that sidestream smoking can impair normal functions of vascular wall endothelial cells by decreasing blood vessel dilation and increasing vessel contraction [5]. Sidestream smoking can also induce endothelial cell thrombotic and inflammatory responses [6]. Endothelial cells play important roles in regulating hemostasis and maintaining vascular integrity. Endothelial cells are constantly exposed to shear stress induced by blood flow. Altered shear stress is a risk factor for cardiovascular inflammation and atherosclerosis initiation [7]. Under physiological blood flow, hemodynamic shear stress helps to maintain the normal functions of endothelial cells; while pathological shear stress (low or elevated) can lead to endothelial cell activation. Shear stress activated endothelial cells produce intercellular adhesion molecule-1 (ICAM-1) and release many chemo-attractant proteins to promote inflammation [8,9]. Endothelial cell surface thrombomodulin, which has anti-thrombogenic and anti-inflammatory properties, can be down-regulated by pathological shear stress [10].

Complement activation is an important component of local and systemic inflammatory response. We reported previously that mainstream cigarette smoke extract and shear stress can activate endothelial cells and lead to complement activation [11]. In that study, complement component C4d, iC3b and SC5b-9 deposition was observed on mainstream smoke and shear stress treated endothelial cells [11,12]. Complement activation on endothelial cells could be either protective or pathogenic. Under physiological conditions, it may help to clear apoptotic cells to prevent local vascular damage [13]; while under pathological conditions, endothelial complement activation may enhance inflammation in the arterial wall, leading to atherosclerosis [14,15]. Maresh et al. demonstrated that a mixture of mainstream and sidestream smoke can regulate a number of endothelial genes, including vasoregualtory genes and complement factor H [16]. However, there is no evidence if sidestream smoke can directly regulate complement activation.

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Atherosclerosis could disturb local blood flow significantly and cause altered local shear stress. Patients with cardiovascular disease (such as atherosclerosis) may be exposed to secondhand smoke, or mostly, sidestream smoke. It was reported that sidestream smoke can accelerate the development of arteriosclerotic plaques [2]. But how endothelial cells respond to sidestream smoke under altered shear stress conditions was not fully understood. In the present study, we aimed to investigate the combined effect of sidestream smoke and shear stress on endothelial cell activation and associated complement activation *in vitro*. To improve the physiological relevance of the *in vitro* model, human coronary artery endothelial cells were subject to dynamic shear stress waveforms (associated with coronary artery disease) obtained from computational fluid dynamics models [17,18], and sidestream smoke extract simultaneously.

Materials and Methods

Sidestream Smoke Extract

Sidestream smoke extract was prepared from Marlboro 100's (16 mg tar, 1.2 mg nicotine, Philip Morris). The setup used to collect sidestream smoke was described previously [19]. Briefly, the filter of a cigarette was blocked and the non-filtered smoke from the smoldering end of the cigarette was collected and extracted into hepes buffered saline solution (pH 7.4), at a puffing rate mimicking that of a smoker.

Cell Culture

Human coronary artery endothelial cells (HCAEC) and all culture reagents were purchased from ScienCell Research Laboratories (Carlsbad, CA). Cells were maintained in endothelial cell media supplied with 5% fetal bovine serum, endothelial cell growth factors, and penicillin/streptomysin. HCAEC were used between passage 1 and 7.

Platelet Poor Plasma

Fresh human rich plasma (PRP), anticoagulated with 0.32% sodium citrate, was obtained from Oklahoma Blood Institute (Oklahoma City, OK). Platelet poor plasma (PPP) was prepared by centrifugation of PRP at room temperature as described before [12]. PPP was then aliquoted and frozen at −80 °C until use.

Antibodies

Murine anti human ICAM-1 antibody (1 μg/mL, Abcam, Cambridge, MA) was used to measure HCAEC surface ICAM-1 expression. Murine anti human thrombomodulin antibody (1 μg/mL, Abcam) was used to measure endothelial cell surface thrombomodulin expression. Endothelial cell associated complement activation was measured by C1q, C4d and iC3b deposition on cell surface, using murine anti human C1q, C4d and iC3b antibodies (5 μg/mL, Quidel Cooperation, San Diego, CA). C1 inhibitor released by stimulated endothelial cells was measured using a commercial EIA kit from Quidel. Murine anti human CR1 antibody (2 μg/mL, Ancell Cooperation, Bayport, MN) was used to measure endothelial cell surface complement receptor type 1 (CR1, CD35) expression. Endothelial cell surface C1q receptors, cC1qR (bind to the collagen tail of C1q) and gC1qR (bind to the globular head of C1q) were detected using a rabbit polyclonal anti-cC1qR peptide and two murine monoclonal gC1qR antibodies (clone number 74.5.2 and 60.11) respectively [12,20–22]. All peptides and antibodies against C1q receptors were developed in Dr. Ghebrehiwet's laboratory. All antibodies used in this study were diluted in hepes buffered modified Tyrode's solution (HBMT, pH 7.4).

Sidestream Smoke and Shear Stress Treatment

Confluent HCAEC monolayer was treated with sidestream smoke extract at the final concentration of 1 cigarette/5 L overnight at 37 °C. It was assumed that the total blood volume of an individual was 5 L, and this dose was used to mimic the blood concentration of sidestream smoke when one individual was exposed to one cigarette.

Following sidestream smoke treatment, HCAEC were exposed to dynamic shear stress waveforms (Fig. 1) in a cone and plate shearing device for one hour at 37 °C [23]. These waveforms were obtained from a human coronary artery computational fluid dynamics model [17,18]. The normal shear stress waveform mimics physiological pulsatile shear stress (0.1 – 1.2 Pa) imposed on coronary artery endothelial cells, and the low shear stress waveform (0.05 – 0.3 Pa) represents pathological low pulsatile shear stress that often develops in recirculation zones in a stenosed coronary artery. Low amplitude shear stress is usually considered atherogenic for vascular wall endothelial cells [24,25].

ICAM-1, Thrombomodulin, gC1qR, cC1qR and Complement Inhibitor Expression

After sidestream smoke and dynamic shear stress treatment, endothelial cell activation and inflammatory responses were measured by

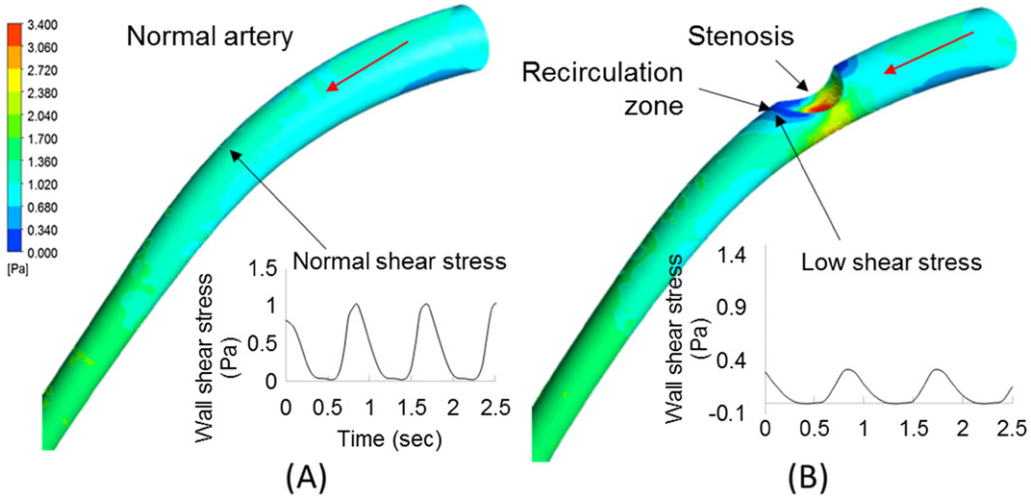

Fig. 1. Normal and low pulsatile shear stress waveforms applied to HCAEC in a cone and plate shearing device. Red arrows indicate flow direction in the (A) normal or (B) stenosed coronary artery.