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S-nitrosocaptopril interrupts adhesion of cancer cells to vascular endothelium by suppressing cell adhesion molecules via inhibition of the NF-κB and JAK/STAT signal pathways in endothelial cells

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ABSTRACT

Inflammatory cytokines can induce the expression of cell adhesion molecules (CAMs) in endothelial cells. The induction may play an important role in attracting circulating tumor cells (CTCs) to endothelial cells. S-nitrosocaptopril (CapNO) is known to produce vasorelaxation and interfere the hetero-adhesion of CTCs to vascular endothelium via down-regulating the expression of CAMs. To elucidate the mechanisms underlying the inhibition of CapNO on CAMs, in this study, we examined the relationship between cytokines and CAMs expression and investigated the effects of CapNO on cytokine-induced NF-κB and JAK/STAT signal pathways. The activation of CAMs by cytokines was dependent on concentrations and reaction time of cytokines, and the combination of cytokines could produce a strong synergistic effect. IL-1β induced the expression of CAMs on endothelial cells by activating NF-κB and JAK/STAT pathways. CapNO inhibited IL-1β-stimulated NF-κB pathway by down-regulating IKK-α and inducing IκB-α directly. CapNO also inhibited JAK/STAT pathway by inhibiting JAK2 and STAT3 expressions. These effects bring about down-regulating CAMs expression on endothelial cells. These results suggest that CapNO may interrupt adhesion of cancer cells to endothelium by suppressing CAMs via inhibiting the NF-κB and JAK/STAT pathways in endothelial cells.

1. Introduction

Cancer is recognized to be a worldwide serious public health problem. A total of 14.1 million new cancer cases and 8.2 million cancer deaths occurred all around the world in 2012 (Torre et al., 2015). Metastasis is the principal cause of cancer-associated death (Nguyen et al., 2009; Sethi and Kang, 2011). Indeed, metastasis is the daily threat to the cancer survivors, among them 30–70% will eventually face the metastatic nightmares within 2–5 years after surgery or local irradiation (Wan et al., 2015). Current therapies against malignant tumor metastasis are unsatisfactory (DeSantis et al., 2014).

Distant metastasis relies on hematogenous dissemination via the blood circulation. This process is very complex and cancer cells must complete a series of sequential steps (Chambers et al., 2002). Survival of the circulating tumor cells (CTCs) within the bloodstream and then adhesion to the vascular endothelium at the metastatic sites are crucial for initiating metastatic cascade (Labelle and Hynes, 2012; Lu et al., 2015; Plaks et al., 2013). According to the widely accepted "seed and soil" hypothesis, CTCs (the "seeds") succeed in forming metastasis at a distant site if it provides a hospitable "soil" (Fidler, 2003; Hiratsuka et al., 2011). Vascular endothelium "soil" may be "cultured" by microenvironmental changes in target organs (Quail and Joyce, 2013). The high expression of cell adhesion molecules (CAMs; including VCAM-1, ICAM-1, E-selectin, et al.) on endothelial cells may play an important role in attracting CTCs to endothelial cells (Honn and Tang, 1992; Lauri et al., 1991; Rice and Bevilacqua, 1989). Meanwhile, inflammation is also a critical reason for tumor metastasis progression, especially CAMs expression (Coffelt and de Visser, 2014; Coussens and Werb, 2002; Mantovani et al., 2008). Inflammatory cells release cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1α and IL-1β (Coussens and Werb, 2002). These cytokines up-regulate the expression of CAMs in endothelial cells

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(Meager, 1999), and CAMs mediate adhesion of CTCs to the vascular endothelium. The causal relationship between inflammatory responses and CAMs expression is widely accepted, but the cellular and molecular mechanisms mediating this relationship remain unresolved.

Based on the understanding of cancer metastatic processes especially in CTCs adhesion to vascular endothelium process, we proposed that chemically to interfere the adhesion of CTCs to vascular endothelial cells (the most important and first step of metastatic cascade) may efficiently prevent cancer metastatic cascade from initiation. S-nitrosocaptopril (CapNO) is the S-nitrosylated captopril (Cap) that possesses dual pharmacological properties of both nitric oxide (NO) and angiotensin-converting enzyme inhibitor (Jia and Blantz, 1998; Jia et al., 1999). CapNO exhibits many NO-like activities such as direct vasorelaxation in vivo and in vitro (Jia and Blantz, 1998; Jia and Wong, 2001) and inhibition of platelet aggregation (Jia et al., 2000; Loscalzo, 2001). CapNO also enhances the transcellular permeability of Taxol (Jia et al., 2003) and has been shown to be a potent radiosensitizer in a syngeneic mouse tumor model (Jordan et al., 2010). Moreover, CapNO has weak cytotoxicity which is similar to Cap (Jia et al., 2001). We recently demonstrated that CapNO produced direct vasorelaxation through the cGMP-dependent mechanism, and interfered with hetero-adhesion of cancer cells to vascular endothelium via down-regulating cytokine-induced expression of CAMs (Lu et al., 2014). However, the potential molecular mechanisms of CapNO inhibition on CAMs is not clear.

Based on the above-mentioned beneficial effects of CapNO, we have reasons to believe that CapNO may be a safe and efficient cancer metastatic chemopreventive. In the present study, we examined the relationship between inflammatory responses and CAMs expression, and investigated the mechanisms by which CapNO inhibits CAMs expression.

2. Material and methods

2.1. Antibodies, chemicals and human samples

Mouse anti-human CD54-PE (P-phycoerythrin), mouse anti-human CD106-PE, mouse anti-human CD62E-APC (Allophycocyanin) and CS&T beads were obtained from Becton Dickinson (BD) Pharmingen™. Rabbit anti-human VCAM-1 (CD106) antibody, mouse anti-human ICAM-1 (CD54) antibody and mouse anti-human E-selectin (CD62E) antibody were obtained from Abcam. Rabbit anti-human AKT antibody was obtained from Santa Cruz Biotechnology, Inc. Rabbit anti-human NF-κB p50 antibody, rabbit anti-human NF-κB p65 antibody, rabbit anti-human IκB-α antibody, rabbit anti-human IKK-α antibody, rabbit anti-human p38 antibody, rabbit anti-human JNK antibody, rabbit anti-human ERK1/2 antibody, rabbit anti-human p-AKT antibody, rabbit anti-human JAK2 antibody and rabbit anti-human STAT3 antibody were obtained from Wanlei Biotechnology, Inc. Mouse anti-human beta-actin (β-actin) antibody and human lymphotoxin (LT, TNF-β) were purchased from Cell Signaling Technology, Inc. Recombinant human interleukin-1 beta (IL-1β), recombinant human tumor necrosis factor alpha (TNF-α), recombinant human interferon gama (IFN-γ) and recombinant human interleukin-6 (IL-6) were purchased from Peprotech, Inc. Fibronectin and ECGS were obtained from BD Biocoat™. The preparation and physicochemical characterization of crystalline CapNO were described previously (Jia et al., 2000, 1999). Protocols involving human samples were approved by the local ethics committee and conducted in compliance with the Declaration of Helsinki.

2.2. Cell culture

Human colorectal cancer cells HT29 were obtained from Cell Bank of Chinese Academy. HT29 cells were cultured in Mc COY's 5A medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, obtained from Gibico), 100 units/ml penicillin and 100 μg/ml streptomycin. Human umbilical vein endothelial cells (HUVECs) were isolated from human umbilical veins as our described previously (Lu et al., 2014), and cells were cultured in 1% gelatin-coated tissue culture flasks in M199 medium (Gibico) supplemented with 20% fetal bovine serum (FBS, obtained from Gibico), 8 units/ml heparin, 100 mg/ml ECGS, 100 units/ml penicillin and 100 μg/ml streptomycin. The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and harvested with 0.25% trypsin (GenView) before using. HUVECs were used for these studies from 3 to 6 passages.

2.3. Flow cytometry

Flow cytometric analysis was performed on BD FACSAriaIII (BD Biosciences) cell sorter with the laser excitation set at 488 and 633 nm. The optical alignment and the functional stability tests were carried out with the use of the Cytometer Setup and Tracking system (CS&T, Becton Dickinson). Forward versus side scatter histograms were used to gate on single intact cells. PE dye was excited by 488 nm laser, and the derived fluorescence was first separated through longpass filter of 556 nm. The signals were detected through bandpass filter of 585/42 nm. APC dye was excited by 633 nm laser, and the derived fluorescence was detected through bandpass filter of 660/20 nm. Immunoglobulin conjugates matched for the isotype and fluorochrome were used as the control to assess autofluorescence and non-specific staining. The BD FACSDiva software provided with the system was employed for data acquisition and initial data analysis.

2.4. Activation of adhesion molecules effects of cytokines on HUVECs

The relationship between cytokine doses and CAMs expression was examined by the flow cytometry. The HUVECs were seeded in 6-well culture plates at a density of 5×10^5 cells/well for 24 h. Then the supernatant was replaced with the fresh medium containing different concentrations (0, 0.1, 1, 2, 5, 10, 20, 30 and 40 ng/ml) of IL-1β or TNF-α. After 4 h incubation, cells were collected. The HUVECs were respectively stained with mouse anti-human CD54 (PE-labeled), mouse anti-human CD62E (APC-labeled) and mouse anti-human CD106 (PE-labeled) antibody and incubated at 4 °C for 30 min in the dark. Background staining was performed by staining cells with isotype-matched control. After staining, cells were washed with the staining buffer (2% FBS, 2 mM EDTA in PBS) and resuspended in 500 μL of the staining buffer. Flow cytometric analysis was carried out on the BD FACSAriaIII (BD Biosciences), and the obtained data were analyzed with FlowJo software.

In addition, the relationship between cytokine-induced time and CAMs expression was also examined. HUVECs were incubated with IL-1β (10 ng/ml) or TNF-α (10 ng/ml) for 0, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h, respectively. The cells were collected and stained as mentioned above. The expression of cell-surface ICAM-1, VCAM-1 and E-selectin was measured by flow cytometry.

The activation of CAMs on HUVECs caused by different cytokines was evaluated by flow cytometry. The HUVECs were grown to confluence on 6-well culture plates in fresh medium (without ECGS). After that each well was incubated respectively with IL-1β (10 ng/ml), TNF-β (10 ng/ml), TNF-α (10 ng/ml), IL-6 (10 ng/ml), IFN-γ (10 ng/ml), endothelial cell growth supplement (ECGS, 100 mg/ml) and fibronectin (100 ng/ml) for 4 h. In addition, synergism experiments of cytokines on CAMs expression were