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NF-κB and Cytokines in Pancreatic Acinar Cells

Hyeyoung Kim, Jeong Yeon Seo, Kyung Hwan Kim

Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea

Reactive oxygen species (ROS), generated by infiltrating neutrophils, are considered as an important regulator in the pathogenesis and deveolpment of pancreatitis. A hallmark of the inflammatory response is the induction of cytokine gene expression, which may be regulated by oxidant-sensitive transcription factor, nuclear factor-κB (NF-κB). Present study aims to investigate whether neutrophils primed by 4β-phorbol 12β-myristate 13α-acetate (PMA) affect the productions of H_2O_2 and lipid peroxide (LPO), NF-κB activation and cytokine production in pancreatic acinar cells, and whether these alterations were inhibited by N-acetylcysteine (NAC) and superoxide dismutase (SOD). ROS generation in neutrophils increased by PMA, which was inhibited by NAC and SOD. The productions of H_2O_2, LPO and TNF-α were increased with the amounts of PMA-primed neutrophils added to acinar cells while the productions of H_2O_2, LPO and cytokines increased with time. PMA-primed neutrophils resulted in the activation of two species of NF-κB dimers (a p50/p65 heterodimer and a p50 homodimer). Both NAC and SOD inhibited neutrophil-induced alterations in acinar cells. In conclusion, ROS, generated by neutrophils, activates NF-κB, resulting in upregulation of inflammatory cytokines in acinar cells. Antioxidants such as NAC might be clinically useful antiinflammatory agents by inhibiting oxidant-mediated activation of NF-κB and decreasing cytokine production.

Infiltration of inflammatory cells, such as neutrophils, lymphocytes and monocytes, is quite common in damaged pancreatic glands of models of acute and chronic pancreatitis (1). These phenomenon was also shown in those patients with acute pancreatitis (2) and chronic pancreatitis at early stage (3). Neutrophils are known to be the highest producer of reactive oxygen species (ROS) among those inflammatory cells. Actually ROS production of neutrophils obtained from the patients with acute pancreatitis was enhanced (4). Even though ROS alone cannot initiate experimental pancreatitis, ROS are still considered as an important regulator in the pathogenesis and development of pancreatitis.

Clinical studies have shown the inflammatory cytokines such as IL-1β, IL-6 and TNF-α in the serum of patients with acute pancretitis. The degree of cytokine elevation correlated with disease severity and overall morbidity (5). A key regulator of cytokine induction is the pleiotropic transcription factor nuclear factor-κB (NF-κB). NF-κB represent a family of proteins sharing the Rel homology domain, which bind to DNA as homo- or hetero-dimers, and activates a multitude of cellular stress-related and early response genes such as the genes for cytokines, growth factors, adhesion molecules, and acute phase proteins (6). Several antioxidants such as N-acetylcysteine (NAC) and pyrrolidine dithiocarbamate potentially inhibit NF-κB activation and/or NF-κB interaction with its upstream regulatory binding site thereby preventing NF-κB-mediated transcriptional activation (7, 8). These studies suggest the hypothesis that antioxidants might inhibit cytokine production by inhibiting oxidant-mediated activation of transcription factors. Recently, involvement of NF-κB activation in pancreatitis was proposed in experimental pancreatitis model using cerulein (9, 10). We have shown that NF-κB regulates IL-8 production in *Helicobacter pylori*-stimulated gastric epithelial cells and hydroxyl radical scavengers (mannitol and dimetylthiourea) inhibit IL-8 production (11). Consequently, NF-κB represents a potential target for pharmacological therapy of inflammation. We investigated whether neutrophils primed with 4β-phorbol 12β-myristate 13α-acetate (PMA) affect the productions H_2O_2 and lipid peroxide (LPO) as indices of oxidative stress, NF-κB activation and cytokine production, and whether these alterations are inhibited by an antioxidant NAC and superoxide dismutase (SOD).

Chemiluminecence (CL) assay

The CL value of neutrophils was increased by PMA treatment. Treatment of SOD and NAC inhibited increase in CL values by PMA treatment. These results show that neutrophils, isolated in the present study, have ability to generate ROS. No detectable ROS generation from neutrophils was observed when PMA was omitted, indicating that neutrophils were not activated by purification procedure.

Key Words: *NF-κB; Cytokines; Pancreatic Acinar Cells*

Address for correspondence: Hyeyoung Kim, Ph.D.
Department of Pharmacology, Yonsei University College of Medicine, Seoul 120-752, Korea, Tel: +82.2-361-5232, Fax: +82.2-313-1894,
E-mail: kim626@yumc.yonsei.ac.kr

Oxidative indices and cytokine production in acinar cells

Neutrophils alone did not increase the productions of H_2O_2, LPO and cytokines in acinar cells. However, PMA-primed neutrophils highly increased the productions of these oxidative stress indices and cytokines in the cells. For concentration response of acinar cells to PMA-primed neutrophils, the productions of H_2O_2, LPO and TNF-α were increased with the ratio of PMA-primed neutrophils to acinar cells at 2 hr-culture. For the time response of acinar cells to PMA-primed neutrophils (at the ratio of neutrophils: acinar cell, 10:1), the productions of LPO, IL-1β, IL-6 and TNF-α were increased with time. Further studies on NAC and SOD for the productions of LPO and cytokines, 2 hr-culture time and the ratio of PMA-primed neutrophils: acinar cells, 10:1 were used. Treatments of NAC and SOD inhibited neutrophil-induced increases in LPO and cytokines dose-dependently.

NF-κB activation in acinar cells

Two different NF-κB bands were shown in the cells treated with PMA-primed neutrophils, while the cells at the start of incubation contained a little activated NF-κB. An increased amount of activated NF-κB was detected at 1 hr and even higher levels of activated NF-κB were observed at 2 hr after treatment with PMA-primed neutrophils. Two different NF-κB bands reflects the presence of two species of activated NF-κB dimer; the classic p50/p65 NF-κB heterodimer and a p50 homodimer. Treatment of both ROS scavengers decreased NF-κB complex formation.

The main finding of the present study is that (1) PMA-primed neutrophils stimulate the productions of H_2O_2 and LPO, NF-κB activation and the productions of inflammatory cytokines (IL-1β, IL-6, TNF-α) in pancreatic acinar cells and (2) an antioxidant NAC inhibit LPO production and oxidant-mediated activation of NF-κB and thereby decrease cytokine production by PMA-primed neutrophils similar to SOD.

In conclusion, we hypothesize that ROS, generated by infiltrating neutrophils into pancreas, activate NF-κB in acinar cells, resulting in upregulation of certain cytokines, like IL-1β, IL-6 and TNF-α, which may mediate pancreatic inflammation. We believe that inhibition of a number of inflammatory molecules by targetting NF-κB system represents an exciting and promising approach to the treatment of pancreatitis. By reducing oxidant-mediated NF-κB activation, antoxidants such as NAC might be endowed with clinically useful antiinflammatory effect.

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