Cytokine Regulation by Hypoxia and Ischemia in Lung

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Introduction

Transplantation is one of greatest challenging achievements and saves thousands of lives each year. It also improves the quality of patients who present each year with organ failure. However, the transplanted organs are very insufficiency owing to the overwhelming demands for donated organs. Xenotransplantation may be one of the best possible approaches to solving the severe shortage of human donors, which greatly limits progress in clinical transplantation. Therefore, xenotransplantation is currently a hot topic in bio-organ of biomedical research. Among many species, pigs are suitable animals due to easier animal husbandry, comparatively similar anatomical and physiological similarities to human organs (Hughes, 1986; Pereira-Sampaio et al, 2004; Vodicka et al, 2005).

Lung transplantation is an effective therapeutic option for patients with end-stage pulmonary disease. Ischemia-reperfusion (IR) injury is a major complication of the early postoperative period after lung transplantation and occurs in up to 20% of transplants, resulting in primary graft dysfunction with about 60% mortality (Sun J, 2008). I/R-induced lung microvascular injury ultimately depends upon some balance between lung metabolic stress, the extent of the I/R-induced inflammatory response, endogenous antioxidant levels, and the timing, magnitude, and duration of oxygen free radical generation during both periods of ischemia and reperfusion (Moore TM, 1996).

Ischemic preconditioning may be a model of transient ischemic attack. Numerous laboratory studies have shown that a sublethal ischemic event primes the brain to resist subsequent severe ischemic injury (Miller et al., 2001). Also, recently clinical studies suggest that transient ischemic attack may improve stroke outcome by serving as a preconditioning stimulus and activation neuroprotective processes (Moncayi et al., 2000; Wegener et al., 2004). However, effects of ischemic preconditioning don`t investgate ischemia/reperfusion-induced proinflammatory molecules in lung.

In the process of I/R injury, diverse inflammatory molecules are released from the lung tissue. cPLA2 hydrolyses the membrane phospholipids, resulting in the release of arachidonic acid (AA). The enzyme cyclooxygenase (COX), which converts arachidonic acid (AA) to a prostaglandin, exists in two distinct isoforms, COX-1 and COX-2. COX-1 is largely a constitutive isoform, whereas COX-2 is induced in response to various stimuli (Simmons et al. 2004). The transcription factor nuclear factor-κB (NF-κB) is a nuclear transcription factor found in all cell types, and is involved in cellular responses to stimuli such as acute inflammation (Ross et al., 2000). NF-κB acts on target genes for proinflammatiory cytokines, chemokines, immunoreceptors, cell adgesion molecules, acute phase proteins, and inducible nitric oxide synthase. NF-κB is expressed in the cytoplasm of virtually all cell types, where its activity is controlled by a family of regulatory proteins, called inhibitors of NF-κB (I-κB). The inflammatory products of NF-κB activation have been demonstrated in several cellular models, and critical role of NF-κB in the inflammatory cascade in vivo is now emerging (Blackwell et al., 1996; Chandrasekar et al., 1998; Lentsch et al., 1998; Liu et al., 1997). However, its role in mediating ischemic response through regulation of COX-2 and NF-kB expression in lung tissues is still unknown.

The accumulation of unfolded proteins in the lumen of the endoplasmic reticulum (ER) produces stress in the ER, which results in the activation of intracellular signal transduction pathways to restore normal ER function. This activation is called the unfolded protein response (UPR). The ER stress response involves three major signaling pathways: the pancreatic double-stranded RNA-activated protein kinase-like ER-associated kinase (ER kinase PERK), activating transcription factor-6 (ATF6), and inositol-requiring ER-to-nucleus signal kinase 1 (IRE1) pathways.

Arginine methylation, which is responsible for the regulation of a variety of biological functions, is mediated by protein arginine methylation transferases (PRMTs). Type I PRMTs, including PRMT 1, 3, and 4, catalyze conversion of arginine to a monomethylarginine (MMA) intermediate, which is subsequently converted to asymmetric dimethylarginine (ADMA). Type II PRMTs, such as PRMT5, catalyze the formation of symmetric dimethylarginine (SDMA). However, the role of PRMTs in hypoxia and ischemia has not been clearly elucidated in lungs.

Therefore, we examined the effect of ischemic condition and preischemic condition on expression of inflammatory cytokines, ER stress, and apoptosis in lungs of miniature pig. Furthermore, we also investigated the role of PRMT in lung hypoxia and ischemic condition

Results

1. The changes of inflammatory cytokine during ischemia, ischemia-reperfusion, and preischemic-reperfusion-ischemia conditions

To study whether cPLA2 phosphorylation is involved in ischemia of lung tissue, the expression of cPLA2 was examined in lung homogenates. Lung ischemia induced the phosphorylation of cPLA2 over 15 min. Next, we measured the expression of COX in ischemia, ischemia-reperfusion, and preischemic-reperfusion-ischemia conditions. Ischemia increased the expression of COX-2, but not COX-1. However, ischemia didn't affect the expression of COX-2 in the condition of preischemia-reperfusion The changes of NF-kB was examined. Lung ischemia induced the phosphorylation of NF-kB over 15 min. However, ischemia didn't affect the phosphorylation of NF-kB in the condition of preischemia. We also checked the levels of IKB in these conditions. Ischemia increased the phosphorylation of IK-B. However, ischemia didn't affect the phosphorylation of IK-B in the condition of preischemia-reperfusion.

The involvement of IL-6 in ischemic condition was investigated. Lung ischemia also increased the expression of IL-6, which was prevented by preischemic condition. JNK expression and bradykinin receptor expression are correlated with the profiling of inflammatory cytokine in ischemic lung tissue and preischemic condition of lung tissue.

2. The changes of apoptotic related proteins during ischemia, ischemia-reperfusion, and preischemic-reperfusion-ischemia conditions

To study whether apoptotic molecules are involved in ischemia of lung tissue, the expression of diverse apoptotic molecules such as Bax, Bcl-2, caspase-9, and caspase-3 was examined in lung homogenates. Lung ischemia induced the phosphorylation of Bax but decreased the expression of Bcl-2. It also increased the expression of caspase-9 and caspase-3 mRNA in lung tissues. These alterations of apoptotic molecules in ischemia-reperfusion injury were prevented in pre-ischemic condition.

We also investigated the involvement of ER stress related proteins such as IRE-1 alpha and PERK in lung ischemia/reperfusion. Lung ischemia increased phosphorylation of IRE-1 alpha and PERK, which was also prevented by preischemic condition.

3. Activation of PRMT1 and PRMT5 mediates hypoxia- and ischemia-induced apoptosis in human lung epithelial cells and the lung of miniature pigs: the role of p38 and JNK mitogen-activated protein kinases

This study examined the role of hypoxia in PRMT activation in A549 human lung epithelial cells, as well as the role of ischemia in PRMT activation in the lung of miniature pigs. In A459 cells, hypoxia increased the expression of PRMT1 and PRMT5, and overexpression of PRMT1 and PRMT5 induced apoptosis. The transfection of PRMT1 and PRMT5 small interfering RNA (siRNA) prevented hypoxia-inducible factor (HIF)-1♋ expression and apoptosis in A549 cells. Hypoxia-induced expression of PRMT1 and PRMT5 was blocked by p38 and JNK mitogen-activated protein kinase (MAPK) inhibitors, but not by an inhibitor of extracellular signal-regulated kinases (ERK) 1/2. In the lungs of miniature pigs, ischemia stimulated PRMT1 and PRMT5 expression and induced phosphoryltion of p38

MAPK (p-p38), phosphorylation of JNK (p-JNK), and apoptotic molecules. These results demonstrate that PRMT1 and PRMT5 are involved in hypoxia and ischemia-induced apoptosis via p-p38 MAPK and p-JNK in in vitro and in vivo models.

Conclusion

These results demonstrate that inflammatory cytokines such as IL-6, COX-1, NF-kB and ER stress related apoptotic molecules such as PERK, IRE-1 alpha, eIF-2 alpha are involved in the events of lung ischemic condition. These signaling events are related to apoptosis in lung ischemia. PRMT1 and PRMT5 are also involved in hypoxia or ischemia -induced apoptosis via p-p38 MAPK and p-JNK in *in vitro* and *in vivo* models.