Journal of Cardiothoracic-Renal Research (2006) 1, 147—152

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DNA damage and mismatch repair pathway in lung ischemia and reperfusion injury☆

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Received 20 May 2006; accepted 20 May 2006

KEYWORDS
DNA damage;
DNA repair;
Apoptosis;
Ischemia—reperfusion injury;
MYH;
Oxidative stress;
Lung transplantation;
8-oxoG

Summary
Background: Oxidative damage induced by reperfusion is responsible for increased morbidity and mortality following lung transplantation. A stable and deleterious DNA adduct, 8-oxogaunine (8-oxoG) results due to oxidative DNA damage. Mut-Y homologue (MYH) is a DNA repair enzyme promoting DNA reconstruction through the mismatch repair pathway to repair 8-oxoG lesion. We investigated the role of DNA mismatch repair pathway mediated by MYH in the setting of lung ischemia and reperfusion.
Methods: Left lungs of the adult Sprague Dawley rats were subjected to 1 h ischemia and 2 and 4 h reperfusion. Un-operated animals served as controls. Quantification of 8-oxoG was performed using immunohistochemistry (IHC) and MYH was analyzed by Western blot. Apoptosis was assessed by caspase-3 levels.
Results: Indices of inflammation and permeability were raised in both reperfusion groups. There was significant increase in DNA damage as reflected by positive 8-oxoG staining in 2 h (22% increase) and 4 h reperfusion (31% increase) compared to control ($p < 0.01$). MYH staining by IHC was significantly reduced in 2 and 4 h reperfusion compared to controls ($p < 0.05$). Down regulation of DNA repair enzyme (MYH) was mirrored functionally by decreased protein levels in lung tissues subjected to reperfusion compared to controls. Increasing apoptosis was detected in the reperfusion groups as reflected by caspase-3 IHC and protein estimation by Western blot.
Conclusion: Reperfusion leads to increased DNA damage and down regulation of DNA mismatch repair pathway in a model of ischemia and reperfusion in lungs. Gene therapy targeted at this pathway may prove an attractive therapeutic intervention to reduce reperfusion injury in lung transplantation.

☆ Presented at 42nd Annual Meeting of the Society of Thoracic Surgeons, January 30—February 1, 2006, Chicago, IL, United States, as a poster.

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1574-0668/$ — see front matter
doi:10.1016/j.jccr.2006.05.007

Introduction

There is increased morbidity and mortality associated with reperfusion injury during lung transplantation. Early graft failure is strongly associated with reperfusion injury during lung transplantation [1]. Reperfusion injury is implicated in chronic sequelae like the bronchiolitis obliterans syndrome [2]. These effects are primarily mediated through generation of reactive oxygen species (ROS) [3,4]. ROS including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and the peroxynitrite radical ($ONOO^-$), play a critical role in the pathogenesis of reperfusion injury and can have a detrimental effect on the stability of the DNA [5].

Interactions between DNA and ROS produce DNA strand breaks and base modifications. Eight DNA lesions are known to be mediated by free radicals. Amongst the various oxidative-damaged DNA products, 8-oxo-7,8-dihydrodeoxyguanine (8-oxoG) 8-oxoG is the most stable and deleterious adduct [6]. Un-repaired 8-oxoG lesions in DNA can lead to A/8-oxoG mismatches during DNA replication, and result in G:C to T:A mutations [7,8]. Mut-Y homologue (MYH) removes the adenine mis-incorporated opposite 8-oxoG, G, or C following DNA replication [9].

The role of DNA damage and its repairing mechanisms, particularly MYH has not been investigated in the context of lung reperfusion injury. We hypothesize that reperfusion injury leads to a disparity between DNA injury and repair mechanisms in the context of lung transplantation. We aimed to study the DNA damage and repair ability by MYH in a model of unilateral normothermic ischemia and reperfusion model of rat lung.

Methods

All procedures were performed under aseptic conditions and anesthesia was achieved using a combination of ketamine and acepromazine. Experimental animals included 10 weeks old, male Sprague Dawley rats (200—250 g, Harlan, Indianapolis, IN). The animals were allowed 2 weeks to acclimatize, and were housed at a temperature of 20—22 °C, humidity of 70% and 12 h alternating light—dark cycle. The rats were allowed water and food ad libitum till the surgery. Animals received humane care in compliance with the ''Guide for Care and use of Laboratory Animals'', published by the National Research Council (National Academy Press, 1996). The Institutional Animal Care and Use Committee (IACUC) approved all the procedures in this study.

Model of ischemia and reperfusion injury

The model is previously reported [10], briefly, after a tracheotomy through cervical incision animals were connected to volume/pressure controlled ventilator (Harvard Apparatus, Holliston, MA). The left pulmonary artery was approached through a left thoracotomy, and dissected from the bronchus and occluded for 1 h and then animals sacrificed after 2 and 4 h. The experiment was conducted at normothermia.

Experimental protocol

The control animals did not undergo any type of surgery and served as controls. There were two reperfusion groups, a 2 and 4 h reperfusion following 1 h of ischemia. There were five animals in each group; animals not surviving the reperfusion period were excluded from the analysis. At the end of the procedure, animals were sacrificed and bronchoalveolar lavage (BAL) performed, the cells were counted immediately, and supernatants saved. For histological sections, the pulmonary artery was flushed with saline to remove any blood in pulmonary tree and the pulmonary architecture maintained by agarose instillation in the bronchial tree prior to preserving in formalin. Routine histological processing was undertaken thereafter.

Determination of DNA damage in lung cells

Formalin-fixed, paraffin-embedded lung sections from ischemia—reperfusion rats were evaluated for 8-oxoG with anti-8-oxoG antibody (Trevigen, Gaithersburg, MD). Briefly, the slides were fixed and washed with PBS, pH 7.4, and incubated for 40 min at 37 °C. The DNA was denatured by soaking the slides in 4N HCl for 7 min. After slides were incubated with 50 mM Tris base for 5 min at room temperature and two washings with PBS, 10% FBS was added for 1 h at room temperature to block nonspecific staining sites. Slides were then incubated with 3% H_2O_2 for 30 min at room temperature to block endogenous peroxidase. Thereafter, slides were incubated with primary anti-8-oxoG monoclonal antibody (diluted 1:100 in 10 mM Tris—HCl, pH 7.5, 10% serum) overnight at 4 °C, rinsed twice with PBS, and incubated with secondary anti-mouse antibody (1:100) conjugated with 20 μg/mL streptavidin—horseradish peroxidase in 1× PBS for 1 h at room temperature. After being stained with diamino benzamide tetrahydrochloride (DAB; DAKO, Carpinteria, CA) and counterstained with methyl green, slides were examined under light microscopy. The percentage of cells staining positive for 8-oxoG was quantified as follows: number of positive hepatocytes/total number of hepatocytes × 100 = percent 8-oxoG-positive cells.

Immunohistochemical staining

Immunohistochemical staining was performed as we have previously described [11]. Briefly, after samples were deparaffinized, sections was rinsed in 0.1 mol/L PBS for 20 min and blocked in 10% normal horse serum for 2 h. The sections then were incubated with primary Cas-3, AIF antibody (1:100) in 10% normal horse serum or 10% normal rabbit serum and 0.3% Triton X-100 for 20 h at 4 °C. After endogenous peroxidase activity was quenched by exposure of the slides to 0.3% H_2O_2 and 10% methanol for 20 min, the sections were washed in PBS and incubated with secondary antibody—horseradish peroxidase conjugate (Amersham Biosciences UN Limited, Little Chalfont Buckinghamshire, UK). The final reaction was achieved by incubating the sections with freshly prepared reagent containing 3-amino-9-ethylcarbazole (Sigma) dissolved in dimethyl-