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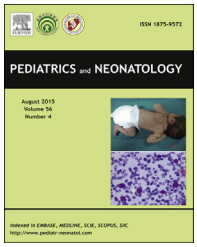

ORIGINAL ARTICLE

Neonatal Hyperoxia Exposure Induces Kidney Fibrosis in Rats

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Key Words
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Background: Human and animal studies have demonstrated that neonatal hyperoxia increases oxidative stress and adversely affects glomerular and tubular maturity. This study was undertaken to determine how exposure to neonatal hyperoxia affected kidney morphology and fibrosis and to elucidate the relationship between connective tissue growth factor (CTGF) and collagen expression in rat kidneys.

Methods: Sprague–Dawley rat pups were exposed to either hyperoxia or ambient air. The control groups were maintained in ambient air for 1 week and 3 weeks. The hyperoxia groups were exposed to >95% O_2 for 1 week and subsequently placed in an environment of 60% O_2 for an additional 2 weeks. The animals were euthanized on Postnatal Day 7 or 21 and the kidneys underwent histological analyses and oxidative stress and total collagen measurements.

Results: The rats reared in O_2-enriched air exhibited significantly higher tubular injury scores (1.4 ± 0.5 vs. 0.7 ± 0.7 on Day 7; 1.4 ± 0.5 vs. 0.6 ± 0.5 on Day 21), a larger proportion of the cortex occupied by glomeruli (25.5 ± 4.1 vs. 21.3 ± 3.1% on Day 7; 20.1 ± 3.5 vs. 17.1 ± 1.7% on Day 21), larger glomerular sizes (84.7 ± 5.8 vs. 77.5 ± 6.1 μm on Day 7; 88.4 ± 2.9 vs. 84.9 ± 3.1 μm on Day 21), and higher total collagen content (54.1 ± 27.5 vs. 18.3 ± 6.3 μg/mg protein on Day 7; 397.4 ± 32.8 vs. 289.5 ± 80.0 μg/mg protein on Day 21) than did rats reared in ambient air. Immunohistochemical expressions of oxidative stress marker 8-hydroxy-2′-

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deoxyguanosine and CTGF immunoreactivities were significantly higher in the rats reared in O_2-enriched air compared with the rats reared in ambient air on Postnatal Days 7 and 21.
Conclusion: Neonatal hyperoxia exposure contributes to kidney fibrosis, which is probably caused by activated CTGF expression.

1. Introduction

Oxygen therapy is often used to treat newborns that present respiratory disorders; however, administering supplemental oxygen yields toxic effects on the glomerulus and proximal tubules of the kidney, causing renal damage. Human and animal studies have demonstrated that neonatal hyperoxia increases oxidative stress and adversely affects glomerular and tubular maturity. This is manifested by enlarged renal corpuscles, renal tubular necrosis, and interstitial inflammation during the perinatal period.[1–4] These detrimental effects may extend into adulthood, presenting as hypertension.[4,5] Furthermore, rabbit kidneys respond to environmental hyperoxia by exhibiting augmented tissue oxygenation.[6] Various studies have suggested that neonatal hyperoxia exposure increases oxidative stress in the kidneys, inducing kidney injury. However, the effects and exact mechanisms of hyperoxia exposure on kidney injury and fibrosis remain unknown.

Connective tissue growth factor (CTGF) is part of the CTGF-Cyr61/Cef10-Nov family, playing a crucial role in kidney pathogenesis, and enabling functions including migration, hypertrophy, and fibronectin production in mesangial cells; the epithelial–mesenchymal transition and fibronectin production of tubular epithelial cells; and collagen production by renal interstitial fibroblasts.[7–12] CTGF was originally identified in conditioned media from human umbilical vein endothelial cells and was implicated in fibroblast proliferation, cellular adhesion, and angiogenesis.

It is now recognized that short exposure to high-oxygen concentrations causes increases in oxidative stress in preterm infants.[2] Elmarakby and Sullivan[13] postulated that increased oxidative stress during diabetes stimulated CTGF fibrotic signaling in the kidneys. Increased CTGF expression also occurred in ureteral obstruction-induced kidney fibrosis.[14,15] Few studies have explored how neonatal hyperoxia affects kidney morphology and fibrosis and CTGF expression. We hypothesized that neonatal hyperoxia exposure induced structural kidney changes and fibrosis, accompanied by increased CTGF expression in rats. This study explored how neonatal hyperoxia exposure affected kidney morphology and fibrosis, examining the relationship between CTGF and collagen expression in hyperoxia-exposed rat kidneys.

2. Materials and methods

2.1. Animals and hyperoxia exposure

This study was performed in accordance with the guidelines provided and approved by the Animal Care Use Committee of Taipei Medical University. Time-dated pregnant Sprague–Dawley rats were housed in individual cages. Within 12 hours of their birth, the rat litters were pooled, randomly redistributed to the newly delivered mothers, and subsequently exposed to either hyperoxia or ambient air. The nursing mothers were rotated between the experimental and control litters every 24 hours to avoid oxygen toxicity. The control groups were maintained in normoxia for 1 week and 3 weeks. The hyperoxia groups were exposed to >95% O_2 for 1 week and subsequently placed in an environment of 60% O_2 for an additional 2 weeks (NexBiOxy, Hsinchu, Taiwan). The animals were euthanized using intraperitoneal injections of pentobarbital (100 mg/kg) on Postnatal Day 7 or 21, and their body and kidney weights were recorded. One kidney per animal underwent histological analysis, and the second kidney underwent oxidative stress and total collagen measurements.

2.2. Histological examination

The kidney was placed in 4% paraformaldehyde, washed in phosphate-buffered saline, and serially dehydrated in increasing concentrations of ethanol prior to being embedded in paraffin. Five-micrometer tissue sections were stained with hematoxylin, eosin, and Masson's trichrome; examined using light microscopy; and subsequently assessed for kidney morphology and fibrosis. The histological analysis of the kidney was modified according to the suggestions of Toledo-Rodriguez et al.[16] The fraction of the cortex occupied by glomeruli was calculated as the ratio of the grid points that touched the cortex to the grid points that touched glomeruli. The sizes of the individual glomeruli located in the middle cortex and juxtamedullary zone were calculated as the average of the largest and smallest glomerular diameters within a field of view; the calculations involved 10 ± 5 glomeruli per kidney. Kuruş et al[17] defined tubular injury as tubular dilation, tubular atrophy, vacuolization, the degeneration and sloughing of tubular epithelial cells, or thickening of the tubular basement membrane. Only cortical tubules were used in the scoring system, where 0 = no tubular injury; 1 = <10% of tubules injured; 2 = 10–25% of tubules injured; 3 = 26–50% of tubules injured; 4 = 51–75% of tubules injured; and 5 = >75% of tubules injured.

2.3. Immunohistochemistry for 8-hydroxy-2′-deoxyguanosine and CTGF

Immunohistochemical staining was performed on 7-μm paraffin sections by using immunoperoxidase visualization.