Pediatric RESEARCH

BASIC SCIENCE ARTICLE

Maternal inflammation exacerbates neonatal hyperoxia-induced kidney injury in rat offspring

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BACKGROUND: Preclinical studies have demonstrated that maternal inflammation or neonatal hyperoxia adversely affects kidney maturation. This study explored whether prenatal lipopolysaccharide (LPS) exposure can augment neonatal hyperoxia-induced kidney injury.

METHODS: Pregnant Sprague–Dawley rats received intraperitoneal injections of LPS (0.5 mg/kg) in normal saline (NS) or NS on 20 and 21 days of gestation. The pups were reared in room air (RA) or 2 weeks of 85% O_2, creating the four study groups, NS + RA, NS + O_2, LPS + RA, and LPS + O_2. Kidneys were taken for oxidase stress and histological analyses.

RESULTS: The rats exposed to maternal LPS or neonatal hyperoxia exhibited significantly higher kidney injury score, lower glomerular number, higher toll-like receptor 4 (TLR4), myeloperoxidase (MPO), and 8-hydroxy-2′-deoxyguanosine (8-OHdG) expressions, and higher MPO activity compared with the rats exposed to maternal NS and neonatal RA. The rats exposed to both maternal LPS and neonatal hyperoxia exhibited significantly lower glomerular number, higher kidney injury score, TLR4, MPO, and 8-OHdG expressions compared with the rats exposed to maternal LPS or neonatal hyperoxia.

CONCLUSION: Maternal inflammation exacerbates neonatal hyperoxia-induced kidney injury and the underlying mechanism may be related to activation of TLR4 and increased oxidative stress.

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INTRODUCTION

Chorioamnionitis is an intrauterine inflammation in either mixed fetal–maternal tissues or fetal tissue origin.[1] Pregnant Sprague–Dawley rats received intraperitoneal injections of lipopolysaccharide (LPS) on gestation day 18 or 20 induced proinflammatory cytokines in the maternal and fetal serum, amniotic fluid, chorioamnion, and placenta, and renal cortex in rat offspring.[2–4] Maternal inflammation induced by an intraperitoneal injection of LPS also increases oxidative stress, decreases glomerular numbers and creatinine clearance rates, and leads to hypertension in the adult offspring rats.[5] These results indicate that intraperitoneal administration of LPS can model maternal infection that lead to a fetal inflammatory response.

Human and animal studies have demonstrated that neonatal hyperoxia increases oxidative stress and induces glomerular and tubular damage. These are manifested as enlarged renal corpuscles, renal tubular necrosis, interstitial inflammation, and kidney fibrosis during the perinatal period.[6–10] Chorioamnionitis is a common cause of preterm birth.[11] Oxygen therapy is often used to treat preterm newborns that present respiratory disorders. This may predispose the preterm infants exposed to chorioamnionitis to impair renal structure and function during the neonatal period. However, the effects and exact mechanisms of chorioamnionitis and neonatal hyperoxia exposure on kidney injury remain unknown.

Human kidney development is completed in utero by gestational week 36.[12] The nephrogenesis of rats begins on embryonic day 12 and is completed between 10 and 15 days after birth.[13] Rats are born with immature kidneys, and the first 2 postnatal weeks correspond to the second and third trimesters of kidney development in the human fetus. Therefore, the neonatal rat is a useful model for studying the mechanisms of kidney development in the human fetus. We hypothesized that maternal inflammation could augment neonatal hyperoxia-induced kidney injury in rats. The purposes of our study were to determine the effects of maternal inflammation and neonatal hyperoxia exposure on kidney injury and to elucidate the mechanisms that mediate maternal inflammation and neonatal hyperoxia-induced kidney injury in neonatal rats.

METHODS

Animal model

Our study was approved by the Animal Care and Use Committee at Taipei Medical University (LAC-2017-0191). Time-dated pregnant Sprague–Dawley rats were housed in individual cages with 12-h light–dark cycles. Laboratory food and water were available ad libitum. The rats received LPS treatment that consisted of an intraperitoneal injection of LPS (0.5 mg/kg) from *Escherichia coli* serotype 0111:B4 (Sigma-Aldrich, St. Louis, MO, USA) in normal saline (NS) on gestation days 20 and 21. The rat dams were allowed to deliver vaginally at term. Within 12 h of birth, litters were pooled and randomly redistributed to the newly delivered mothers, and the pups were then randomly assigned to room air (RA) or oxygen-enriched atmosphere (85% O_2) treatment from postnatal days 1 to 14. The litter size was adjusted according to the recommendation for testing maternal effects on reproductive/developmental toxicity.[14] Thus, creating four study groups, NS +

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Table 1. Body weights, kidney weights, and kidney-to-body-weight ratios of LPS-treated rats reared in ambient or O_2-enriched air

Treatment	*n*	Body weight (g)	Kidney weight (g)	Kidney-to-body-weight ratio (%)
Normal saline + room air	16	22.68 ± 2.51	0.29 ± 0.03	1.29 ± 0.11
Normal saline + hyperoxia	9	18.84 ± 5.71	0.29 ± 0.08	1.58 ± 0.14***
LPS + room air	14	22.32 ± 3.60	0.28 ± 0.05	1.23 ± 0.07
LPS + hyperoxia	9	17.40 ± 3.11*,**	0.29 ± 0.04	1.69 ± 0.17***

Values represent means plus/minus SDs
LPS lipopolysaccharide
*$P < 0.001$, compared with the normal saline + room air and LPS + room airgroups
**$P < 0.05$, compared with the LPS + room airgroups
***$P < 0.01$, compared with the normal saline + room air group

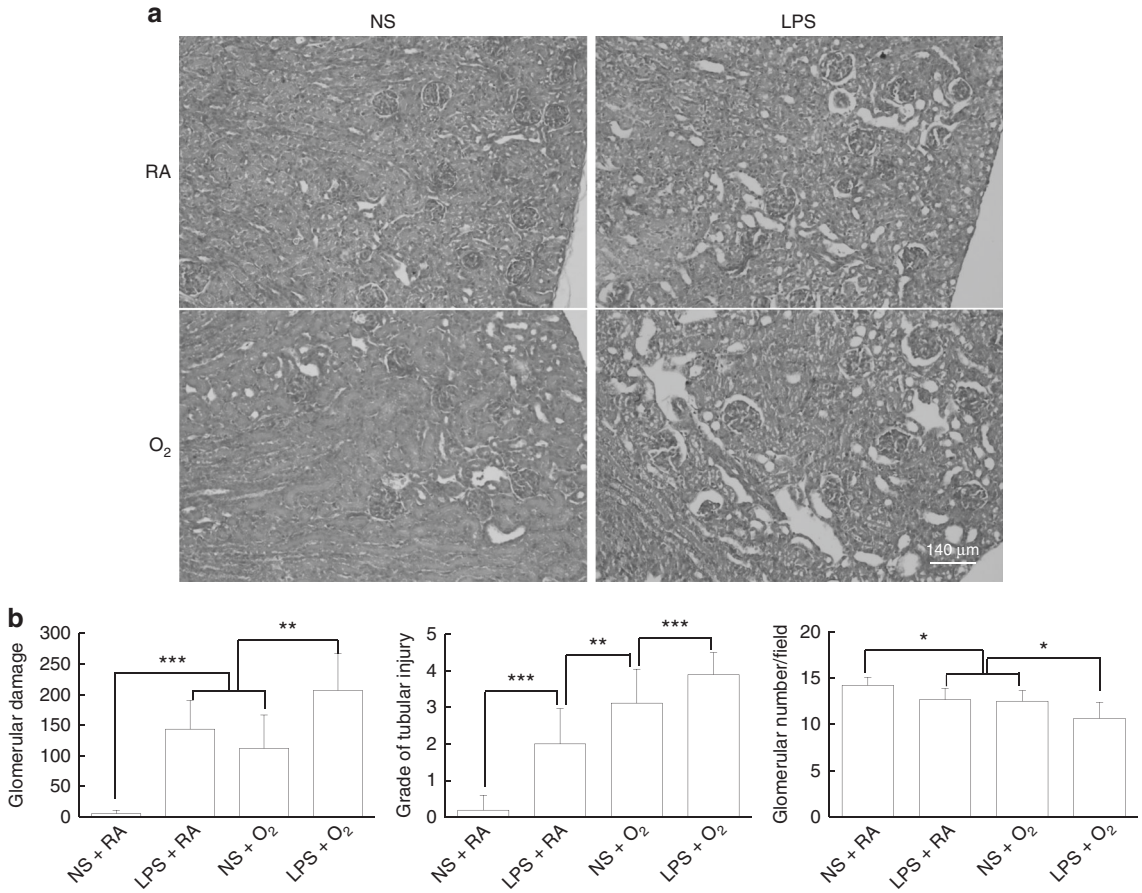

Fig. 1 **a** Representative histology (hematoxylin and eosin (H&E) staining) and **b** injury score in 14-day-old rats exposed to prenatal lipopolysaccharide (LPS) or normal saline (NS) and postnatal room air (RA) or hyperoxia. Tubular atrophy, dilatation of the tubular lumen, increased space between the renal tubules, and decreased glomerula number were observed in the maternal LPS- or neonatal hyperoxia-exposed groups. The rats exposed to maternal LPS and neonatal hyperoxia exhibited higher tubular and glomerular injury score and lower glomerular number than the rats exposed to maternal LPS or neonatal hyperoxia (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, two-way analysis of variance (ANOVA) followed by Bonferroni post-test). $n = 8$–10 rats

RA, NS + O_2, LPS + RA, and LPS + O_2. To avoid oxygen toxicity in the nursing mothers, they were rotated between the O_2 treatment and RA control litters every 24 h. An oxygen-rich atmosphere was maintained in a transparent 40 × 50 × 60-cm plexiglass chamber receiving O_2 continuously at 4 L/min. Oxygen levels were monitored using a ProOx P110 monitor (BioSpherix, Redfield, NY, USA). The animals were euthanized using intraperitoneal injections of pentobarbital (100 mg/kg) on postnatal day 14, and their body and kidney weights were recorded.

Histological examination

The kidney was fixed in 4% paraformaldehyde, after serially dehydrated in increasing concentrations of ethanol prior to being embedded in paraffin. Five-micrometer tissue sections were stained with hematoxylin and eosin or periodic acid-Schiff's (PAS). All of the stained slides were examined and photographed using Olympus BX43 light microscopy for subsequently assessed for kidney morphology. According to a modification of the method of Pichler et al.,[15] a semiquantitative analysis of the

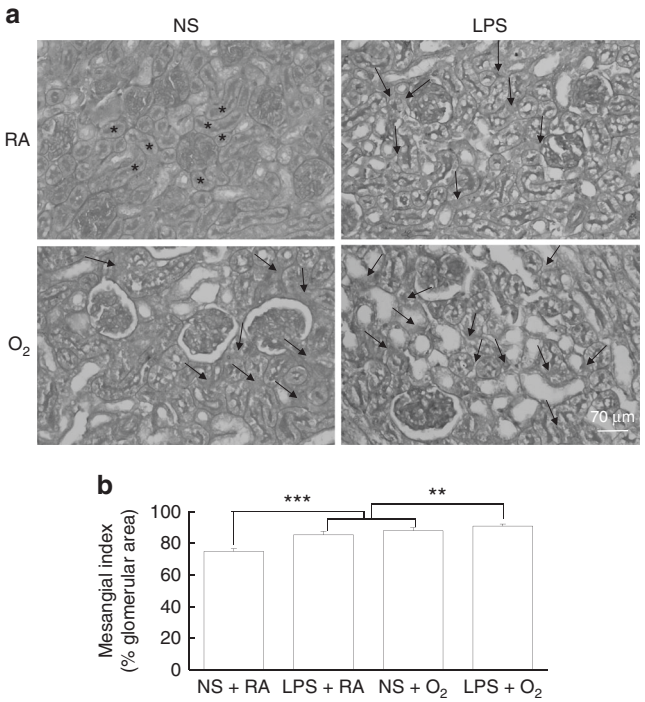

Fig. 2 **a** Representative microphotographs of periodic acid-Schiff's (PAS) staining and **b** mesangial matrix expansion in 14-day-old rats exposed to prenatal lipopolysaccharide (LPS) or normal saline (NS) and postnatal room air (RA) or hyperoxia. Strong positive PAS reaction was seen within the brush border (asterisk) of proximal tubules of NS + RA group and was lost after maternal LPS and/or neonatal hyperoxia exposure. Mesangial matrix (arrow) surrounded the renal tubules was increased in the rats exposed to maternal LPS or neonatal hyperoxia. The rats exposed to maternal LPS and neonatal hyperoxia exhibited higher mesangium compared with the rats exposed to maternal LPS or neonatal hyperoxia (**$P < 0.01$, ***$P < 0.001$, two-way analysis of variance (ANOVA) followed by Bonferroni post-test). $n = 8$–10 rats

tubular injury was performed. Ten fields in the renal cortex were evaluated per section (×200 magnification). The tubular injury was scored as: 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. The degree and the severity of glomerular damage in each glomerulus were scored from 0 to 4 as Raij et al.[16] described: 0 = no glomerulus damaged; 1 = 25% of the glomerulus damaged; 2 = 50% of glomerulus damaged; 3 = 75% of glomerulus damaged; 4 = 100% of the glomerulus damaged. The number of glomeruli per field was counted in 10 fields per kidney at ×200 magnification. Glomeruli were counted at the cortex included nephrogenic zone and the rest of the cortex (excluding nephrogenic zone).[17] Ten randomly selected glomeruli from each animal were quantified in PAS-stained sections using Image Pro Plus 6.0 (Media Cybernetics, Silver Spring, MD, USA) to assess the percentage of PAS-positive area in the mesangium of glomerulus, which was denoted as mesangial index.[18]

Immunohistochemistry for TLR4, MPO, and 8-OHdG

Immunostaining was performed on 5-μm paraffin sections with immunoperoxidase visualization. After the routine deparaffinization, heat-induced epitope retrieval was done by immersed slides in 0.01 M sodium citrate buffer (pH 6.0). To block endogenous peroxidase activity and nonspecific binding of antibody, sections were first pre-incubated for 1 h at 37 °C with 0.1 M phosphate-buffered saline (PBS) containing 10% normal goat serum and 0.3% H_2O_2 before being incubated for 20 h at 4 °C with primary antibodies mouse monoclonal anti-toll-like receptor 4 (TLR4) (1:50; Santa Cruz Biotechnology Inc.) and rabbit polyclonal anti-myeloperoxidase (MPO) (ab 9535, 1:25, Abcam, Cambridge, MA, USA). The sections were then treated for 1 h at 37 °C with biotinylated rabbit anti-mouse immunoglobulin G (IgG) (1:200; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for the anti-8-hydroxy-2′-deoxyguanosine (8-OHdG) antibody. The fluorochrome-conjugated secondary antibodies used were Rhodamine (TRITC)-conjugated AffiniPure Donkey anti-mouse IgG (H+L) for the anti-TLR4 antibody, and Rhodamine Red-X-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) for the anti-MPO. Nuclei were detected using 4′,6-diaminidino-2-phenylindole (1:1000; Sigma-Aldrich). The sections were reacted with fluorochrome-conjugated secondary antibodies and then washed with PBS, mounted, and examined under a fluorescence microscope. The sections were treated with biotinylated IgG followed by reaction with reagents from an ABC kit (Avidin-Biotin Complex, Vector), and the reaction products were visualized using a diaminobenzidine substrate kit (Vector) according to the manufacturer's recommendations. All immunostained sections were viewed and photographed using a Nikon Eclipse E600. The positive immunoreactivity of TLR4 and MPO cells in 10 randomly selected high-power fields (HPFs) from each section at ×400 magnification were analyzed,[19] and the positively stained 8-OHdG cells were counted in five fields randomly selected from each section using a light microscope (magnified at ×400) were analyzed.[20]

Western blot analysis

Protein levels of interleukin-1β (IL-1β) were measured by Western blot using anti-IL-1β (GTX55675, 1:1000; GeneTex, San Antonio, TX, USA). Mouse anti-β-actin monoclonal antibody (C4, 1:1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was used as an internal control. The intensity of IL-1β and β-actin bands was measured through densitometric analysis using the AIDA software. The densitometry unit of the protein expression in the NS-treated rats was assigned as 1 after normalizing with β-actin.

MPO activity and TLR4 assay

Kidney tissues were homogenized, sonicated, and centrifuged. Supernatants were collected for determination of MPO activity using MPO colorimetric activity assay kit (Bio-vision, Milpitas, CA, USA). TLR4 protein was measured using a commercial ELISA kit (MyBioSource Inc., San Diego, CA, USA).

Statistical analysis

The data are presented as the mean ± standard deviation. Statistical analyses were performed using two-way analysis of variance with a Bonferroni post hoc test for multiple group comparisons. The differences were considered statistically significant at $P < 0.05$.

RESULTS

Three NS-treated and three LPS-treated dams gave birth to a total of 31 and 30 pups, respectively. Nearly half of the pups were randomly distributed to RA and hyperoxia groups. Six rats exposed to maternal NS and neonatal hyperoxia and seven rats exposed to maternal LPS and neonatal hyperoxia died.

Body and kidney weight and kidney-to-body weight ratio

Table 1 shows how maternal LPS and neonatal hyperoxia exposure affected body weight, kidney weight, and the kidney-to-body-weight ratio on postnatal day 14. Neonatal hyperoxia-exposed rats exhibited significantly lower body weights and higher kidney-to-body-weight ratios than did RA-exposed rats. Maternal inflammation did not influence body weights or kidney-to-body-weight ratios.

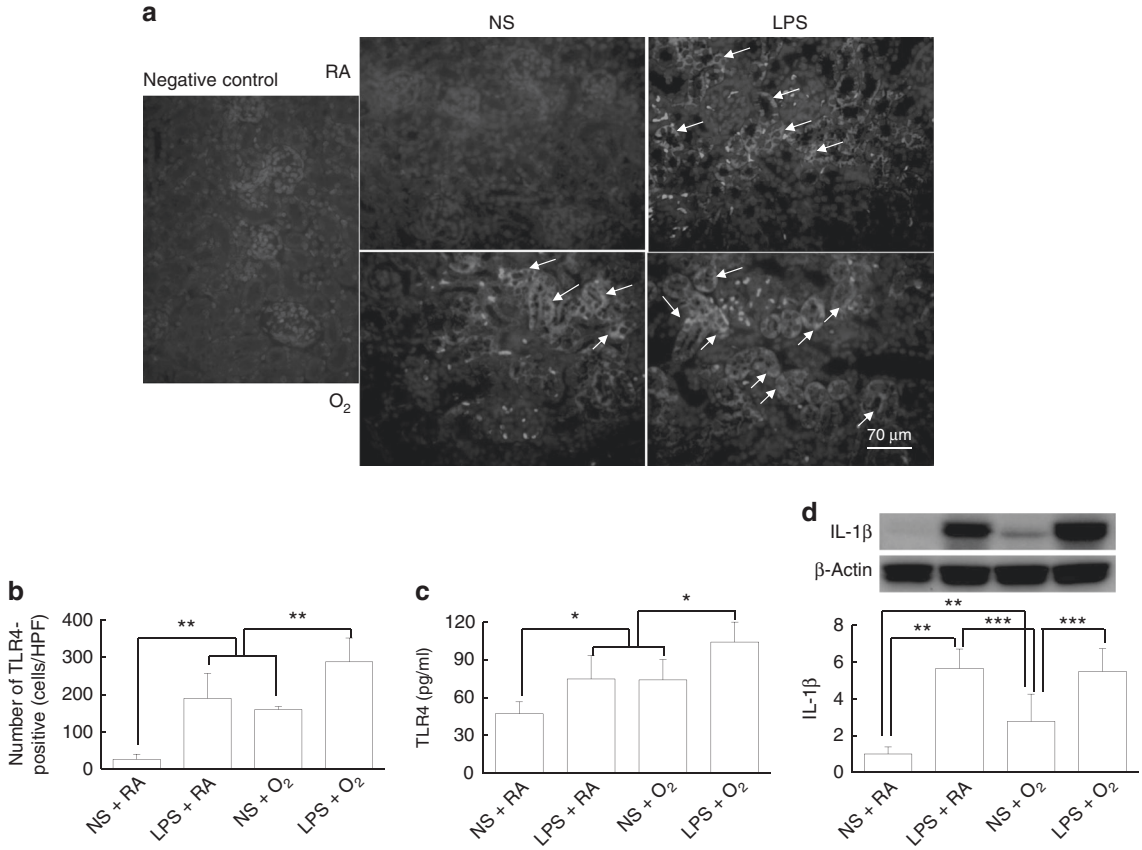

Fig. 3 **a** Representative immunofluorescence images of toll-like receptor 4 (TLR4), **b** positive TLR4 cells/high-power field (HPF), **c** TLR4 protein, and **d** interleukin-1β (IL-1β) expressions in 14-day-old rats exposed to prenatal lipopolysaccharide (LPS) or normal saline (NS) and postnatal room air (RA) or hyperoxia. The rats exposed to maternal LPS and neonatal hyperoxia exhibited significantly higher positive TLR4 cells/HPF (arrow) and TLR4 protein compared with the rats exposed to maternal LPS or neonatal hyperoxia. The LPS + RA and NS + O_2 groups exhibited significantly higher IL-1β expressions than the NS + RA group. The LPS + RA and LPS + O_2 exhibited significantly higher IL-1β expressions than the NS + O_2 group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, two-way analysis of variance (ANOVA) followed by Bonferroni post-test). $n = 8$–10 rats

Histology

Figure 1 shows representative kidney sections stained with hematoxylin and eosin from the maternal LPS- and neonatal hyperoxia-exposed rats on postnatal day 14. The rats exposed to maternal NS and neonatal RA displayed normal kidney structure, and there was no evidence of tissue injury (Fig. 1a). Tubular atrophy, dilatation of the tubular lumen, increased space between the renal tubules, and enlarged corpuscle profile were observed in the maternal LPS- or neonatal hyperoxia-exposed groups. The rats exposed to maternal LPS and neonatal hyperoxia exhibited significantly higher tubular and glomerular injury score and lower glomerular number compared with the rats exposed to maternal LPS or neonatal hyperoxia (Fig. 1b).

PAS staining is used to assess accumulation of polysaccharides in microvilli, basement membranes, and mesangium. Widespread PAS reaction in the brush border of proximal tubules, which indicates a relatively intact kidney structure, was observed in the rats exposed to maternal NS and neonatal RA (Fig. 2a). Whereas weak positive PAS reaction was observed in the tubular brush border of the rats exposed to maternal LPS or neonatal hyperoxia. PAS-positive material revealed that the percentage of mesangial expansion area was markedly increased in the rats exposed to maternal LPS or neonatal hyperoxia compared with the rats exposed to maternal NS and neonatal RA. The rats exposed to maternal LPS and neonatal hyperoxia exhibited significantly higher mesangial index compared with the rats exposed to maternal LPS or neonatal hyperoxia (Fig. 2b).

Immunofluorescence of TLR4 and TLR4 assay and cytokine

Immunofluorescence staining of TLR4 expression in kidney tissues is presented in Fig. 3. TLR4 immunofluorescence was scattered in renal tubules and the positive fluorescence cells were markedly increased after maternal LPS or neonatal hyperoxia exposure (Fig. 3a). The rats exposed to maternal LPS and neonatal hyperoxia exhibited significantly higher positive TLR4 cells/HPF and TLR protein compared with the rats exposed to maternal LPS or neonatal hyperoxia (Fig. 3b, c). The rats exposed to maternal LPS or neonatal hyperoxia exhibited significantly higher IL-1β expressions than those exposed to maternal NS and neonatal RA (Fig. 3d). The rats exposed to maternal LPS exhibited significantly higher IL-1β expressions than those exposed to neonatal hyperoxia alone.

Immunohistochemistry for MPO and 8-OHdG and MPO activity

To investigate whether maternal inflammation and neonatal hyperoxia increased macrophage infiltration, we used immunohistochemical assays for MPO in the kidneys. The MPO immunoreactivity was primarily detected in the interstitial area of the renal cortex (Fig. 4a). The rats exposed to maternal LPS or neonatal hyperoxia exhibited significantly higher MPO-positive cells/HPF and MPO activity in kidney tissues compared with the rats exposed to maternal NS and neonatal RA (Fig. 4b, c). The rats exposed to maternal LPS and neonatal hyperoxia exhibited significantly higher MPO-positive cells/HPF and MPO activity compared with the rats exposed to maternal LPS or neonatal hyperoxia.

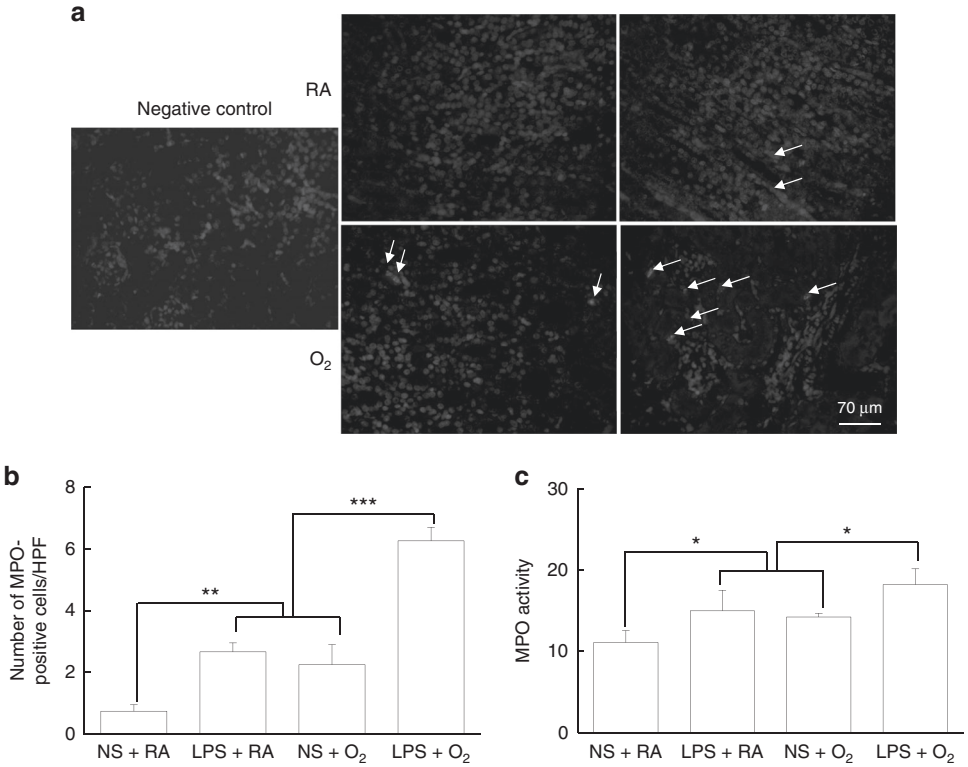

Fig. 4 **a** Representative immunofluorescence images of myeloperoxidase (MPO) and **b** positive MPO cells/high-power field (HPF) and **c** MPO activity in 14-day-old rats exposed to prenatal lipopolysaccharide (LPS) or normal saline (NS) and postnatal room air (RA) or hyperoxia. The rats exposed to maternal LPS or neonatal hyperoxia exhibited significantly higher MPO-positive cells/HPF (arrow) in kidney tissues compared with the rats exposed to maternal NS and neonatal RA. The rats exposed to maternal LPS and neonatal hyperoxia exhibited significantly higher MPO-positive cells/HPF and MPO activity compared with the rats exposed to maternal LPS or neonatal hyperoxia (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, two-way analysis of variance (ANOVA) followed by Bonferroni post-test). $n = 8$–10 rats

Immunohistochemistry was used to detect the oxidative stress marker 8-OHdG, which was primarily found in glomerular cell and some tubular cell nuclei (Fig. 5a). The rats exposed to maternal LPS or neonatal hyperoxia exhibited significantly more 8-OHdG-positive cells than did the rats exposed to maternal NS and neonatal RA (Fig. 5b). The rats exposed to maternal LPS and neonatal hyperoxia exhibited significantly more 8-OHdG-positive cells than did the rats exposed to maternal LPS or neonatal hyperoxia.

DISCUSSION

Our in vivo model revealed that maternal LPS exposure or neonatal hyperoxia exposure induced kidney injury in neonatal rats, as evidenced by higher glomerular and tubular injury scores and higher inflammatory cells than those of the normal rats. The major findings are that maternal inflammation induces kidney injury and exacerbates hyperoxia-induced kidney injury in the rat offspring. These exacerbation effects on kidneys were associated with the increased expression of TLR4 and oxidative stress. These results suggest that maternal inflammation during pregnancy may exacerbate hyperoxia-induced kidney injury and TLR4 may be involved in the pathogenesis.

In this study, we found that rats exposed to postnatal hyperoxia significantly decreased body-weight and increased kidney-to-body-weight ratios irrespective of maternal NS or LPS treatment on postnatal day 14. These results suggest that maternal inflammation did not influence body-weight gain. The rats reared in O_2-enriched air exhibited proportionally greater body-weight loss than kidney weight loss, yielding an increased kidney-to-body-weight ratio on postnatal day 14.

In this study, we found that rats reared in O_2-enriched air exhibited lower glomerular number than did rats reared in ambient air. This result was in contrast to a study in a mouse model (mice exposed to 65% O_2 from birth to postnatal day 7) revealed no changes in nephron number.[9] The discrepancy may be due to differences in the duration and concentration of oxygen used and vulnerabilities of the animal model. Prenatal LPS administered at a higher dose (0.79 mg/kg) on 8th, 10th, and 12th day of gestation showed decreased glomerular number at 7 weeks of age in offspring rats.[21] Their finding is consistent with this study suggesting that maternal LPS and neonatal hyperoxia may accelerate loss of nephrons or impair nephrogenesis and maternal inflammation during pregnancy may exacerbate neonatal hyperoxia-induced kidney injury.

LPS is a well-characterized pathogen-associated molecule present in the outer membrane of most gram-negative bacteria. LPS initiates a strong immune response and activates TLR4. TLRs are crucial constituents of the innate immune system that identify pathogens and elaborate inflammatory responses.[22] Recent studies have demonstrated that TLR4 activation contributes to the pathogenesis of renal inflammation.[23–25] Based on the aforementioned reports, this study was designed to explore the effect of maternal LPS treatment on TLR4 expression in the kidney of rat pups. In this study, we demonstrated that prenatal LPS-treated rats exhibited high TLR4 expression in the kidney. This finding is consistent with recent studies suggesting that TLR4 activation contributes to the pathogenesis of renal inflammation and fibrosis.[23–25] Our results suggest that prenatal LPS-induced kidney injury is probably controlled by TLR4.

In this study, we demonstrated that neonatal hyperoxia increased TLR4 and IL-1β expressions, and maternal LPS and

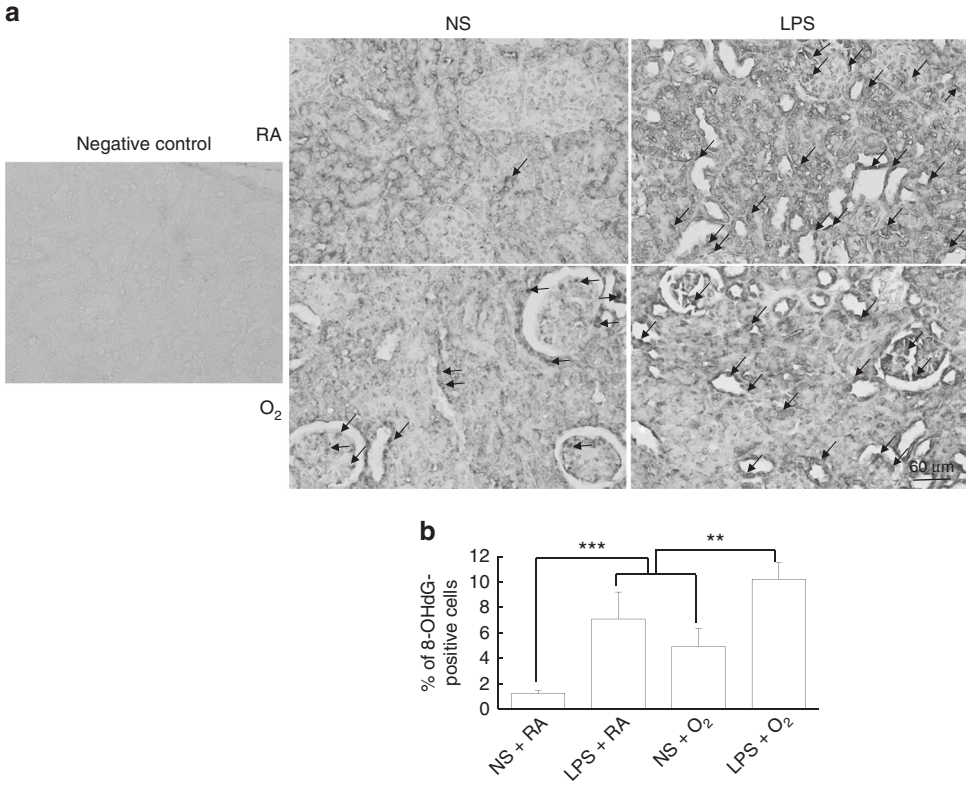

Fig. 5 **a** Representative immunohistochemistry images of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and **b** 8-OHdG-positive cells in 14-day-old rats exposed to prenatal lipopolysaccharide (LPS) or normal saline (NS) and postnatal room air (RA) or hyperoxia. The rats exposed to maternal LPS or neonatal hyperoxia exhibited significantly more 8-OHdG-positive cells (black arrow) than did the rats exposed to maternal NS and neonatal RA. The rats exposed to maternal LPS and neonatal hyperoxia exhibited significantly more 8-OHdG-positive cells than did the rats exposed to maternal LPS or neonatal hyperoxia (**$P < 0.01$, ***$P < 0.001$, two-way analysis of variance (ANOVA) followed by Bonferroni post-test). $n = 8$–10 rats

neonatal hyperoxia did not further increase IL-1β expressions than those exposed to maternal LPS alone in the kidney. Hyperoxia increases oxidative stress and enhances inflammation in the organs. TLRs link the oxidative stress and inflammation and TLR4 is known to be involved in the inflammatory cascade in response to hyperoxia in the lung, heart, and intestine of neonatal rats.[26–29] Although TLR4 activation is known to be a first-line response of the innate immune system, these results suggest that LPS recognition by TLR4 initiates IL-1β expression via alterative pathways.[30]

The MPO examination is widely used to quantify the number of neutrophils in a tissue because MPO is an enzyme that is released mostly from neutrophils.[31] In this study, we used the MPO-positive cells as an index of the accumulation of activated neutrophils in the kidneys. We found that maternal LPS injection or neonatal hyperoxia exposure increased the MPO expression and activity in the kidneys of rat offspring and combined exposure further increased MPO expression and activity. These results indicate that maternal inflammation exacerbated renal inflammation in the rats reared in hyperoxia.

Human and animal studies have demonstrated that maternal LPS administration and neonatal hyperoxia separately increases oxidative stress and induces glomerular and tubular damage.[4–10] The effects of combined maternal inflammation and neonatal exposure on oxidative stress were unknown. Oxidative stress leads to lipid peroxidation and induces DNA oxidative damage by generating 8-OHdG.[32] 8-OHdG is a DNA base-modified product generated by reactive oxygen species as a marker of oxidative DNA damage.[33] In this study, we used immunohistochemistry to detect the renal oxidative stress marker 8-OHdG. We found that the rats exposed to maternal LPS and neonatal hyperoxia exhibited significantly more 8-OHdG-positive cells than did the rats exposed to maternal LPS or neonatal hyperoxia alone. These results indicate that maternal inflammation exacerbates neonatal hyperoxia-induced oxidative stress in rat offspring kidney.

In conclusion, we showed that maternal LPS treatment and neonatal hyperoxia increased inflammatory cells and oxidative stress in the kidney of neonatal rats on postnatal day 14. The development of maternal LPS- and/or neonatal hyperoxia-induced kidney injury was associated with increase in TLR4 protein expression in kidney tissues. These results suggest that TLR4 may be involved in the pathogenesis of kidney injury induced by maternal systemic inflammation and/or neonatal hyperoxia, and that targeting TLR4 strategies may attenuate kidney injury. These results are relevant to neonates who were exposed to chorioamnionitis and required supplemental oxygen during infancy. Further studies are needed to evaluate the effects of maternal inflammation and neonatal hyperoxia exposure on kidney injury in rat offspring in knock-out animal models or used antibody inhibition experiments.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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REFERENCES

1. Hagberg, H., Wennerholm, U. B. & Savman, K. Sequelae of chorioamnionitis. *Curr. Opin. Infect. Dis.* **15**, 301–306 (2002).

2. Gayle, D. A. et al. Maternal LPS induces cytokines in the amniotic fluid and corticotropin releasing hormone in the fetal rat brain. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **286**, R1024–R1029 (2004).
3. Beloosesky, R., Gayle, D. A. & Ross, M. G. Maternal *N*-acetylcysteine suppresses fetal inflammatory cytokine responses to maternal lipopolysaccharide. *Am. J. Obstet. Gynecol.* **195**, 1053–1057 (2006).
4. Wang, J. et al. Prenatal exposure to lipopolysaccharide alters renal DNA methyltransferase expression in rat offspring. *PLoS ONE* **12**, e0169206 (2017).
5. Wang, X. et al. Prenatal lipopolysaccharide exposure results in dysfunction of the renal dopamine D1 receptor in offspring. *Free Radic. Biol. Med.* **76**, 242–250 (2014).
6. Torbati, D. et al. Multiple-organ effect of normobaric hyperoxia in neonatal rats. *J. Crit. Care* **21**, 85–94 (2006).
7. Vento, M., Sastre, J., Asensi, M. A. & Viña, J. Room-air resuscitation causes less damage to heart and kidney than 100% oxygen. *Am. J. Respir. Crit. Care Med.* **172**, 1393–1398 (2005).
8. Perrone, S. et al. Oxidative kidney damage in preterm newborns during perinatal period. *Clin. Biochem.* **40**, 656–660 (2007).
9. Sutherland, M. R. et al. Neonatal hyperoxia: effects on nephrogenesis and long-term glomerular structure. *Am. J. Physiol. Ren. Physiol.* **304**, F1308–F1316 (2013).
10. Jiang, J. S., Chou, H. C., Yeh, T. F. & Chen, C. M. Neonatal hyperoxia exposure induces kidney fibrosis in rats. *Pediatr. Neonatol.* **56**, 235–241 (2015).
11. Lahra, M. M., Beeby, P. J. & Jeffery, H. E. Maternal versus fetal inflammation and respiratory distress syndrome: a 10-year hospital cohort study. *Arch. Dis. Child Fetal Neonatal Ed.* **94**, F13–F16 (2009).
12. Zoetis, T. & Hurtt, M. E. Species comparison of anatomical and functional renal development. *Birth Defects Res. B* **68**, 111–120 (2003).
13. Seely, J. C. A brief review of kidney development, maturation, developmental abnormalities, and drug toxicity: juvenile animal relevancy. *J. Toxicol. Pathol.* **30**, 125–133 (2017).
14. OECD. *Draft OECD Guideline for Testing of Chemicals on Reproduction/Developmental Toxicity Screening Test* No. 421 (Organization for Economic Co-operation and Development, Paris, 2015).
15. Pichler, R. H. et al. Pathogenesis of cyclosporine nephropathy: roles of angiotensin II and osteopontin. *J. Am. Soc. Nephrol.* **6**, 1186–1196 (1995).
16. Raij, L., Azar, S. & Keane, W. Mesangial immune injury, hypertension, and progressive glomerular damage in Dahl rats. *Kidney Int.* **26**, 137–143 (1984).
17. Sánchez, S. I., Seltzer, A. M., Fuentes, L. B., Forneris, M. L. & Ciuffo, G. M. Inhibition of angiotensin II receptors during pregnancy induces malformations in developing rat kidney. *Eur. J. Pharmacol.* **588**, 114–123 (2008).
18. Ding, T. et al. Kidney protection effects of dihydroquercetin on diabetic nephropathy through suppressing ROS and NLRP3 inflammasome. *Phytomedicine* **41**, 45–53 (2018).
19. Liu, C. et al. Mefunidone attenuates tubulointerstitial fibrosis in a rat model of unilateral ureteral obstruction. *PLoS ONE* **10**, e0129283 (2015).
20. EJ, Kim et al. Involvement of oxidative stress and mitochondrial apoptosis in the pathogenesis of pelvic organ prolapse. *J. Urol.* **189**, 588–594 (2013).
21. Hao, X. Q., Kong, T., Zhang, S. Y. & Zhao, Z. S. Alteration of embryonic AT(2)-R and inflammatory cytokines gene expression induced by prenatal exposure to lipopolysaccharide affects renal development. *Exp. Toxicol. Pathol.* **65**, 433–439 (2013).
22. Medzhitov, R. Origin and physiological roles of inflammation. *Nature* **454**, 428–435 (2008).
23. Anders, H. J., Banas, B. & Schlöndorff, D. Signaling danger: toll-like receptors and their potential roles in kidney disease. *J. Am. Soc. Nephrol.* **15**, 854–867 (2004).
24. El-Achkar, T. M. et al. Sepsis induces changes in the expression and distribution of Toll-like receptor 4 in the rat kidney. *Am. J. Physiol. Ren. Physiol.* **290**, F1034–F1043 (2006).
25. Yiu, W. H., Lin, M. & Tang, S. C. Toll-like receptor activation: from renal inflammation to fibrosis. *Kidney Int. Suppl.* **4**, 20–25 (2014).
26. Gill, R., Tsung, A. & Billiar, T. Linking oxidative stress to inflammation: Toll-like receptors. *Free Radic. Biol. Med.* **48**, 1121–1132 (2010).
27. Chen, Y. et al. Attenuation of hyperoxia-induced lung injury in neonatal rats by 1α,25-dihydroxyvitamin D3. *Exp. Lung Res.* **41**, 344–352 (2015).
28. Chen, C. M., Lin, W., Huang, L. T. & Chou, H. C. Human mesenchymal stem cells ameliorate experimental pulmonary hypertension induced by maternal inflammation and neonatal hyperoxia in rats. *Oncotarget* **8**, 82366–82375 (2017).
29. Chou, H. C. & Chen, C. M. Neonatal hyperoxia disrupts the intestinal barrier and impairs intestinal function in rats. *Exp. Mol. Pathol.* **102**, 415–421 (2017).
30. Joshi, V. D. et al. A role for Stat1 in the regulation of lipopolysaccharide-induced interleukin-1beta expression. *J. Interferon Cytokine Res.* **26**, 739–747 (2006).
31. Odobasic, D., Kitching, A. R. & Holdsworth, S. R. Neutrophil-mediated regulation of innate and adaptive immunity: the role of myeloperoxidase. *J. Immunol. Res.* **2016**, 2349817 (2016).
32. Toyokuni, S. et al. Quantitative immunohistochemical determination of 8-hydroxy-2′-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrilotriacetate-induced renal carcinogenesis model. *Lab. Invest.* **76**, 365e74 (1997).
33. Vijayaraghavan, M. et al. Dimethylarsinic acid induces 8-hydroxy-2′-deoxyguanosine formation in the kidney of NCI–Black–Reiter rats. *Cancer Lett.* **165**, 11e7 (2001).