Received: 21 February 2022 | Revised: 18 April 2022 | Accepted: 4 May 2022

DOI: 10.1111/1348-0421.12984

ORIGINAL ARTICLE

Microbiology and Immunology

Hyperoxia combined with the B-cell antagonist rituximab led to intestinal dysbiosis in neonatal mice

Kun Yang[1] | Ya-Ji Xu[1,2] | Yan He[1] | Cheng-Xin Duan[1] | Huai-Fu Wang[1] | Wei-Jun Ding[1]

[1]Department of Fundamental Medicine, Chengdu University of Traditional Chinese Medicine, Chengdu, China

[2]School of Preclinical Medicine, Department of Preclinical Medicine, Chengdu University, Chengdu, China

Correspondence

Wei-Jun Ding, Department of Fundamental Medicine, Chengdu University of Traditional Chinese Medicine, 1166 Liutai Avenue, Chengdu 611137, China.
Email: Dingweijun@cdutcm.edu.cn

Funding information

Experimental Formulary Sichuan Youth Science and technology Innovation research team, Grant/Award Number: 2020JDTD0022; National Natural Science Foundation of China, Grant/Award Number: NO. 82074151; Experimental Formulary Sichuan Youth Science and Technology Innovation research team; Chengdu University Research Fund and State Key Laboratory of Ophthalmology; Zhongshan Ophthalmic Center

Abstract

The adverse factors impacting the intestinal microbiota of newborns remain to be elucidated. We put forward a hypothesis that hyperoxia in combination with rituximab exhibits a synergistic effect that interferes with neonatal intestinal microbiota. Six C57BL/6J mice, aged 12 weeks and pregnant 18 days, were purchased. Their pups were breastfed and raised under a 75% oxygen or conventional environment. Low- (20 mg/kg) and high-dose (40 mg/kg) rituximab were intraperitoneally administered. Fecal genomic DNA was extracted and sequenced by a 16S rRNA platform. Severe intestinal dysbiosis in newborns were observed, whereas mild dysbiosis was caused by inducing hyperoxia alone, confirming the synergistic interference of the combination of hyperoxia and B-cell antagonist (rituximab) in neonatal intestinal microbiota disruption. Slight dysbiosis was observed in the intestinal microbiota of dams, indicating their much robust ability to confront hyperoxic conditions. The abundance of *Akkermansia muciniphila* was significantly and extensively altered in both pups and dams after being subjecting them to hyperoxic conditions with or without rituximab administration. In conclusion, this work demonstrated that the synergistic effect of hyperoxia and rituximab led to severe intestinal dysbiosis in newborns. More studies are recommended to explore the precise regulatory mode between hyperoxia and rituximab in intestinal microbiota.

KEYWORDS

Akkermansia muciniphila, hyperoxia, immunology, intestinal microbiota, newborn, rituximab

INTRODUCTION

The intestinal microbiota of newborns undergoes a rapid and important process of maturation, which substantially impacts mucosal immune,[1] blood–brain barrier maturation,[2] social behavioral development,[3] and pathogenesis of a variety of diseases.[4] Healthy intestinal flora is mainly composed of anaerobes, which are highly susceptible to local levels of oxygen concentration. Oxygen therapy has been widely applied to improve the blood oxygen saturation of patients suffering from hypoxia caused by various diseases, such as coronavirus infectious disease 2019 (COVID-19),[5] chronic obstructive pulmonary disease,[6] bronchial asthma,[7] and neonatal critical disease.[8] According to a report released by the World Health Organization (WHO) in 2013, the global preterm infants account for about 10% of newborns.[9] Hence the complications of neonatal hypoxia and oxygen therapy gain increasing attention. Premature retinopathy is a common neonatal disease characterized by retinal ischemia and massive neovascularization that occurs in premature infants or

Abbreviations: AKK, *Akkermansia muciniphila*; COVID-19, coronavirus infectious disease 2019; CTL, received placebo and reared under conventional condition; HDR, high dosage of rituximab and reared under hyperoxic condition; LDA, linear discriminant analysis; LCE, lactating mice reared under conventional environment; LDR, low dosage of rituximab and reared under hyperoxic condition; LHE, lactating mice reared under hyperoxic environment; ns, not significant; PBS, received placebo and reared under hyperoxic condition; UPGMA, unweighted pair-group method with arithmetic means; WHO, World Health Organization.

low-weight full-term babies.[10] The oxygen concentration is thought to be an important cause of premature retinopathy. Recently, rituximab has been used to treat retinopathy, such as retinal vasculitis,[11] retinal occlusive vasculopathy,[12] granulomatosis with polyangiitis,[13,14] and thyroid eye disease.[15] However, the effect of hyperoxia combined with B-cell antagonist rituximab on intestinal flora remains unclear. The fetal intestine is usually considered sterile. After delivery, multiple antigens continuously challenge newborns' intestine,[16] and the neonatal intestine will be rapidly and heavily colonized by various microbes within a few days.[17] A stable intestinal microbiota is usually established several weeks after birth.[18] During this period, the intestinal flora of newborns is extremely vulnerable to a variety of interferences, particularly hypoxia, hyperoxia, breastfeeding,[19] and antibiotics.[20] However, the profound impact of hypoxia and oxygen therapy (hyperoxia), on the construction and functions of neonatal intestinal microbiota remains to be determined.

Intestinal B lymphocytes are among the main factors that trim pathological bacteria located in the intestinal microbiota by synthesis and secretion of secretory IgA.[1] The intestine is always considered as the largest immune organ of human beings, on account of the largest number of lymphocytes which restrain intestinal microbiota, the largest microbiota in human beings. Actually, mature B cells are observed in the fetal intestine since the 14th week of embryonic development[8] and $CD20^+$ B cells are present in all specimens of fetal intestines.[21] Previous studies have observed that the colonization pattern of gut bacteria disturbed the maturation of human B cells[22]; conversely, B cells may perturb the functions and construction of intestinal microbiota. However, only few studies focused on the mutual regulations between intestinal B cells and intestinal microbiota, let alone at the neonatal stage.

Rituximab is a chimeric murine/human monoclonal antibody extensively used in selectively deleting CD20 located at the surface of normal and malignant B cells.[23,24] As an effective CD20 antagonist, rituximab impacts B cells in mucosa-associated lymphoid tissues as well as B-cell non-Hodgkin lymphoma.[24] Limited cases[25] have reported that rituximab can slightly injure digestion functions, such as dyspepsia and diarrhea. However, the cross-talking between intestinal B cells and gastrointestinal microbiota and rituximab still needs to be elucidated. Herein we put forward a hypothesis that hyperoxia combined with rituximab perform synergistic interference to impact the vulnerable intestinal microbiota at the neonatal stage, and applied these factors to intervene in newborns' intestinal microbiota, to reveal their adverse effect on the intestinal microbiota at the neonate stage.

MATERIALS AND METHODS

Animals

The Animal Ethics Committee of Chengdu University of Traditional Chinese Medicine approved this study (2021-05). Six C57BL/6J mice, aged 12 weeks and pregnant 18 days, were purchased from Chengdu Dossy Biological Technology Co. Ltd. After being fed under conventional conditions for 3 days, these dams delivered 6–12 litters. The pups were breastfeeding and raised in the conventional environment (~21% oxygen) for 7 days. These newborns were then randomly divided into four groups and received breastfeeding from dams within a hyperoxic chamber customized for the animal experiment (ProOx-820; Ta Wang Technology Co. Ltd). The maintaining conditions were as follows: light/dark period 12 h/12 h, ambient noise 40 ± 10 dB, temperature 22 ± 2℃, humidity 60 ± 3%, and 75% oxygen. All pregnant and lactating mice were reared with *ad libitum* access to tap water and chow. The dams were shifted between the hyperoxic and room air environment every 24 h to avoid oxygen toxicity.[26]

Grouping and animal model

Grouping

The neonates were randomly divided into four groups:

(1) Low dosage of rituximab (20 mg/kg) and reared under hyperoxic condition (LDR).
(2) High dosage of rituximab (40 mg/kg) and reared under hyperoxic condition (HDR).
(3) Received placebo (PBS) and reared under hyperoxic condition (PBS)
(4) Received placebo (PBS) and reared under conventional condition (CTL).

The lactating mice were randomly divided into two groups:

(1) Lactating mice reared under hyperoxic environment (75% oxygen [LHE]).
(2) Lactating mice reared under conventional environment (21% oxygen [LCE]).

Animal model

All pups were first reared under the conventional environment for 7 days after birth. The oxygen concentration at this period was about 21%. The 75% oxygen concentration was maintained according to the manufacturer's instruction. Breastfeeding was performed over a layer of spongy shavings within the hyperoxic chamber (feeding environment: Figure S1B). The newborns were then divided into groups LDR, HDR, and PBS, and transferred into a hyperoxic chamber (ProOx-820; Ta Wang Technology Co. Ltd) for 5 days (oxygen room equipment diagram: Figure S1C). The neonates in groups LDR, HDR, and PBS were daily intraperitoneally injected rituximab 20, 40, or 0 mg/kg,

respectively.[27] The reagent rituximab (Roche h0284/sh0166, Switzerland) was purchased from the Affiliated Hospital, Chengdu University of Traditional Chinese Medicine. Comparatively, the pups within the CTL group were reared under a conventional environment. The experimental flowchart is shown in Figure 1.

All pups were weighed on postnatal days 7, 12, and 17. The general condition of both dams and pups was daily recorded. At the time points of the experiment, pups were anesthetized and the colon contents were collected for 16S rRNA analysis.

Fecal collection and pretreatment

A fecal genomic DNA extraction kit was purchased from TIANGEN Biochemical Technology Co. Ltd. The intestinal tracts of all anesthetized mice were immediately dissected, colonic contents carefully collected. The intestinal tracts were opened with sterile scissors, and the colonic contents flushed several times by sterile PBS and centrifuged at 4000 rpm (1078×g) for 10 min. Finally, the precipitates were resuspended in 1 mL sterile PBS and stored at −80℃ for sequencing.

16S rRNA sequencing and data analysis

For 16S rRNA gene sequencing and data analysis, the V4 regions of the 16S rRNA genes were PCR amplified using barcoded universal primers (515FGTGCCAGCMGCCGCGGTAA; 806RGGACTACHVGGGTWTCTAAT) by applying 30 ng of genomic DNA. The PCRs were set up in triplicate, and the PCR product was purified using a QIAquick PCR purification kit (QIAGEN). The purified PCR product was pooled in equal molar concentrations quantified by a library quantification kit (KK4824; Kapa Biosystems) and sequenced by Laragen, using the Illumina MiSeq platform. A reagent kit was used for paired-end sequencing. Operational taxonomic units were chosen based on 97% sequence similarity to the Greengenes 13_5 database. Taxonomy assignment and rarefaction were performed by QIIME1.8.0 using about 80,000 reads per sample.[28]

Statistical methods

SPSS software (V16.0; SPSS Inc.) was used for statistical analysis. Data were expressed as mean ± SD. ANOVA is used for testing for differences in the means of the groups PBS, LDR, and HDR. Group comparisons were conducted using Student's *t*-test. Differences were considered significant at $P < 0.05$ or $P < 0.01$. Bacterial compositions among samples were visualized using bar plots.

RESULTS

General situation of newborns raised under a hyperoxic environment

There were 48 pups delivered by six pregnant mice, ranging from 6 to 12 per litter. As shown in Table S1, the bodyweight of newborns in groups LDR ($P < 0.05$) and HDR ($P < 0.01$) was significantly decreased after rituximab injection. The general situation of groups LDR and HDR was worse than that of the group CTL or PBS, characterized by poor mental status and weak activities. However, hyperoxic stress alone did not show significant bodyweight reduction in dams or pups. Unfortunately, three fragile pups were killed during this experiment, one in each group CTL, HDR, and PBS.

Hyperoxia combined with rituximab lead to severe dysbiosis in newborns

Results displayed that the α diversities of intestinal microbiota were significantly decreased in both HDR and LDR animals, compared with those of the PBS group (Figure 2a). The unweighted pair-group method with arithmetic means (UPGMA) cluster displayed the similarity classification of intestinal composition between samples, indicating statistical differences in groups LDR, HDR, and PBS (Figure 2c). The Venn diagram shows marked reduction in species diversity caused by rituximab and hyperoxia (Figure 2d).The constructions of intestinal microbiota were globally altered after hyperoxia in combination with rituximab therapy. Rituximab dosage dependently altered the abundance of intestinal

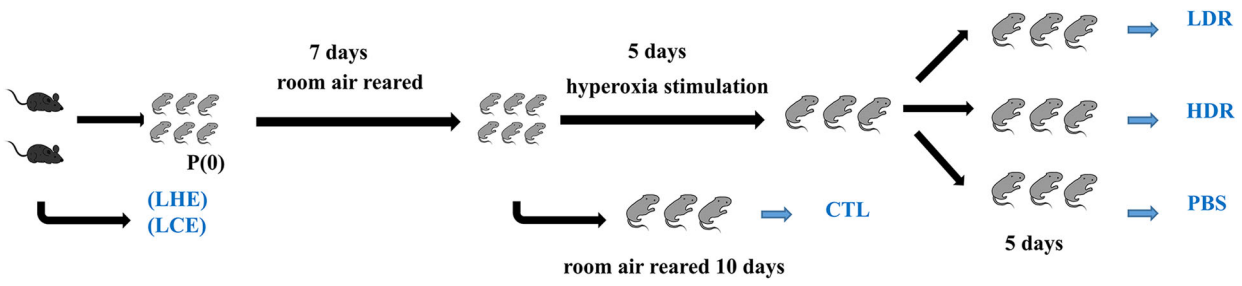

FIGURE 1 The experimental flowchart.

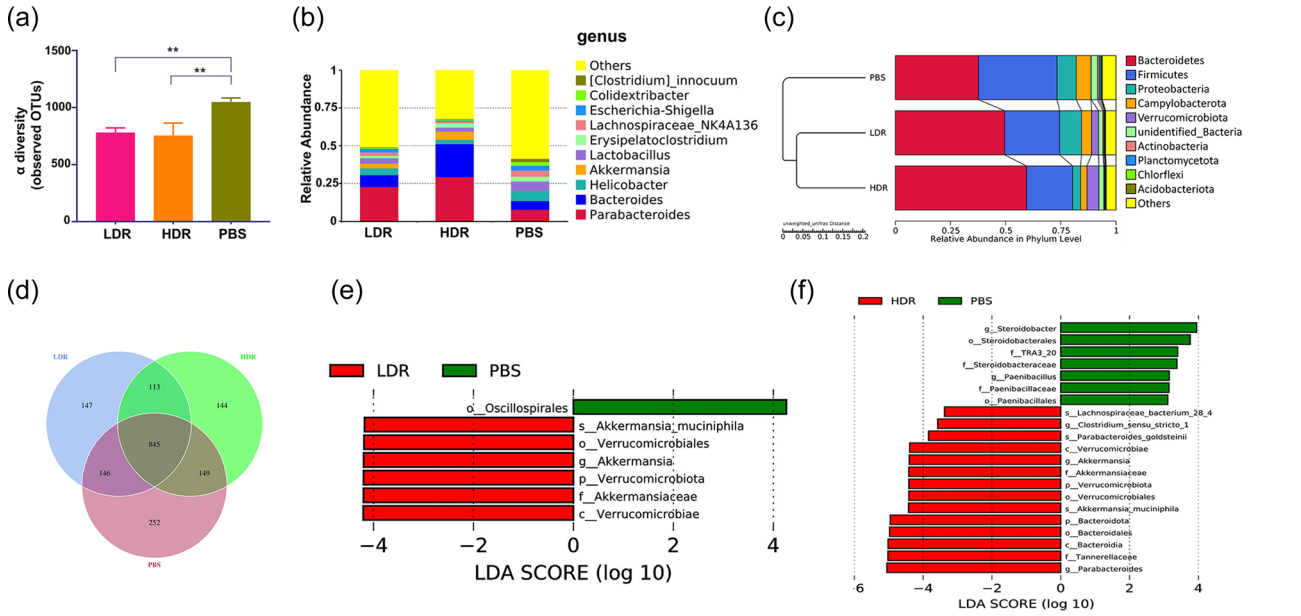

FIGURE 2 (a) Hyperoxia combined with rituximab significantly altered the gut microbiota of newborns. The α diversity of intestinal microbiota in LDR, HDR, and PBS groups. (b) The proportion abundance of the top 10 components in LDR, HDR, and PBS groups. (c) UPGMA clustering tree shows the similarity classification among LDR, HDR, and PBS groups. (d) Venn diagram displays the shared and different species between groups. (e,f) Histogram of the LDA scores; $^{**}P < 0.01$. The length of the histogram indicates the difference in the impact of species

microbiota in levels phylum, class, order, family, genus, and species (Table S2). For instance, the combined therapy of hyperoxia with rituximab significantly increased phyla Bacteroidetes and deleted Firmicutes, agreeing with the results of UPGMA analysis. We applied linear discriminant analysis (LDA) for quantitative analysis of biomarkers between groups and demonstrated main abundant differences (Figure 2e,f). *Akkermansia muciniphila* (AKK) derived from groups LDR and HDR was the most abundant species, compared with the PBS group. The abundances of Akkermansiaceae, Verrucomicrobiales, and Verrucomicrobiae detected in LDR pubs were significantly enriched (Figure 2e). Tannerellaceae, Bacteroidales, Bacteroidia, AKK, Verrucomicrobiales, and Verrucomicrobiae were significantly enriched in HDR neonates, compared with PBS counterparts (Figure 2f). The heatmap demonstrated statistical differences in intestinal flora structure among the three groups (PBS, LDR, and HDR) at the genus level (Figure S2). Compared with the PBS group, low- and high-dose rituximab disturbed intestinal flora. For example, AKK increased significantly, while *Lactobacillus murinus* and *Helicobacter hepaticus* decreased significantly.

Preliminary construction of intestinal flora against hyperoxic stimulation in neonatal mice

The intestinal microbiota between CTL and PBS were compared to reveal the adverse effects of hyperoxia at the neonatal stage. Results of α-diversity analysis did not demonstrate a statistical difference between groups CTL and PBS ($P > 0.05$; Figure 3a), nor the abundance of key phyla such as Bacteroidetes and Firmicutes in mammal intestine (Figure 3d), indicating relatively mild changes in intestinal microbiota confronting hyperoxia alone. Significantly, partial change of the structure and composition is existed in the intestinal flora of group CTL and group PBS, such as that in Firmicutes-Clostridia and Bacteroidia which are bacteria at the class level, in Parabacteroides, *Akkermansia*, Bacteroides, and *Helicobacter* at the genus level. And AKK and *L. murinus* have changed at the species level (Table S2).The adverse effect of hyperoxia was further demonstrated in a clustering heatmap (Figure S3). It is an unexpected but interesting discovery that hyperoxia significantly decreased the abundance of probiotics such as AKK, and enriched pathological species such as *H. hepaticus*. Six downregulated and one upregulated species were identified after hyperoxic culture (Figure 3c). The evolutionary branch diagram displayed that Verrucomicrobiae, Akkermansiaceae, Verrucomicrobiales, and Oscillospirales had statistical differences after being raised under a hyperoxic environment.

Stable intestine of dams resists hyperoxic stimulation

The α diversities of intestinal microbiota derived from lactating mice did not display significant changes between groups LHE and LCE (Figure 4a), nor in the core phyla Bacteroidetes and Firmicutes (Figure 4b). Negative results of principal components analysis using Euclidean distance were obtained with or without hyperoxic stimulation (Figure 4d). However, some statistical differences were observed in the lower taxa (Figure 4c,e). At the genus level,

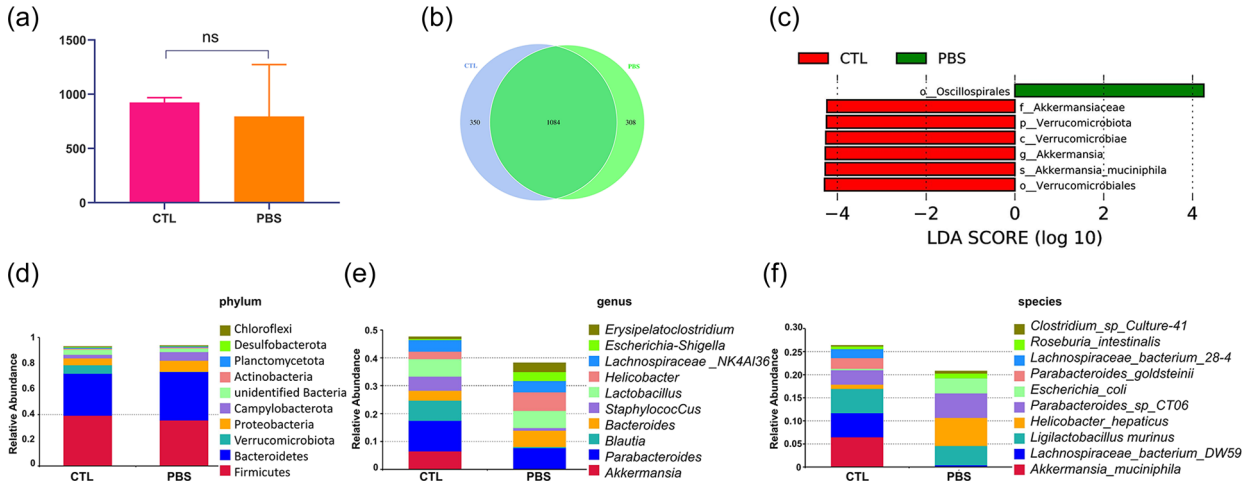

FIGURE 3 (a) Preliminary construction of intestinal flora against hyperoxic stimulation in neonatal mice. The α diversity of intestinal microbiota of the CTL and PBS groups. (b) Venn diagram presents the shared and different species between the CTL and PBS groups. (c) Histogram of the LDA scores. (d–f) Top 10 relative abundance in the phylum, genus, and species levels

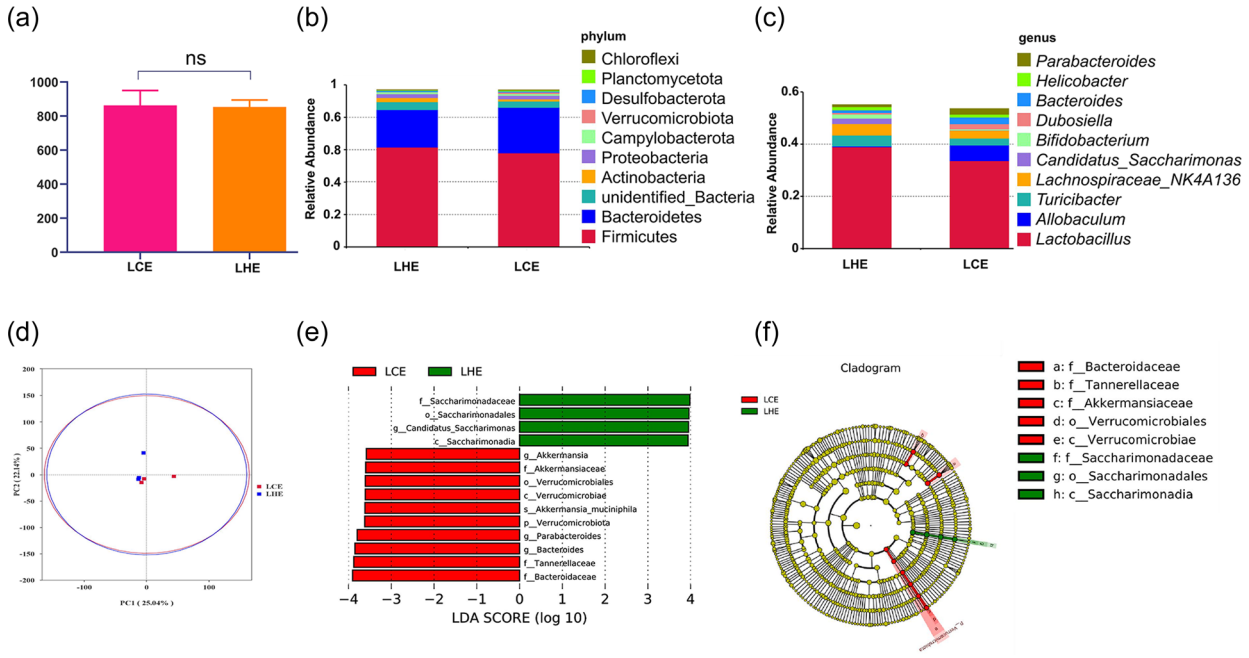

FIGURE 4 (a) Stable intestine of dams resists hyperoxic stimulation. The α diversity of intestinal microbiota of mice in the LHE and LCE groups. (b, c) Relative abundance in the phylum and genus levels. (d) The major different elements identified by principal components analysis. (e) Histogram of the LDA scores (the threshold of the LDA score was 3.0). (f) Cladogram of the intestinal microbiota between LCE and LHE.

Lactobacillus (*LCE* 33.57%; *LHE* 38.89%) was enriched, whereas Parabacteroides and *Akkermansia* were down-expressed in the intestinal microbiota of LHE mice. Notably, the abundance of *Lactobacillus* in the neonatal intestinal microbiota was markedly lower than that of adult mice; less than 10% of *Lactobacillus* were detected in groups CTL (6.24%), LDR (3.53%), HDR (2.63%), and PBS (6.15%). A remarkable difference between groups LHE and LCE is shown in the clustering heatmap and evolutionary branch diagram (Figures 4e,f and S4). For instance, Saccharimonadaceae, Saccharimonadales, and Saccharimonadia are considered potential species responsible for the hyperoxic stress of adult mice.

DISCUSSION

Despite booming publications concerning the interactions of microbiota with their hosts, only few have focused on the impact factors involved in the development and maturation

of newborns that may dominate their lifelong health and pathogenesis. Hyperoxia might be one of these factors in pediatric disease, because hyperoxic therapy is becoming an effective strategy for millions of preterm infants. Hyperoxia is considered an important cause of retinopathy in preterm infants, and rituximab is used to treat retinopathy. We aimed to investigate the intestinal dysbiosis of newborns encountering hyperoxic exposure and rituximab. This is the first known research to reveal that hyperoxia combined with B-cell selective depletion using rituximab can severely alter neonatal intestinal microbiota. Several studies reported that hyperoxia and the B-cell antagonist rituximab could provoke intestinal dysbiosis,[22,29] yet controversial results about the impacts of hyperoxia on intestinal microbiota were reported.[26,30] Xing and colleagues[31] detected the adverse effect of hyperoxia by exposing rats to 80% oxygen and observed that hyperoxia provoked gut dysbiosis in a complex manner. Pescovitz *et al.*[29] found that a four-dose course of rituximab partially preserved beta-cell function, yet increased adverse events in the intestine. Moreover, Althouse *et al.*[26] reported that neonatal hyperoxia combined with antibiotics altered the β diversity and relative abundance of commensal bacteria. Antibiotics are notorious reagents that disturb the intestinal microbiota in an intricate manner.[26] This is the first study to demonstrate that the combined effects of hyperoxia and an extensively used B-cell antagonist, rituximab, disturbed the intestinal microbiota in neonatal mice.

Our study has demonstrated that both high and low dosage of rituximab combined with about 75% oxygen stimulation significantly decreased the α diversities of intestinal microbiota (Figure 2a). A total of 141 species were diminished in both LDR and HDR groups (Figure 2d). Table S2 presents systematic alterations from phylum to species. It demonstrated interesting results that exposure to hyperoxia combined with different dosages of rituximab caused dosage-dependent changes in the abundance of intestinal microbiota, compared with hyperoxic exposure alone (Table S2). In addition, LDA scores and proportional abundances showed significantly enriched microbiota taxa between LDR and HDR groups. Taken together, hyperoxia combined with rituximab caused global and dosage-dependent dysbiosis, confirming the hypothesis that hyperoxia and rituximab exhibit synergistic interference to disturb the vulnerable intestinal microbiota at the neonatal stage.

In contrast to the severe intestinal dysbiosis caused by the combination of hyperoxia and rituximab, a mild degree of intestinal dysbiosis was demonstrated in neonates encountering hyperoxia alone. First, the α diversity between the CTL and PBS groups has not changed significance ($P > 0.05$; Figure 3a). Second, the structure of intestinal imbalance changes slightly while confronting hyperoxic stress. For instance, the relative abundance of Bacteroidetes and Firmicutes, key phyla in the mammal intestinal microbiota, did not display statistical differences (Figure 3d). The significant differences at the levels of class, genus, and species between the CTL and PBS groups also indicate mild dysbiosis in intestinal microbiota (Table S2). The third aspect deals with dysregulated abundance of representative AKK and *L. murinus*, and increase in pathological species such as *H. hepaticus*, which directly indicated intestinal dysbiosis of neonates raised under 75% oxygen condition. Finally, the adverse effect of hyperoxia was further demonstrated by clustering heatmap, LDA scores, and LEfSe analysis. Similar results were obtained when neonates were exposed to 80% oxygen.[32] These results reconfirmed the synergistic effect of hyperoxia in combination with a B-cell antagonist (rituximab).

Unlike neonates,[33] relatively slight dysbiosis in the intestinal microbiota of dams was investigated after hyperoxic stress. On the one hand, there were no statistical alterations in α diversities of dams' intestinal microbiota with or without hyperoxic stimulation (Figure 4a). Between the LHE and LCE groups, no obvious difference was observed in the key phylum of the gut (Bacteroidetes and Firmicutes; Figure 4b). Negative principal components analysis scores were obtained among lactating mice irrespective of whether hyperoxic stimulation was induced (Figure 4d). On the other hand, some statistical differences were observed in the lower taxa (Table S2). Significant differences were noted between the LHE and LCE groups, as shown by the abundance cluster heatmap and LDA score. From the aforementioned results, it can said that a slight intestinal disorder exists in lactating mice exposed to hyperoxia. Finally, adult mice are richer in the probiotic *Lactobacillus* than the newborn mice. Theoretically, a mature ecosystem such as the intestinal microbiota of adult mice should have stronger tolerance and resilience while encountering hyperoxia and other adverse factors.[34] Our data confirmed these reports that, in contrast to newborns, adult mice do have much more robust ability to confront hyperoxic stress. Disturbance of intestinal flora due to hyperoxia remains controversial. Lo *et al.*[35] found a significant decrease in intestinal flora diversity in mice after 7 days of postnatal hyperoxic stimulation; however, this effect disappeared after 42 days of continuous postnatal hyperoxic stimulation. It was shown that the effect of hyperoxia on the structure and composition of the intestinal flora was related to the time of hyperoxic intervention. At 7 days (lactation), the intestinal flora of mice is fragile and vulnerable to hyperoxia, which led to intestinal flora imbalance and reduced intestinal tight junction protein expression.[36–38] By contrast, at 42 days, equivalent to human puberty, hyperoxic stimulation did not cause significant changes in intestinal diversity in newborn mice. In this study, we collected the intestinal contents of mice on day 17, which may explain why the α diversity analysis did not demonstrate a statistical difference between the CTL and PBS groups; however, the apparent decrease in the abundance of AKK at the low genus level was of interest to us. It is an interesting observation that the relative abundance of *AKK* was significantly altered in pups and dams impacted by hyperoxia and/or rituximab. *AKK* is a

promising probiotic that is extensively distributed in the intestinal tract. *AKK* can ameliorate obesity[39] and inflammatory bowel diseases[40] through regulation of hosts' metabolism,[41] immunity,[6] and intestinal barrier.[31] In this study, we observed that hyperoxia and/or rituximab can intensively disturb the abundance of *AKK* in both pups and dams, suggesting *AKK* is a core species while encountering adverse factors in intestinal microbiota.

This study did not thoroughly determine the intestinal dysbiosis pattern or pathological mechanisms. Besides, several shortcomings should be noted. The first drawback was the lack of detecting the quantity or distribution of intestinal B cells, which might profoundly impact and trim the construction of intestinal microbiota. Second, long-time effects of hyperoxia and/or rituximab against the intestinal microbiota of either pups or dams were not investigated. Third, limited sample sizes, particularly in LHE and LCE groups, did not help us to completely determine the pathological characteristics of intestinal dysbiosis. Finally, we focused only on exploring the impact of hyperoxia in combination with rituximab on neonates' intestinal microbiota, without considering the clinic dosage or therapy course. Notwithstanding these limitations, this work does indicate the primary pattern of intestinal dysbiosis in both pups and dams while encountering hyperoxia and rituximab. These shortcomings could be conquered in future studies.

In conclusion, the present study demonstrated that the synergistic effect of hyperoxia and rituximab led to severe intestinal dysbiosis in newborns, while adult mice possess a more resilient ability to rescue intestinal dysbiosis. The precise regulatory mode between hyperoxia and rituximab and intestinal microbiota remains to be elucidated.

ACKNOWLEDGMENT

This research was financially supported by the National Natural Science Foundation of China (No. 82074151) and the Experimental Formulary Sichuan Youth Science and Technology Innovation research team (No. 2020JDTD0022). This study was also supported by the Chengdu University Research Fund and State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

ORCID

Wei-Jun Ding http://orcid.org/0000-0002-4933-7347

REFERENCES

1. Moreau MC, Raibaud P, Muller MC. Relationship between the development of the intestinal IgA immune system and the establishment of microbial flora in the digestive tract of young holoxenic mice. Ann Immunol (Paris). 1982;133d(1):29–39.
2. Braniste V, Al-Asmakh M, Kowal C, et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med. 2014;6(263):263ra158.
3. Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. Mol Psychiatry. 2014;19(2):146–8.
4. Young VB. The intestinal microbiota in health and disease. Rev Gastroenterol Mex. 2012;77(Suppl 1):23–5.
5. Pan W, Li J, Ou Y, et al. Clinical outcome of standardized oxygen therapy nursing strategy in COVID-19. Ann Palliat Med. 2020;9(4):2171–7.
6. Brill SE, Wedzicha JA. Oxygen therapy in acute exacerbations of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2014;9:1241–52.
7. Rubin M. Bronchial asthma; therapeutic by oxygen and technique of its application. Sem Med. 1946;53(1):754–7.
8. Goldstein RF, Malcolm WF. Care of the neonatal intensive care unit graduate after discharge. Pediatr Clin North Am. 2019;66(2):489–508.
9. Blencowe H, Cousens S, Chou D, et al. Born too soon: the global epidemiology of 15 million preterm births. Reprod Health. 2013; 10(Suppl 1):S2.
10. Dammann O, Rivera JC, Chemtob S. The prenatal phase of retinopathy of prematurity. Acta Paediatr. 2021;110(9):2521–8.
11. Rosenbaum JT, Sibley CH, Lin P. Retinal vasculitis. Curr Opin Rheumatol. 2016;28(3):228–35.
12. Conrady CD, Shakoor A. Rituximab-associated retinal occlusive vasculopathy: a case report and literature review. Ocul Immunol Inflamm. 2020;28(4):622–5.
13. Murthy SI, Shah S, Bagga B, Dudam R. Rituximab therapy combined with methotrexate for severe necrotizing scleritis in a case of granulomat osis with polyangiitis. Indian J Ophthalmol. 2020;68(9):1981–3.
14. Starr MR, Garrity JA, Tooley AA, Salomão DR. Clinical and histopathological findings of patients with orbital granulomatosis with polyangiitis cases refractory to rituximab. Can J Ophthalmol. 2019;54(6):682–7.
15. Ostrowski RA, Bussey MR, Shayesteh Y, Jay WM. Rituximab in the Treatment of Thyroid Eye Disease: A Review. Neuroophthalmology. 2015;39(3):109–15.
16. Dai D, Walker WA. Protective nutrients and bacterial colonization in the immature human gut. Adv Pediatr. 1999;46:353–82.
17. Goldmanln DA. Bacterial colonization and infection in the neonate. Am J Med. 1981;70(2):417–22.
18. Fanaro S, Chierici R, Guerrini P, Vigi V. Intestinal microflora in early infancy: composition and development. Acta Paediatr Suppl. 2003;91(441):48–55.
19. Gorodnichenko LI, Prozorovskaia KN, Artemenko LV. Role of mother's milk in the development of intestinal microflora in newborn infants. Zh Mikrobiol Epidemiol Immunobiol. 1977;2:100–4.
20. Dierikx TH, Visser DH, Benninga MA, et al. The influence of prenatal and intrapartum antibiotics on intestinal microbiota colonisation in infants: a systematic review. J Infect. 2020;81(2):190–204.
21. Golby S, Hackett M, Boursier L, Dunn-Walters D, Thiagamoorthy S, Spencer J. B cell development and proliferation of mature B cells in human fetal intestine. J Leukoc Biol. 2002;72(2):279–84.
22. Lundell AC, Björnsson V, Ljung A, et al. Infant B cell memory differentiation and early gut bacterial colonization. J Immunol. 2012;188(9):4315–22.
23. Nakamura M, Takahashi T, Matsui H, et al. New pharmaceutical treatment of gastric MALT lymphoma: anti-angiogenesis treatment using VEGF recepto r antibodies and celecoxib. Curr Pharm Des. 2014;20(7):1097–103.
24. Jiang L, Li N. B-cell non-Hodgkin lymphoma: importance of angiogenesis and antiangiogenic therapy. Angiogenesis. 2020;23(4):515–29.
25. Mallepally N, Abu-Sbeih H, Ahmed O, et al. Clinical features of rituximab-associated gastrointestinal toxicities. Am J Clin Oncol. 2019;42(6):539–45.
26. Althouse MH, Stewart C, Jiang W, Moorthy B, Lingappan K. Impact of early life antibiotic exposure and neonatal hyperoxia on the murine microbiome and lung injury. Sci Rep. 2019;9(1):14992.

27. Gupta P, Shayota BJ, Desai AK, et al. A race against time-changing the natural history of CRIM negative infantile pompe disease. Front Immunol. 2020;11:1929.
28. Huang L, Duan C, Xia X, et al. Commensal microbe-derived propionic acid mediates juvenile social isolation-induced social deficits a nd anxiety-like behaviors. Brain Res Bull. 2021;166:161–71.
29. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med. 2009;361(22):2143–52.
30. Makanyengo SO, Carroll GM, Goggins BJ, Smith SR, Pockney PG, Keely S. Systematic review on the influence of tissue oxygenation on gut microbiota and anastomotic healing. J Surg Res. 2020;249:186–96.
31. Xing Z, Li Y, Liu G, He Y, Tao Y, Chen M. Hyperoxia provokes gut dysbiosis in rats. Crit Care. 2020;24(1):517.
32. Liang ZJ, Wu QP, Chen BT, Lin ZL, Lin J, Chen SQ. Postnatal hyperoxia or DEHP exposure leads to growth restriction and delayed lung development in newb orn rats. Pediatr Neonatol. 2018;59(1):24–30.
33. Rossi JF, Céballos P, Lu ZY. Immune precision medicine for cancer: a novel insight based on the efficiency of immune effector cells. Cancer Commun (Lond). 2019;39(1):34.
34. Spencer J, MacDonald TT, Finn T, Isaacson PG. The development of gut associated lymphoid tissue in the terminal ileum of fetal human intestine. Clin Exp Immunol. 1986;64(3):536–43.
35. Lo YC, Chen KY, Chou HC, Lin IH, Chen CM. Neonatal hyperoxia induces gut dysbiosis and behavioral changes in adolescent mice. J Chin Med Assoc. 2021;84(3):290–8.
36. Chou HC, Chen CM. Neonatal hyperoxia disrupts the intestinal barrier and impairs intestinal function in rats. Exp Mol Pathol. 2017;102(3):415–21.
37. Chen CM, Chou HC. Hyperoxia disrupts the intestinal barrier in newborn rats. Exp Mol Pathol. 2016;101(1):44–9.
38. Liu DY, Lou WJ, Zhang DY, Sun SY. ROS plays a role in the neonatal rat intestinal barrier damages induced by hyperoxia. BioMed Res Int. 2020;2020:8819195.
39. van der Lugt B, van Beek AA, Aalvink S, et al. *Akkermansia muciniphila* ameliorates the age-related decline in colonic mucus thickness and attenuates immune activation in accelerated aging Ercc1-/Δ7 mice. Immun Ageing. 2019;16:6.
40. Wang L, Tang L, Feng Y, et al. A purified membrane protein from *Akkermansia muciniphila* or the pasteurised bacterium blunts colitis associated tumourigenesis by modulation of CD8+ T cells in mice. Gut. 2020;69(11):1988–97.
41. Wedgwood S, Warford C, Agvatisiri SR, et al. The developing gut-lung axis: postnatal growth restriction, intestinal dysbiosis, and pulmonary hyper tension in a rodent model. Pediatr Res. 2020;87(3):472–9.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Yang K, Xu Y-J, He Y, Duan C-X, Wang H-F, Ding W-J. Hyperoxia combined with the B-cell antagonist rituximab led to intestinal dysbiosis in neonatal mice. Microbiol Immunol. 2022;66:353–360.
https://doi.org/10.1111/1348-0421.12984