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Effects of celecoxib on AQP-1, NF-κB and apoptosis in lung tissue of neonatal rats with hyperoxia-induced lung injury

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ABSTRACT

To analyze the effects of AQP-1, NF-κB and apoptosis in lung tissue of neonatal rats with hyperoxia-induced lung injury (HILI).162 neonatal SD rats were divided into control group (COG), model group (MOG) and celecoxib group (CEG), 54 rats in each group. Each group was subdivided into 1st, 5th and 10th-day groups with 18 rats in each group. The neonatal rat model of HILI was established. Nine rats in each group were randomly selected on the 3rd, 6th and 12th day respectively. The level of oxidative stress (OS) in bronchoalveolar lavage fluid (BALF), the pathological changes of lung tissue, the ratio of lung wet weight to dry weight (W/D), the expression of inflammatory factors in lung tissue, the l AQP-1 and NF-κB level and apoptosis in lung tissue were tested. In comparison with COG, the level of MDA in BALF, the ratio of lung W/D, the expression level of IL-6, TNF- α, NF- κB and the number of apoptotic cells in lung tissue were significantly increased, while the level of SOD in BALF and AQP-1 in lung tissue notably decreased in the MOG (P<0.05). The level of malondialdehyde (MDA) in BALF, the ratio of lung W/D, IL-6, TNF-α, NF-κB and plenty of apoptotic cells in lung tissue in CEG were notably decreased than MOG(P<0.05), while the level of SOD in BALF and AQP-1 in lung tissue were raised in CEG. In the COG, the lung tissue structure was complete, the arrangement was neat, the alveolar cavity was clear, and there was no inflammatory infiltration. With the extension of time, the inflammatory infiltration of lung tissue in the MOG gradually increased, the plenty of red blood cells gradually increased, and the size of the alveolar cavity varied; compared with the MOG, the inflammatory infiltration in the CEG decreased and the plenty of red blood cells decreased. Celecoxib can improve oxidative injury, inhibit inflammation, up-regulate AQP-1 and down-regulate NF-κB, inhibit apoptosis, and have a certain protective effect on HILI in neonatal rats.

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Introduction

Acute lung injury is a kind of acute and progressive respiratory distress and refractory hypoxaemia., which is characterized by high mortality and high disability rate. The development of neonatal lung organs is not yet mature, and the occurrence of hypoxemia is not uncommon. As an important method for the treatment of hypoxemia, high-concentration oxygen therapy plays an important role in preventing multiple organ failure, striving for treatment time and saving patients' lives. However, high concentrations of oxygen inhalation for a long time will have a serious impact on the organs and systems of the body (1,2). This may be because hyperoxia can promote the expression of many inflammatory factors in the lung, induce the accumulation of excessive oxygen free radicals, reduce the number of alveoli and obvious apoptosis in lung tissue (3). Celecoxib, a selective inhibitor of cyclooxygenase-2 (COX-2), is a non-steroidal anti-inflammatory drug and has certain anti-inflammatory and analgesic effects. Some studies have discovered that celecoxib could inhibit the development of gastric cancer (4); in addition, celecoxib can also induce apoptosis of laryngeal cancer cells (5). It can have an important effect on many kinds of cells. Nevertheless, there are few reports on the effects of celecoxib in hyperoxia-induced lung injury (HILI). In this paper, we studied whether celecoxib can have a certain therapeutic effect on hyperoxia lung injury in neonatal rats.

Materials and Methods

Experimental animal

Randomly selected 162 neonatal SD rats [Zhejiang Weitong Lihua Experimental Animal Technology Co., Ltd., production license SCXK (Zhejiang) 2020-0002, use license SYXK (Zhejiang) 2019-0003], weight (55 ± 11) g, 3 weeks old, all young rats were free to eat and drink at laboratory temperature (22 ± 2) ℃, humidity (56 ±11)%, day and night for 12 hours.

Main instruments and reagents

Biological microscope (Shenzhen Chensheng Optical Instrument Co., Ltd., model: SC-Y409A); oxygen box (Guangzhou Huayuexing Instrument Co., Ltd., model: H135); ultra-low temperature refrigerator (Shanghai Tianfeng Industrial Co., Ltd., model: TF-86-200-WA); low temperature and high speeds centrifuge (Beijing Times Beili centrifuge Co., Ltd., model: GT16-3); MDA test kit

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(Hefei Lyle Biotechnology Co., Ltd.). SOD test kit (Suolaibao Technology Co., Ltd.); TNF- α test kit (Shanghai Jimi Industrial Co., Ltd., specification: 96T); IL-6 test kit (Beijing Rejing Biotechnology Co., Ltd., specification: 40T); rabbit anti-mouse AQP-1 polyclonal antibody (Wuhan Aimijie Technology Co., Ltd.); rabbit anti-mouse NF-κB antibody (Nanjing Lefusai Biotechnology Co., Ltd.). Celecoxib (Pfizer Pharmaceuticals LLC, production batch number: 20190063, specification: 0.2g*6 granules).

Establishment of a neonatal rat model of HILI

The neonatal rats were randomly divided into control, model and celecoxib groups of 54 rats each, and each group was further divided into 3rd, 6th and 12th-day groups, with 18 rats in each group.

The oxygen box has an outlet hole and an intake hole, and the rats in the MOG and CEG are continuously fed in the oxygen box so that the oxygen concentration in the oxygen box is maintained at about 85%. The rats in the COG were fed in natural air under the same conditions as those in the MOG and CEG. All groups were fed for 3 days, 6 days and 12 days respectively.

Observation index

Nine rats in each group were randomly selected on the 3rd day, 6th day and 12th day respectively. The bronchoalveolar lavage fluid (BALF) of each group was centrifuged and the supernatant was taken. The expression level of malondialdehyde (MDA) in BALF was determined by the thiobarbituric acid method, and the expression level of superoxide dismutase (SOD) in BALF was measured by the xanthine oxidase reduction method.

Nine rats in each group were randomly selected on the 3rd day, 6th day and 12th day respectively. The rats were killed under anesthesia, and the lung tissues were taken, and the pathological changes in the lung tissue of each group were determined by HE staining. The tissue of the upper lobe of the right lung was fixed in formalin solution, and 4 μ m thick paraffin sections were routinely made, xylene dewaxing, gradient alcohol dehydration, hematoxylin staining, ammonia returning to blue, eosin staining, tap water rinsing, alcohol dehydration, xylene transparent and neutral gum sealing tablets, and the changes of lung tissue in each group were observed by microscope.

The tissue of the lower lobe of the left lung was taken, the surface water was absorbed and recorded its weight as wet weight. After weighing, it was dried in an oven at 65℃ for 48 hours, and the weight was recorded as dry weight. The ratio of lung wet weight to dry weight (W/D) was calculated.

The upper lobe of the left lung was taken, lysate was added, homogenized and centrifuged, and the supernatant was taken. The expression levels of Tumor necrosis factor-α (TNF-α) and Interleukin-6, IL-6 in lung tissue of rats in each group were measured by enzyme-linked immunosorbent assay (Elisa). The testing steps are carried out in complete accordance with the operating procedures.

Aquaporin-1 (AQP-1) and nuclear factor kappa B (NF-κB) in the lung tissue of rats in each group were determined by Western blotting. The lung tissue protein was extracted from the lower lobe of the right lung according to the operation kit, then the separation gel and concentrated gel were arranged successively, the protein was boiled and denatured, samples were taken and electrophoretic, the protein on the gel was transferred to PVDF membrane, incubated overnight at 4℃, AQP-1 and NF-κB first antibody was added, diluted first antibody, closed at 37℃ for 1 hour, washed the membrane 3 times, each time 10 min, diluted second antibody, and washed the membrane again. A chemiluminescence imager was used for image quantitative analysis.

Determine the apoptosis of lung tissue of rats in each group on the 12th day: take the middle lobe of the right lung, make paraffin section routinely, and detect the apoptosis of lung tissue of rats in each group by TUNEL staining. Take paraffin sections, add protease K working solution, fix at room temperature, rinse with phosphate buffer, add Dnsael reaction solution, rich at room temperature, rinse with deionized water, add 0.3% hydrogen peroxide to rinse closed phosphate buffer, develop color with DAB chromogenic solution, seal film with neutral gum, and observe under the microscope. Five sections were selected from each group and four fields of view were randomly selected from each section to calculate the quantity of apoptotic cells in the lung tissue.

Statistical method

Single factor and multi-sample mean comparisons were used in multi-group comparisons, and independent sample t-test were used for paired comparisons, all expressed by ($x\pm s$). In this study, SPSS20.0 software was used to analyze the statistical data, and the difference was considered to be statistically significant. And in comparison with the control group [a] $P<0.05$; in comparison with model group [b] $P<0.05$.

Results

OS indexes in BALF in various groups of rats

With the extension of time, the level of MDA in BALF of rats in each group gradually increased and the level of SOD decreased gradually. The level of MDA in the BALF of the MOG was raised and the level of SOD was reduced than COG ($P<0.05$). In comparison with the MOG, the levels of MDA and SOD in the BALF of rats in the CEG were reduced and raised ($P<0.05$) (Table 1).

Histopathological changes of lung tissue in various groups of rats

In the COG, the lung tissue structure was complete, the arrangement was neat, the alveolar cavity was clear, and there was no inflammatory infiltration. With the extension of time, the inflammatory infiltration of lung tissue in the MOG gradually increased, red blood cells number gradually increased, and the size of the alveolar cavity varied; compared with the MOG, the inflammatory infiltration in the CEG decreased and red blood cells number decreased.

W/D of lung tissue in each group

The lung W/D ratio of the MOG was raised than COG ($P<0.05$), and that of the CEG was reduced than that of the MOG ($P<0.05$) (Table 2).

Comparison of the levels of inflammatory factors in lung tissue of rats in each group

The levels of IL-6 and TNF-α in the lung tissue of the MOG were raised than COG ($P<0.05$), and those in the lung tissue of the CEG were reduced than MOG ($P<0.05$)

Table 1. OS indexes in BALF of rats ($\bar{x}\pm s$).

Group	Number	3 d	6 d	12 d
COG	9			
SOD (U/mL)		20.58±1.78	18.87±2.00	17.18±2.74
MDA (nmol/mL)		0.96±0.04	1.01±0.07	1.05±0.09
MOG	9			
SOD (U/mL)		12.15±1.37[a]	11.68±1.29[a]	10.46±1.02[a]
MDA (nmol/mL)		1.49±0.09[a]	1.58±0.09[a]	1.74±0.11[a]
CEG	9			
SOD (U/mL)		18.11±0.82[b]	16.17±0.68[b]	14.09±0.88[b]
MDA (nmol/mL)		1.07±0.07[ab]	1.17±0.09[ab]	1.23±0.09[ab]

Table 2. Comparison of W/D of lung tissue ($\bar{x}\pm s$).

Group	Number	3 d	6 d	12 d
COG	9	4.87±0.17	4.92±0.26	4.93±0.17
MOG	9	5.12±0.24[a]	5.43±0.17[a]	5.63±0.14[a]
CEG	9	4.91±0.18[ab]	4.98±0.11[ab]	5.11±0.13[ab]
F		4.10	19.32	54.55
P		0.030	<0.001	<0.001

Table 3. The levels of inflammatory factors ($\bar{x}\pm s$).

Group	Number	3 d	6 d	12 d
COG	9			
IL-6 (pg/mL)		141.87±36.82	157.07±45.48	168.28±36.25
TNF-α (n g/mL)		82.17±16.87	87.28±20.42	91.28±36.58
MOG	9			
IL-6 (p g/mL)		351.74±82.42[a]	362.28±107.18[a]	374.58±99.81[a]
TNF-α (n g/mL)		359.41±32.48[a]	367.72±48.24[a]	375.26±63.14[a]
CEG	9			
IL-6 (p g/mL)		201.56±74.15[b]	214.38±100.49[b]	223.99±111.55[b]
TNF-α (n g/mL)		169.54±32.45[ab]	186.28±40.73[ab]	196.58±48.27[ab]

Table 4. AQP-1 and NF-κB repression.

Group	Number	AQP-1	NF-κB
COG	9	1.05±0.13	0.56±0.12
MOG	9	0.57±0.09[a]	1.76±0.42[a]
CEG	9	0.74±0.11[ab]	0.86±0.23[ab]
F		43.151	41.365
P		<0.001	<0.001

(Table 3).

Comparison of expression levels of AQP-1 and NF- κB in lung tissue of rats in each group

The AQP-1 in the lung tissue of the MOG was reduced and the expression level of NF-κB was raised than COG (P<0.05), and AQP-1 and NF- κ B in the lung tissue of the MOG was raised than that of the MOG(P<0.05) (Table 4).

Comparison of apoptosis in lung tissue of rats in each group

A large number of apoptotic cells in the lung tissue of the MOG was found more than COG (P<0.05), and that of the MOG was reduced than that of the MOG (P<0.05) (Table 5).

Table 5. Apoptosis in lung tissue of rats ($\bar{x}\pm s$).

Group	Number	Apoptotic cells number
COG	9	7.95±1.43
MOG	9	85.79±7.32[a]
CEG	9	18.78±3.62[ab]
F		698.37
P		<0.001

Discussion

The pathogenesis of hyperoxia lung injury is complex. Most scholars believe that oxidative stress and inflammation may be implicated in the onset and development of hy-

peroxic lung injury. (6). Oxygen is an essential element for the body. in the process of metabolism, a variety of active oxides are produced, and the active oxides and antioxidant systems work together to maintain the balance of oxides in the body. MDA is the end product of lipid peroxides, which can cross-link lipids and proteins, denature the biofilm, destroy the cell structure and affect the cell function. The level of MDA was strongly related to the degree of lipid peroxidation (7). As an antioxidant cytokine, SOD can scavenge lipid peroxidation products. The level of SOD can have an important effect on the antioxidant capacity of the reaction body (8). The results of this study showed that when neonatal rats were exposed to high oxygen concentration, there was obvious OS, and the inflammatory infiltration of lung tissue gradually increased, the plenty of red blood cells gradually increased, and the ratio of W/D increased significantly. Celecoxib can significantly improve oxidative stress response and lung injury in rats.

The inflammatory reaction is an important pathology of hyperoxia lung injury. When hyperoxia lung injury occurs, large amounts of inflammatory factors, such as TNF-α and IL-6, are released that damage the barrier function of vascular endothelial cells and epithelial cells, amplify the cascade of cytokines and chemokines, and further aggravate lung injury (9). The findings of this study suggest that when neonatal rats were exposed to high oxygen concentration, there was an obvious inflammatory reaction in the lung tissue, which was aggravated with time. This may be due to the fact that hyperoxia can lead to excessive accumulation of reactive oxygen species in the body, promote the inflammatory factors expression, such as TNF-α, IL-1 hormone and IL-6 in the lung, and activate related pathways. Celecoxib can significantly inhibit pulmonary inflammatory factors expression (10).

This surface of the alveoli is covered by epithelial cells, and the cells are closely linked to each other, forming a barrier of alveolar epithelial cells. AQP is a small molecular membrane channel protein specifically permeable to whom, including 13 kinds of AQP0-12, among which AQP-1 is predominantly expressed in alveolar epithelial cells. The study found that (11), in the mouse model of acute lung injury, AQP-1 expression levels were dramatically reduced, and the mice showed obvious pulmonary edema. After knocking out the AQP-1 gene, the water permeability of the alveolar-capillary cavity decreased significantly, and the level of AQP-1 was significantly correlated with lung capillary water permeability (12). It has been found that redox-sensitive transcription factors also play an important role in HILI. NF-κB and signal transduction and transcription or honeysuckle may regulate cell necrosis and apoptosis in HILI. NF- κB is an important transcriptional regulator, which can regulate the expression of many inflammatory and immune genes (13). When the body is stimulated by pathogens, NF-κB inhibitory protein kinase is activated, which is hydrolyzed after ubiquitin modification, thus activating NF- κB, while NF-κB can activate the expression of inflammatory factors such as TNF-an and IL-6, leading to cascade amplification, resulting in lung injury (14). This research proves that hyperoxia could significantly inhibit the AQP-1 and promote NF- κB. Celecoxib could up-regulate AQP-1 and down-regulate NF- κB, and improve lung injury.

It is currently believed that lung injury caused by various causes is always accompanied by lung cell apoptosis in the development of the disease. With the continuous study of hyperoxia lung injury, it is considered that apoptosis is not only its histological feature but also one of its mechanisms. Apoptosis is a basic biological phenomenon of cells, which can stabilize the internal and external environment and eliminate aging cells in vivo. The study found that (15) apoptosis may run through the whole process of lung tissue cell injury and repair. In this study, neonatal rats were exposed to high oxygen concentration, and apoptosis was detected by the TUNEL method. The findings indicated that there was obvious apoptosis in rat lung tissue, and celecoxib could inhibit apoptosis. This may be related to the fact that celecoxib can inhibit oxidative stress, inhibit inflammation and stabilize the structure of alveoli.

To sum up, celecoxib can improve oxidative injury, inhibit inflammation, up-regulate AQP-1 and down-regulate NF-κB, inhibit apoptosis, and have a definite protective effect on HILI in neonatal rats.

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