The role of annexin A1 peptide Ac2-26 in regulating PI3K/Akt signaling pathway to reduce lung injury after cardiopulmonary bypass in rats

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Research article

Keywords: Ac2-26, cardiopulmonary bypass, lung injury, PI3K/Akt, inflammatory factor

Posted Date: April 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-391368/v1>

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Version of Record: A version of this preprint was published at Perfusion on December 24th, 2021. See the published version at <https://doi.org/10.1177/02676591211052162>.

Abstract

Background: The main causes of lung injury after cardiopulmonary bypass (CPB) are systemic inflammatory response syndrome (SIRS) and lung ischemia–reperfusion (IR) injury. SIRS activates the complement system, releases a variety of inflammatory factors, causes accumulation of neutrophils in the lung tissue, and produces a large amount of oxygen free radicals, which are the main causes of CPB lung injury.

Results: Treatment with Ac2-26 improves oxygenation index, reduces the degree of lung pathological damage and lung inflammation, while LY294002 shows the opposite effect.

Conclusions: In short, AnxA1 peptide Ac2-26 activates PI3K /Akt signaling pathway, upregulates the phosphorylation levels of PI3K and Akt, inhibits the expression of p-NF- κ B(p65), and reduces the release of inflammatory factors and lung injury after CPB in rats.

1. Background

With the improvement of cardiac surgery and cardiopulmonary bypass (CPB) technology, postoperative complications in patients with heart disease have been significantly reduced, but almost all patients have varying degree of lung injury after surgery^[1], and the serious manifestations are acute respiratory distress syndrome (ARDS) and even multiple organ failure, the fatality rate can be as high as 40%–70%^[2]. The systemic inflammatory response induced after CPB activates the complement system, releases a variety of inflammatory factors, accumulates neutrophils in the lung tissue, and produces a large amount of oxygen free radicals, which are the main causes of CPB lung injury^[3]. In order to reduce complications and improve the quality of life of patients after surgery, clinically or experimentally, researchers have been looking for best lung-protection measures.

AnxA1 is a type of calcium-dependent phospholipid binding protein, which plays an important role in a variety of cell pathophysiological processes, such as cell anti-inflammatory response, cell proliferation, differentiation, death, apoptosis, and apoptotic cell phagocytosis clearance^[4,5]. After proteolysis of the full-length AnxA1, the N-terminus is broken, and a peptide chain fragment Ac2-26 is formed by the 26 amino acids of the N-segment^[6]; its N-terminus mimics the peptide Ac2-26 and has similar biological effects^[7]. Experiments have found that in the LPS-induced pulmonary endotoxemia model, pretreatment with AnxA1 peptide Ac2-26 can reduce the leakage of leukocytes into the connective tissue and alveolar cavity and reduces the release of TNF- α , IL-6, and IL-1, thereby regulating the ammoniation of endotoxin, which has a protective effect on the lung^[8]. Nair et al confirmed that AnxA1 controls inflammation by inducing neutrophil apoptosis in mouse pleurisy model. The above studies show that AnxA1 and Ac2-26 play an important role in anti-inflammatory and lung protection^[9].

The anti-inflammatory effect of AnxA1 mainly limits the initial steps of inflammation, especially white blood cell replenishment and inflammatory mediator production^[10]. In addition, AnxA1 can also reduce

the adhesion and transendothelial migration of neutrophils to endothelial cells^[11] and promote the secretion of anti-inflammatory cytokine IL-10 by macrophages to reduce inflammation, but the specific molecular mechanism of the effect is not very clear. The PI3K/Akt signaling pathway is widely present in a variety of cells throughout the body and participates in regulating various pathophysiological processes of the body. Studies have found that AnxA1 peptide Ac2-26 can activate PI3K/Akt signaling pathway, downregulate NF- κ B activity, reduce TNF- α level, inhibit h9c2 cardiomyocyte caspase-3/8 activity, reduce organ pathological damage, and have a protective effect on the myocardium^[12].

Therefore, this study intends to establish a CPB left lung IR injury model to observe the effects of AnxA1 peptide Ac2-26 on rat lung tissue injury and PI3K/Akt signaling pathway, and to explore its effect on CPB rat lung injury protection and mechanism.

2. Experimental Animals And Methods

2.1 Materials

Ac2-26 (Tocris Bioscience, UK. Cat. No. 1845)

BCA protein concentration determination kit (MDL,China);

Rat IL-6 ELISA KIT (J&L Biological, China. Cat. No.JL20896)

Rat TNF- α ELISA KIT(J&L Biological, China. Cat. No.JL4559

4)

Rat Total protein ELISA KIT(J&L Biological, China. Cat. No.JL21491)

2.2 Experimental animals and groupings

Adult male SD rats, 350~450g, Specific Pathogen Free(SPF) level, were obtained from Changsha Tianqin Biotechnology Co., Ltd. (LICENSE number: SCXK (Xiang) 2019-0015) raised in Zunyi Medical University animal experiment center). The rats were kept in cages in a temperature controlled environment ($25 \pm 2^{\circ}\text{C}$) and humidity (50%–60%) with water and food ad libitum. The breeding environment was maintained at a constant temperature and exposed to a 12-hour light/dark cycle. All animal experiments were approved by the Animal Investigation Committee of Zunyi Medical University, Zunyi, Guizhou, China. According to the "Guidelines for the Care and Use of Laboratory Animals" (eighth edition, 2011) published by the National Institutes of Health, all test animals received humane care. The SD rats were fasted for 8 hours before the study, but could drink water. The animals were divided into 5 groups ($n = 6$ per group): Sham operation group (S group), Left lung IR injury group (IR group), Dimethyl sulfoxide group (D group), Ac2-26 group (A group), Ac2-26 + LY294002 group (AL group). Except for the S group, the other 4 groups had their chests opened 10 minutes after the establishment of CPB, their left hilum was blocked, and lung IR in rats was simulated.

In the S group, after vascular catheterization, only mechanical ventilation was performed, and the rat did not receive any other treatment.

In the IR group, a model of CPB IR in the left lung was established, and an equal volume of normal saline was injected 10 minutes before the left hilum block.

In the D group, a model of CPB IR in the left lung was established, and an equal volume of DMSO was injected 10 minutes before the left hilum block. In the A group, a model of left lung CPB IR was established, and $1 \text{ mg} \cdot \text{kg}^{-1}$ Ac2-26^[13] was administered through the tail vein 10 minutes before the left hilum was blocked. In the AL group, to establish a model of left lung CPB IR, LY294002 of $0.3 \text{ mg} \cdot \text{kg}^{-1}$ ^[14] was administered through the tail vein 10 minutes before left hilum occlusion, and $1 \text{ mg} \cdot \text{kg}^{-1}$ of Ac2-26 was administered simultaneously.

2.3 Preparation of the CPB pipeline^[15]

CPB tubing consists of a blood reservoir (20 ml syringe), silicone tubing, peristaltic pump, arteriovenous tubing, and rat lung membranes. Before the animal experiment, the CPB pipeline was connected, oxygen was connected, and the CPB pipeline was prefilled with 1 ml of sodium potassium magnesium calcium glucose injection, 9 ml of hydroxyethyl starch, 1 ml of 5% sodium bicarbonate and 1 ml of mannitol.

2.4 Establishment of a model of CPB IR injury in the left lung of rats

This experimental model establishes a model of extracorporeal circulation IR injury using the protocol of He et al. ^[16]. Rat anesthesia was injected intraperitoneally with 1% sodium pentobarbital $50 \text{ mg} \cdot \text{kg}^{-1}$. After sufficient anesthesia was administered, the tail vein was placed in the catheter, and the rats were placed in a fixed supine position. The right femoral artery and the left femoral vein were separated, and the catheter was inserted. Trachea was intubated by visualization with a laryngoscope, and the right common carotid artery was punctured and placed. The tracheal tube was connected to a small animal ventilator for mechanical ventilation, maintaining a respiratory rate of $60 \text{ times} \cdot \text{min}^{-1}$, a tidal volume of $15 \text{ ml} \cdot \text{kg}^{-1}$, I:E=1:2.5, and FiO₂: 99%. The right femoral artery was connected to a biosignal acquisition and processing system to monitor the vital signs, including heart rate and blood pressure. The tail vein was injected with $3 \text{ mg} \cdot \text{kg}^{-1}$ heparin. After systemic heparinization, the bilateral femoral veins were used for venous drainage, the right common carotid artery was refluxed at the arterial end, and the CPB pipeline was connected. After ACT ≥ 480 seconds, the transfer was started. After 10 minutes, the left lung was closed with a noninvasive vascular clamp, and single-lung ventilation was performed for the right lung. The tidal volume and respiratory rate were appropriately reduced to meet the needs of the body. After 45 minutes of circulation, the noninvasive vascular forceps were released, and the left lung was released. The door restored lung ventilation, increased the tidal volume and reduced the respiratory rate. CPB was stopped after 30 minutes and the experiment ended after 90 minutes. During the CPB period, the anal temperature was maintained at 32°C – 34°C , the mean arterial pressure was maintained at 50–80 mmHg, and the perfusion flow rate was $40 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Before the experiment ended, protamine was injected

into the tail vein to neutralize excess heparin. The neutralized machine blood was recovered and input into the rat from the tail vein. Intraoperative arterial blood gas analysis was used to stabilize the rat internal environment. Fentanyl, midazolam, and pentobarbital sodium were used to maintain the depth of anesthesia with vecuronium. When necessary, vital signs of the animal were maintained using vasoactive drugs. During the surgery, a stomach tube was inserted into the rats for gastrointestinal decompression, and an indwelling catheter was used for intraoperative urine drainage.

2.5 Specimen collection

A total of 0.5 ml of femoral artery blood from each group was taken before CPB (T1), before open left hilum occlusion (T2), and at the end of the experiment (T3). Intact left lung tissue was also removed at the end of the experiment. Blood gas analysis of arterial blood and calculation of the oxygenation index (OI), respiratory index (RI), and alveolar–arterial oxygen gradient ($P(A-a)O_2$) were performed. The left lung bronchoalveolar lavage fluid (BALF) and left lung tissue were taken. After centrifugation, the collected BALF was centrifuged and the supernatant was stored in a refrigerator at -80°C for testing. The lung tissue was divided into two parts and stored in a refrigerator at -80°C . The morphological changes of lung tissue were observed under light microscope and electron microscope. The levels of TNF- α and IL-6 and total protein in BALF were detected by ELISA. The expression of PI3K, Akt, NF- κB (p65), p-PI3K, p-Akt, p-NF- κB (p65) and AnxA1 were detected by Western-blot.

2.6 Indicator detection

1. Pulmonary function measurement

OI and RI were calculated based on the results of arterial blood gas analysis.

$$\text{OI} = \text{PaO}_2/\text{FiO}_2 \quad \text{RI} = P(A-a)O_2/\text{PaO}_2$$

$$\text{Remarks: Corrected OI} = \text{PaO}_2/[\text{FiO}_2 \times (P/760)]$$

$$P(A-a)O_2 = (P - \text{PH}_2\text{O}) \times \text{FiO}_2 - \text{PaO}_2 - \text{PaCO}_2/0.8;$$

PH₂O is the saturated water vapor pressure, which is 47 mmHg under standard conditions.

FiO₂ (%) is the concentration of inhaled oxygen, and the experimental model of this animal is 99%.

P is the actual atmospheric pressure, Zunyi area is 680 mmHg.

2. pathological observation

At the end of the experiment, the left lung tissue was taken, and the left lung tissue was divided into 3 parts, one of which was stored at -80°C for inspection. A part of the left lung tissue was fixed with formaldehyde solution, embedded in paraffin, dehydrated, and deparaffinized. It was further stained by hematoxylin and eosin (HE), mounted, and the morphology of the lung tissue was observed using a light

microscope. Three high-powered visual fields were randomly selected to observe the lung tissue morphology, and pictures were taken. The average value was taken using protocol from Cheng et al. [17] Scoring standard, which can reflect the degree of lung injury is shown in Table 1. A portion of the left lung tissue was placed in 2% glutaraldehyde for 24 hours, washed with phosphate buffer, fixed with 2% osmium acid for 1 hour, dehydrated, embedded and stained. Cell morphology and ultrastructure were observed under an electron microscope.

3. BALF

At the end of the experiment, the trachea and lungs were stripped, the right main bronchus was blocked, and 5ml of 0.9% sodium chloride injection was injected through the trachea to the left main bronchus and left lung. Isolation of lavage of the left lung 3 times was repeated, at least 3ml of lavage fluid was recovered, centrifuged at 3000 r/min for 10 minutes, supernatant was removed and stored at -80°C for testing.

4. ELISA

The frozen lung tissue was thawed in a refrigerator at 4 °C, and the specimen was maintained at a temperature of 2-8 °C after thawing. PBS (pH 7.4) was added to the sample, and the specimen was thoroughly ground until homogenized. Standard dilution: Follow the instructions of the ELISA kit (Jiang's biological, China), 10 Wells are set on the enzyme label well plate as standard Wells, and the standard with the corresponding concentration gradient is added to the 10 Wells according to the instructions.

A) Sample adding: Set blank control well and test sample well, add 50ul dilution to the blank control well, add dilution 40μl to the test sample well and 10μl to the test sample well, the total is also 50μl.

B) Incubation: Gently mix the added sample, seal it with a sealing plate membrane and put it into a 37°C incubator for incubation for 30min.

C) Washing plate: tear off the sealing plate membrane and pour out the liquid in the hole. Wash each hole with detergent solution for 5 times, 30S each time.

D) Enzyme reaction: Add 50μl HRP-Conjugate reagent to all the Wells except the blank well. PBS wash the board and then add enzyme reaction test

Incubate again and wash the plate with PBS.

E) Color development: Add chromogen A and B 50μl to each well after shaking, and put them into A 37°C incubator to avoid

The reaction time was 15 min.

F) Termination: Add 50μl Stop Solution to each well after color development, and observe that the color changes from blue to yellow, indicating the termination of reaction.

G) Determination: 30min after the reaction was terminated, the wavelength of 540nm was set on the enzyme plate and pushed into the tray to measure the absorbance value of each hole optical density(OD).

H) Calculation: all OD values are measured by the enzyme plate analyzer, and the standard curve is calculated according to the concentration of the standard substance, according to the sample to be tested. The OD value of the product is substituted into the formula to obtain the concentration of the sample to be tested.

5. Western-blot

Immediately after the experiment, lung tissue was excised and frozen at -80 °C. The samples were then centrifuged in ice-cold lysis buffer at 12,000 g for 5 minutes at 4 °C. and then the whole samples was separated by SDS-PAGE and further transferred onto nitrocellulose membranes. After blocking with TBST containing 5% (w/v) non-fat milk, the membranes were incubated with specific primary antibodies against

PI3K(1:1000,Abcam,Cambridge,UK.No.ab191606),Akt(1:1000,Abcam,Cambridge,UK.No.ab8805),NF- κ B(p65)(1:1000,Abcam,Cambridge,UK.No.ab16502),p-

PI3K(1:1000,Abcam,Cambridge,UK.No.ab182651),p-Akt(1:1000,Abcam,Cambridge,UK.No.ab8805),p-NF- κ B(p65)(1:1000,Abcam,Cambridge,UK.No.ab16502)and AnxA1(1:1000,Abcam,Cambridge

,UK.No.ab214486) at 4°C overnight in blocking solution, all antibodies were diluted at 1:1,000. Following three times of washed with TBST, the membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 1h. The chemiluminescence was detected using the ECL-chemiluminescent kit with Protein Simple .The protein content of the supernatant was determined by a BCA protein assay kit.

2.7 Statistical methods

Data analysis and statistical evaluation were performed using IBM SPSS 18.0. All results are mean standard deviations for at least 3 experiments conducted in triplicate. Data are shown as mean $\pm$ mean standard error (SEM). Repeated measurements were performed within this group using analysis of variance. One-way analysis of variance was used among the groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Changes in OI, RI and P(A-a)O₂ in rats

3.1.1 changes in OI

Comparison of OI of rats in each group at different times: Compared with T1, the OI of rats in each group decreased at T2 and T3 ($P < 0.05$). Compared with the time point of T2, the OI at the time point of T3 in the IR, D, A, and AL groups decreased ($P < 0.05$). Simultaneous comparison of the OI of rats in each group: At

T1, there was no significant difference in OI of rats in each group ($P>0.05$). At T2, compared with the S group, the OI of rats in the IR, D, and AL groups was significantly reduced ($P<0.05$), while the OI of the A group was not significantly different from that of the S group ($P>0.05$); compared with the IR group, the D, AL The OI of group was significantly reduced ($P<0.05$), and the OI of group A was not significantly different from that of IR group ($P>0.05$); compared with group D, the OI of group A was significantly higher ($P<0.05$), and the OI of group AL was higher than that of group D There was no significant difference ($P>0.05$); compared with group A, the OI of group AL was significantly lower ($P<0.05$). At T3, the OI of the IR, D, A, and AL groups was significantly lower than that of the S group ($P<0.05$); the OI of the A group was significantly higher than that of the IR and AL groups ($P<0.05$); the IR, D, and AL groups were larger There was no significant difference in OI between rats ($P>0.05$). (See Table 5, Figure 3). (Table2, Figure 2A).

3.1.2 Changes in RI

Comparison of RI at different times of rats in each group: Compared with T1 time point, RI of rats in each group increased at T2 and T3 time points ($P<0.05$). Compared with the time point of T2, the RI at the time point of T3 increased in the IR, D, A, and AL groups ($P<0.05$). Simultaneous comparison of RI of rats in each group: At T1, there was no significant difference in RI of rats in each group ($P>0.05$). At T2, compared with the S group, the RI of the rats in the D and AL groups increased significantly ($P<0.05$), and the RI in the IR and A groups were not significantly different from the S group ($P>0.05$); compared with the IR group, D, There was no significant difference in RI between group A and AL ($P>0.05$); compared with group A, RI in group AL increased significantly ($P<0.05$). At T3, the RI of the IR, D, A, and AL groups were significantly higher than that of the S group ($P<0.05$); the RI of the A group was significantly lower than that of the IR and AL groups ($P<0.05$), and the difference between the IR, D, and AL groups There was no significant difference in RI ($P>0.05$). (Table3, Figure 3A).

3.1.3 changes in P(Aa)O₂

Comparison of P(Aa)O₂ at different times in each group of rats: Compared with T1, the five groups of rats P(Aa)O₂ increased at T2 and T3 ($P<0.05$). Compared with the time point of T2, P(A-a)O₂ increased at the time point of T3 in the four groups of IR, D, A, and AL ($P<0.05$). Comparison of P(A-a)O₂ in each group of rats at the same time: At T1, there was no significant difference in P(A-a)O₂ in the five groups of rats ($P>0.05$). At T2, compared with group S, P(Aa)O₂ of rats in group D and AL increased significantly ($P<0.05$), and P(Aa)O₂ in group IR and A were not significantly different from group S ($P>0.05$)); Compared with the IR group, there was no significant difference in P(Aa)O₂ in the D, A, and AL groups ($P>0.05$); compared with the A group, the P(Aa)O₂ in the AL group was significantly increased ($P<0.05$). At T3, P(Aa)O₂ in the four groups of IR, D, A, and AL was significantly higher than that in group S ($P<0.05$); P(Aa)O₂ in group A was significantly lower than that in IR and AL groups ($P<0.05$) , There is no significant difference in P(Aa)O₂ between IR, D and AL groups ($P>0.05$). (Table 4, Figure4A).

3.2 Lung tissue light microscopy results and pathological damage score

The lung tissue structure of S group was clear, and the alveolar wall was relatively complete (Figure 2A). In the IR group and D group, the lung tissue structure was disordered, some alveolar wall was broken, and the alveolar cavity was filled with edema fluid, showing moderate inflammation. Infiltration of cells and red blood cells and the degree of lung injury was more severe than that in the S group (Figure 2B, Figure 2C). In the A group, the lung tissue structure was complete, and some alveolar walls were broken, and there was a small amount of inflammatory cell infiltration. The degree of lung injury was significantly less than that in the IR group (Figure 2C, 2D). The lung tissue structure of the AL group was obviously damaged, the alveolar wall was severely broken, and a large number of inflammatory cells, red blood cells and edema fluid were seen in the alveolar cavity (Figure 2E) (Table 5, Figure 2).

Compared with the S group, the lung injury scores of the other four groups were higher than those of the S group ($P<0.05$). The lung injury scores of A group were significantly lower than that of the IR group ($P<0.05$), and the lung injury scores of the IR, D, and AL groups were different. There was no statistical significance ($P>0.05$) compared with the A group, the lung injury score of AL group increased ($P<0.05$) (Figure 3).

3.3 Lung tissue electron microscopy results

Under the microscope, the structure of the lamellar bodies in S group was basically intact, and the mitochondria were not swollen (Figure 4A). In IR group and D group, the structure of lamellar corpuscles was damaged, some lamellar corpuscles and mitochondria were swollen, and there was moderate inflammatory exudation in the alveolar cavity, which was significantly worse than that in the S group (Figure 4B, Figure 4C). Under the electron microscope in the A group, it was seen that the lamellar structure was slightly damaged, the mitochondria were not significantly swollen, and there was a small amount of inflammatory exudation in the alveolar cavity, which was significantly less damaged than the IR group (Figure 4D). In the AL group, the lamellar structure was damaged, some mitochondria were swollen, and there was a large amount of inflammatory exudation in the alveolar cavity, which was more severe than that in the A group (Figure 4E).

3.4 Contents of TNF- α in lung tissues and IL-6 and total protein in BALF at T3

At the end of the experiment, compared with S group, the level of TNF- α in lung tissue of IR, D, A, and AL groups increased significantly ($P<0.05$). The level of TNF- α in A group was significantly lower than that of IR and D groups ($P<0.05$). There was no significant difference in TNF- α expression between IR and D groups ($P>0.05$). The level of TNF- α in AL group was significantly higher than that in A group ($P<0.05$) (Table 6, Figure 5).

At the end of the experiment, compared with S group, the levels of IL-6 and total protein in left lung tissue BALF of IR, D, A, and AL groups were significantly increased ($P<0.05$). The level of IL-6 and total protein in A group was significantly lower than that of IR, D, AL groups ($P<0.05$). Levels of IL-6 and total protein concentration had no significant difference in IR, D, AL groups ($P>0.05$) (Table 7, Figure 6A, Figure 6B).

3.5 Expression of PI3K, p-PI3K, Akt, p-Akt (Ser473), NF- κ B(p65), p-NF- κ B(p65), and AnxA1 in rat lung tissue

Detect the expression of p-PI3K and PI3K in the lung tissues in each group. PI3K was set as an internal reference, and the ratio of p-PI3K/PI3K was calculated to reflect the expression level of p-PI3K^[16,17]. At the end of the experiment, the expression of PI3K in the lung tissues of rats in the IR, D, A, and AL groups was upregulated ($P < 0.05$). Compared with S group, the ratio of p-PI3K/PI3K in lung tissue of IR, D, A, and AL groups increased ($P < 0.05$). Compared with the IR group, the ratio of p-PI3K/PI3K in A group increased significantly ($P < 0.05$), the p-PI3K/PI3K ratio in the D and AL groups was not statistically different ($P > 0.05$). Compared with the D group, the p-PI3K/PI3K ratio in the A group increased ($P < 0.05$), while the p-PI3K in the AL group was no significant change in the ratio of /PI3K ($P > 0.05$). Compared with the A group, the ratio of p-PI3K/PI3K in AL group was significantly lower ($P < 0.05$) (Figure 7, Figure 8A). There was no significant difference in Akt expression in lung tissues of five groups of rats ($P > 0.05$). Compared with S group, the p-Akt/Akt ratio of lung tissue in IR, D, A, and AL groups increased ($P < 0.05$). Compared with IR group, the ratio of p-Akt/Akt in A group was significantly increased ($P < 0.05$), the p-Akt/Akt ratio of the D and AL groups was not statistically different ($P > 0.05$). Compared with the A group, the p-Akt/Akt ratio of the AL group was significantly lower ($P < 0.05$) (Table 8, Figure7, Figure 8B).

The expression of NF- κ B(p65) in the lung tissue of rats in the IR, D, A, and AL groups was upregulated ($P < 0.05$). Compared with the S group, the ratio of p-NF- κ B/NF- κ B(p65) increased ($P < 0.05$). Compared with the IR group, the ratio of p-NF- κ B/NF- κ B(p65) in A group was significantly lower ($P < 0.05$), the ratio of p-NF- κ B/NF- κ B(p65) in D and AL groups did not have any significant difference ($P > 0.05$). Compared with the D group, the ratio of p-NF- κ B/NF- κ B(p65) in the A group decreased ($P < 0.05$), while the ratio of p-NF- κ B/NF- κ B(p65) in AL group was not seen Significant change ($P > 0.05$). Compared with the A group, the ratio of p-NF- κ B/NF- κ B(p65) in AL group was significantly higher ($P < 0.05$). (Table 9, Figure 9A, Figure 9B). Compared with the S group, the expression of AnxA1 in the lung tissues of IR, D, A, and AL groups increased ($P < 0.05$). Compared with the IR group, the expression of AnxA1 in A group increased ($P < 0.05$). Compared with A group, in the AL group the expression of AnxA1 decreased ($P < 0.05$). Compared with S group, the expression of AnxA1 (37kD) in the lung tissues of IR, D, A, and AL decreased ($P < 0.05$), while the expression of AnxA1 (33kD) in the lung tissues increased ($P < 0.05$). (Table 9, Figure 10A , Figure 10B).

4. Discussion

CPB lung injury is one of the main complications after cardiovascular surgery, and it is also an important cause of postoperative patient death^[18]. Studies have shown that tumor necrosis factor and interleukin produced after CPB can directly damage endothelial cells, increase capillary permeability, cause pulmonary edema, and can also promote neutrophils, and macrophage Infiltration and release of cytotoxic enzymes aggravate lung injury^[19]. Under the light microscope, this study found that the structure of lung tissue in IR group was disordered, the alveolar wall was ruptured, the alveolar cavity filled with edematous fluid, the infiltration of inflammatory cells and red blood cells were visible, and the pathological injury score of lung tissue in IR group was significantly higher than that in S group. Under the

electron microscope, IR group showed that the structure of lamellar corpuscles was damaged, some lamellar corpuscles and mitochondria swelled, and alveoli were inflamed, which indicated that the structure of lung tissue was damaged after CPB. In addition, OI, RI and PaO₂ in groups were significantly lower and higher than those in groups. In addition, lung function decreased significantly. The content of TNF- α in lung tissue of IR group was lower than that of S group. The contents of total protein and IL-6 in bronchoalveolar lavage fluid were significantly increased, suggesting that the pulmonary inflammation and vascular permeability were increased after CPB. In addition, the lung injury was serious, which indicated that the left lung IR injury model of CPB was successfully established.

AnxA1 is a member of the structurally related calcium-dependent phospholipid binding protein superfamily. It consists of a highly conserved central domain and an N-terminal sequence that bears a unique function^[20]. Its function involves all aspects of cell life activities, including cells secretion, signal transduction, inflammatory response, apoptosis, etc.^[21] Under normal conditions, AnxA1 is mainly located in the cytoplasm and is inactive. Under the action of inflammatory factors, neutrophils and capillary endothelial cells are combined to stimulate a large amount of AnxA1 to move to the cell surface and anchor in the cell in a calcium-dependent manner. The plasma membrane interacts with adhesion molecules to inhibit the migration of leukocytes to the inflammation site and regulate the body's own anti-inflammatory effects^[22].

AnxA1 reduces the formation of inflammatory response complexes by inhibiting the activity of phospholipase A2 (PLA2). Inflammatory cytokines (TNF- α , IL-1 β , etc.) improve the inflammatory response^[23,24]. In the rat lung transplantation model^[25], after the administration of Ac2-26, it was found that the total protein concentration and pro-inflammatory factor levels in the lung tissue were significantly reduced, and PaO₂ increased. Ac2-26 inhibits oxidative stress and inflammation, improves alveolar capillary permeability and reduces tissue damage. It shows that Ac2-26 can improve the circulatory function of rats, reduce the lung injury of CPB in rats, and have a certain protective effect on the lungs.

The PI3K/Akt signaling pathway is a classic signaling pathway in cells, which participates in the body's IR injury, inflammation, and other processes^[26]. PI3K is a heterodimer composed of a catalytic subunit (p110) and a regulatory subunit (P85), extracellular signals, such as growth factors, growth hormones, endotoxins, and IR injury can be activated PI3K, make the cell membrane produce the second messenger PIP3. PIP3 binds to Akt and PDK in the cell, so that Akt is phosphorylated and activated at Ser473 and Thr308. Among them, phosphorylation at Ser473 can make Akt maximize its biological activity^[27], p-Akt can cause the phosphorylation cascade of downstream effector molecules and the interaction between the target protein^[28]. The activation of PI3K/Akt pathway plays a key role in CPB lung injury^[29]. Studies have found^[30] that after acute lung injury in rats, oxidative stress can be inhibited by activating the PI3K/Akt signaling pathway, and the production of inflammatory mediators can be reduced. The lung injury was significantly improved, and it has a certain protective effect on the lung. The results show that LY294002 can counteract the effect of Ac2-26 on lung injury in rats, and further prove that the

mechanism of Ac2-26 on lung protection of CPB in rats is related to the activation of PI3K / Akt signaling pathway.

As the downstream target protein of PI3K/Akt signaling pathway, NF- κ B has the functions of regulating inflammatory mediators, cell adhesion molecules, cytokines, etc.^[31]. Inhibiting the activity of NF- κ B can reduce the transcription of cytokines such as TNF- α , IL-6, etc., and reduce tissue IR injury^[32,33]. It has been reported in the literature^[34] that in COPD patients, elevated AnxA1 can downregulate lung tissue NF- κ B, reduce IL-6, reduce collagen deposition induced by inflammatory cytokines, and have a protective effect on the lung. The results showed that Ac2-26 could up regulate the expression of p-PI3K and p-Akt, decrease the expression of p-NF- κ B(p65), inhibit the production of inflammatory factors, improve the pulmonary vascular permeability, and reduce the pathological damage of CPB lung tissue. Moreover, PI3K specific inhibitor reversed the protective effect of Ac2-26 on rat lung tissue.

The lung protective effect of Ac2-26, a mimetic peptide of ANXA1, on lung IR injury model after CPB was investigated, and the regulation mechanism of Ac2-26 on PI3K/Akt signaling pathway was explored. However, there are still some deficiencies in the experiment. Our research group is continuing to carry out cytological experiments, and the relevant mechanism remains to be further explored and clarified.

5. Conclusion

The AnxA1 peptide Ac2-26 can improve the lung ventilation function of rats after CPB, reduce the degree of lung pathological damage and lung inflammation, and has a certain lung protection effect on CPB rats. The mechanism may be related to activating the PI3K/Akt signaling pathway, upregulating the phosphorylation levels of PI3K and Akt, inhibiting the expression of p-NF- κ B(p65) in lung tissue, and reducing the release of inflammatory factors.

Declarations

1.Ethic approval, guidenlines and consent to participate

All animal experiments were approved by the Animal Investigation Committee of Zunyi Medical University, Zunyi, Guizhou, China. According to the "Guidelines for the Care and Use of Laboratory Animals" (eighth edition, 2011) published by the National Institutes of Health, all test animals received humane care.

2. Consent of publication

All authors read and approved the final manuscript. The authors declare that they have no competing interests

3.Availability of data and materials

Datasets used and/or analyzed in current research may be obtained from the appropriate authors upon reasonable request.

4. Competing interests

The authors declare no financial and personal relationships with other people or organizations that can inappropriately influence this work. Moreover, the authors have no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of, the manuscript. The authors declare no conflict of interest.

5. Funding

National Natural Science Foundation of China, Fund Project Number: 81760079

6. Authors' contributions:

Yuanjie Zhang designed the experimental project, conducted most of the experiments, and prepared the manuscript. Yunzi He participated in the establishment of an in vitro lung IR injury model and participated in the writing and revision of the manuscript. Hanhua Wu and Junli Luo completed the analysis of part of the experimental data, and Chi Cheng, as the instructor, completed most of the experimental operation techniques. Hong Zhang revised the paper and had the primary responsibility for the final content. All authors read and approved the final manuscript.

7. Acknowledgements

I would like to express my gratitude to all those who helped me during the writing of this thesis. I gratefully acknowledge the help of my supervisor, ms. Hong zhang, who has offered me valuable suggestions in the academic studies. In the preparation of the thesis, she has spent much time reading through each draft and provided me with inspiring advice. Without her patient instruction, insightful criticism and expert guidance, the completion of this thesis would not have been possible.

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Tables

Tables 1-9 are available in the Supplementary Files.

Figures

Figure 1

Changes in OIs, RIs, and P(A-a)O(2) in rats. A The PaO(2)/FiO(2) (OI) change in each group at different times. B The P(A-a)O(2)/PaO(2) (RI) change in each group at different times. C The P(A-a)O(2) change in each group at different times. Compared with T1, ▲P < 0.05. Compared with T2, △P < 0.05. Compared with group S, aP < 0.05. Compared with group IR, bP < 0.05. Compared with group D, cP < 0.05. Compared with group A, dP < 0.05.

Figure 2

Morphological changes in lung tissues of rats in each group under the light microscope at T3. A A lung section from the control rats. B A lung section from the rats in group IR. C A lung section from rats in the IR + dimethyl sulfoxide group. D A lung section from rats in the IR + Ac2-26 group. E A lung section from rats in the IR + LY294002 group.

Figure 3

Pathological damage scores of lung tissues of rats in each group under the light microscope at T3 in rats. Compared with group S, $aP < 0.05$. Compared with group IR, $bP < 0.05$. Compared with group D, $cP < 0.05$. Compared with group A, $dP < 0.05$.

Figure 4

Observation results of lung tissues under an electron microscope at the end of the experiment in rats. A Electron microscope picture from the control rats. B Electron microscope picture from the rats in group IR. C Electron microscope picture from rats in the IR + dimethyl sulfoxide group. D Electron microscope picture from rats in the IR + Ac2-26 group. E Electron microscope picture from rats in the IR + LY294002 group. Red arrow lamellar body, yellow arrow mitochondria.

Figure 5

Comparison of TNF-α content in lung tissue of each group at T3. Compared with group S, $aP < 0.05$. Compared with group IR: $bP < 0.05$. Compared with group D, $cP < 0.05$. Compared with group A, $dP < 0.05$.

Figure 6

Comparison of IL-6 and total protein in left lung tissue BALF of each group at T3. Compared with group S, $aP < 0.05$. Compared with group IR, $bP < 0.05$. Compared with group D, $cP < 0.05$. Compared with group A, $dP < 0.05$.

Figure 7

Expression of PI3K in lung tissues of each group at T3. A Western blot of PI3K, p-PI3K, Akt, and p-Akt in lung tissues of five groups of rats at T3. B Comparison of changes of p-PI3K/PI3K in lung tissue of each group at T3. C Comparison of changes of p-Akt/Akt in lung tissue of each group at T3. Compared with group S, $aP < 0.05$. Compared with group IR, $bP < 0.05$. Compared with group D, $cP < 0.05$. Compared with group A, $dP < 0.05$. Figure 7A.

Figure 8

Expression of NF-κB (p65) in lung tissues of rats. A Western blot of NF-κB (p65) in lung tissues of each group of rats at T3. B Comparison of changes of NF-κB (p65) in lung tissue of each group at T3. Compared with group S, aP < 0.05. Compared with group IR, bP < 0.05. Compared with group D, cP < 0.05. Compared with group A, dP < 0.05.

Figure 9

Expression of AnxA1 in lung tissues of rats. A Western blot of AnxA1 in lung tissues of each group of rats at T3. B Comparison of changes of AnxA1 in lung tissues of each group at T3. Compared with group S, aP < 0.05. Compared with group IR, bP < 0.05. Compared with group, cP < 0.05. Compared with group A, dP < 0.05.

Figure 10

caption is not available in this version

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