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Research Article

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Dexmedetomidine Reduce Multiple Organ Injury Induced by Lung Ischemia/Reperfusion of Mice Through Inhibiting Endoplasmic Reticulum Stress Response

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Abstract

To investigate the effect of Dexmedetomidine reduce brain, myocardium and kidney injury induced by lung ischemia/reperfusion of mice through inhibiting endoplasmic reticulum stress response. SPF C57BL/6J male mice were randomly divided into 5 groups (n = 10): the sham operation group (group sham), the ischemia reperfusion Group (Group I/R), the Atipamezole Group (Group Atip), dexmedetomidine Group (Group-DEX) and dexmedetomidine+AtipamezoleGroup (Group DA). Lung ischemia reperfusion models were established with the method of 30min in-vivo left hilus occlusion followed by 180min reperfusion. In addition to the steps same as Group I/R, the mice in Groups Atip, Dex and DA were given Atip(250μg/kg), Dex(20μg/kg), and Dex+ Atip(20μg/kg+250μg/kg) respectively by intraperitoneal injection 30min before hilar occlusion. After reperfusion, the blood was taken from the eye-orbit to determine the activity of serum CK-MB and LDH and the levels of serum creatinine and urea nitrogen. The brain tissue was taken to determine the brain water content. The tissue of myocardium, brain and kidney was taken to observe the cellular morphology change under the light microscope. The expression of Caspase3 activity in heart, brain and kidney was determined with Thermos Scientific Microplate Reader. The TUNEL method was used to detect the apoptotic index of heart, brain and kidney. The Western blot and RT-PCR were used to determine the expression of proteins and mRNA of JNK, Caspase12, CHOP and GRP78. Compared with Group Sham, obvious injury in the tissue of myocardium, brain and kidney was observed in the other four groups under the light microscope. The activity of CK-MB and LDH, and the levels of serum creatinine, urea nitrogen and brain water content were significantly increased as well. The apoptotic index, Caspase3 activity, and the expression of the proteins and mRNA of JNK, Caspase12, CHOP and GRP78 were also significantly elevated. Compared with Group I/R, Atip and DA, the mitigated injury in the myocardium, brain and kidney was observed in Group DEX under the light microscope. The activity of CK-MB and LDH, and the levels of serum creatinine, urea nitrogen and brain water content were decreased and the apoptotic index, Caspase3 activity, and the expression of the proteins and mRNAs of JNK, Caspase12 and CHOP were also significantly decreased in Group DEX, but the proteins and mRNA expression of GRP78 were significantly elevated. For the comparison among theGroup I/R, Atip and DA, there is no significant difference was found.(grp78).The pre-administration of DEX can mitigate the heart, brain and kidney injury induced by lung ischemia reperfusion. The mechanism may be related to the activation of α2-adrenergic receptor and inhibition of endoplasmic reticulum over-response.

Keywords: Multiple Organ Injury; Dexmedetomidine; Lung Ischemia/Reperfusion

Introduction

Lung ischemia reperfusion is a common pathological process of pulmonary thrombolytic therapy, lung transplantation and pneumonectomy, etc. lung ischemia reperfusion injury (LIRI) often causes acute lung injury accompany with distant organ dysfunction and pathological injury, such as in the heart, brain and kidney. For the lung ischemia reperfusion accompany with heart, brain or kidney injury, the mortality can be significantly increased. Ischemia and hypoxia caused by lung ischemia reperfusion may lead to endoplasmic reticulum stress (ERS). It indicates the conditions, caused by ischemia, hypoxia or oxidative stress, of destroyed endoplasmic reticulum homeostasis and a large number of unfolded or misfolded proteins were produced and disordered Ca^{2+} balance [1]. Moderate ERS may restore the intracellular homeostasis and maintain the cell survival, but the over-response to ERS will aggravate the tissue injury. Studies have shown that in some organ such as brain and heart, ERS plays an important role in ischemia reperfusion injury and may induce apoptosis [2, 3].

DEX is a highly selective α_2 adrenergic receptor agonist. Our early studies have shown that administration of DEX prior to lung ischemia may reduce the levels of proinflammatory mediators and inflammatory factors, and thereby mitigate the lung

ischemia reperfusion injury, playing the role of lung protection [4, 5]. At the same time, some studies have shown that DEX also has a protective effect against the ischemia reperfusion injury in the heart, brain, liver and kidney. However, the effect of DEX on lung ischemia reperfusion-induced distant organ injury in heart, brain and kidney injury remains to be discussed. Therefore, this study is to evaluate the protective effect of DEX on myocardium, brain and kidney in lung ischemia reperfusion mice and its relationship with ERS, and to provide new therapeutic ideas and methods for the prevention and treatment of distant organ injury caused by lung ischemia reperfusion.

Materials And Methods

Animal

Fifty healthy SPF C57BL/6J male mice, weight 20g ~ 24g, 8-10 weeks of age, were provided by the Experimental Animal Center of Wenzhou Medical University. C57BL/6J mice were divided into 5 groups(n=10) by random number table method: the sham operation group (group sham), the lung ischemia/reperfusion group (group I/R), the atipamezole group(groupAtip), the dexmedetomidine group (group DEX), dexmedetomidine and atipamezole group (group DA).

Establishment of Lung Ischemia/Reperfusion Model

According to the methods indicated in the literatures, the lung ischemia reperfusion models were established on the C57BL/6J mice through 30min in-vivo left hilus occlusion followed by 180min reperfusion. After the combined intraperitoneal injection of 100mg/kg ketamine + 10mg/kg for anesthesia, apply disinfection to the chest and neck skin. After incision and separation of the subcutaneous tissue and muscle, the trachea was exposed. The T-incision was made for tracheal intubation and ventilator mechanical ventilation (ventilator parameters: respiration ratio of 2:3, respiratory rate of 120 beats/min, oxygen concentration of 100%, and tidal volume of 0.6 ~ 0.8 ml/min). The left chest was opened at the 3rd-5th intercostal spaces and the left hilar was mobilized and blocked with the arterial clip for 30min followed by 180min reperfusion. At the end of reperfusion, the heart was exposed and heart perfusion was conducted before sampling the tissue of heart, brain and kidney. For the mice in Group Sham, the chest was opened with no hilus occsion but 210min of mechanical ventilation. The mice in Group I/R were given the 30min hilus occlusion and 180min reperfusion. In addition to the steps same as Group I/R, the mice in Group Atip, Dex and DA were given Atip(250μg/kg), Dex(20μg/kg), and Dex+Atip(20μg/kg+250μg/kg) respectively by intraperitoneal injection 30min before hilar occlusion.

Morphological Observation of Myocardial, Brain and Kidney Tissue under Light Microscope

The tissue samples of myocardial, brain and kidney were taken with the size of 0.5cm×0.5cm×0.5cm, and fixed with 4% formaldehyde. The routine paraffin-embedded sections and HE staining were conducted for these samples, and the histological changes of the tissues in each group were observed under the light microscope.

ELISA for the Serum CK-MB and LDH

At the end of 180min reperfusion, the blood samples were taken from the eye-orbit and stored in the -80°C refrigerator. Detecting the serum CK-MB and LDH activity by the 7600-020 automatic biochemical analyzer according to the instructions of the CK-MB and LDHkit.

Concentration Detection of The Serum Creatinine and Urea Nitrogen

The blood samples taken at the end of the experiment were centrifuged and the serum was collected to detect concentration of the serum creatinine and urea nitrogen by the COBAS automatic biochemical analyzer.

Brain Water Content Detection

After the experiment, the brain tissue was collected by craniotomy and the wet weight was weighted. After drying in oven for 48h, the dry weight was weighted. Brain water content calculation formula: brain water content (%) = (wet weight - dryweight) /wet weight × 100%.

Apoptotic Index Detected with the Tunel Method

After blood sampling, the mice were sacrificed and the heart was perfused with normal saline. With the heart fixed with tweezers, the aorta, pulmonary artery and the vena cava were cut and then the heart was separated. At the same time, the brain tissue and the kidneys were taken and rinsed with sterile PBS. After fixing with 4% paraformaldehyde, the tissue was dehydrated, waxed, sliced, baked for fixation. The slices underwent the process of dehydration with dimethyl benzene, absolute alcohol, 95%, 90%, 85%, 80% and 75% ethanol, PBS rinsing, protease K solution for removing the tissue protein and distilled water rinsing. After the operation in according to the instructions of the TUNEL kit, the apoptosis was observed under the light microscope. The apoptotic cells were those with brown nucleus. Ten visions at higher magnification (400×) were randomly selected from each slice and the total number of cells and the number of apoptotic cells were counted to calculate the apoptotic index. the formula of the apoptotic index (AI) = number of apoptotic cells/ total number of cells×100%.

Caspase3 Activity Detection

The 100μl lysate was added for each 3-10 mg tissue which was then homogenized on ice and transfer to a 1.5 ml centrifuge tube for cytolysis of 5 minutes. The tube was centrifuged for 10-15 minutes(4°C,20000r) and the supernatant was transferred for the following operation in accordance with the instructions of the Caspase3 activity test kit. The Caspase3 activity in the tissue of myocardial, brain and kidney were detected with Thermos Scientific Microplate Reader.

Protein Detection with Western Blot

The tissue of myocardial, brain and kidney were taken and homogenized under low temperature, and the 400 μl RIPA (containing 4 μl of PMSF) was used for tissue lysis. After mixing, the mixture was centrifuged at 4°C and the supernatant was collected for protein level detection with the BCA protein quantitative kit. The protein samples were diluted to 2 μg/μl and boiled for 10min for denaturation. For gel electrophoresis, the loading amount was of 20μl. The gel was wet transferred to the PVDF membrane and 5% skim milk was used for sealing for 2h. The membrane was rinsed with TBST for 7min×3 times, and the primary antibody was added and incubated under 4°C overnight.

The membrane was then rinsed with TBST again for 7min×3 times, and the secondary antibody was added and incubated under room temperature for 1h. The membrane was rinsed for 5 min × 3 times and reacted with the chemiluminescent for 3 min before exposure by the UV-800 automatic gel imaging analysis system. The protein absorbance value was analyzed with the Quantity One software. The absorbance values of the target protein band and the internal control band were recorded. The ratio of p-JNK to JNK was calculated to reflect the expression level of p-JNK protein.

mRNA Detection with RT-PCR

The tissue of myocardial, brain and kidney were taken and homogenized in liquid nitrogen. The total RNA was extracted with the Trizol method and the RNA concentration was measured. The cDNA was synthesized and amplified according to the instructions of the RT-PCR kit. PCR parameters: pre-denaturation: 3 min; denaturation: 30 s; annealing: 30 s; extension: 1 min; extension termination: 5 min; Cycle: 30 times. GAPDH gene was used as the internal control for RT-PCR. Primer sequence is as shown in Table 1. Results were analyzed with Quantity One. The expression level was reflected by the ratio of the gray scale values of the target gene band and the internal control GAPDH bands.

Statistical Processing

The data were analyzed with SPSS19.0. The measurement datas was detected by normality test and expressed as mean±standard deviation ($\bar{x}$±s). The one-way analysis of variance was used for intergroup comparison. The homogeneity of variance test was applied to the mean comparison of multiple groups of samples. For those with homogeneity of variance, the SNK-q test was applied for pairwise comparison. The $P < 0.05$ was considered as statistically significant.

Results

Morphological Changes of Heart, Brain and Kidney Observed Under Light Microscope

The myocardial cells from Group Sham with neat arrangement, normal morphology and even cytoplasm staining were observed under the light microscope. In contrast, the myocardial cells from Group I/R, Atip and DA had enlarged intercellular space, dissolved, loose and lightly stained partial cytoplasm in addition to the obvious cellular edema and vacuolar degeneration. The myocardial cells from Group DEX had generally neat arrangement, slightly larger volume and mild edema change.

The neurons from Group Sham with round shape, visible nuclei and nucleoli, and generally normal morphological structure were observed under the light microscope. In contrast, the neurons from Group I/R, Atip and DA had slightly contracted cell bodies, slightly dark stained nuclei and less clear nucleoli in addition to severe cellular edema and significantly increased degenerated and necrotic cells. The neurons from Group DEX had slightly swollen cytoplasm and light color nuclei in addition to mild cellular edema and less necrotic cells.

For Group Sham, the glomerular and renal tubular structures were clearly observed under light microscope with no obvious abnormality being found. For Group I/R, Atip and DA, the glomerular contours were basically clear but the renal tubules had loose and disordered arrangement. The obviously swollen epithelial cells, partial vacuolar degeneration, partial lumen expansion in the renal tubules and interstitial edema were also observed. For Group DEX, the renal injury was reduced and the glomerular and renal tubular contours were clear with mitigated epithelial cell edema.

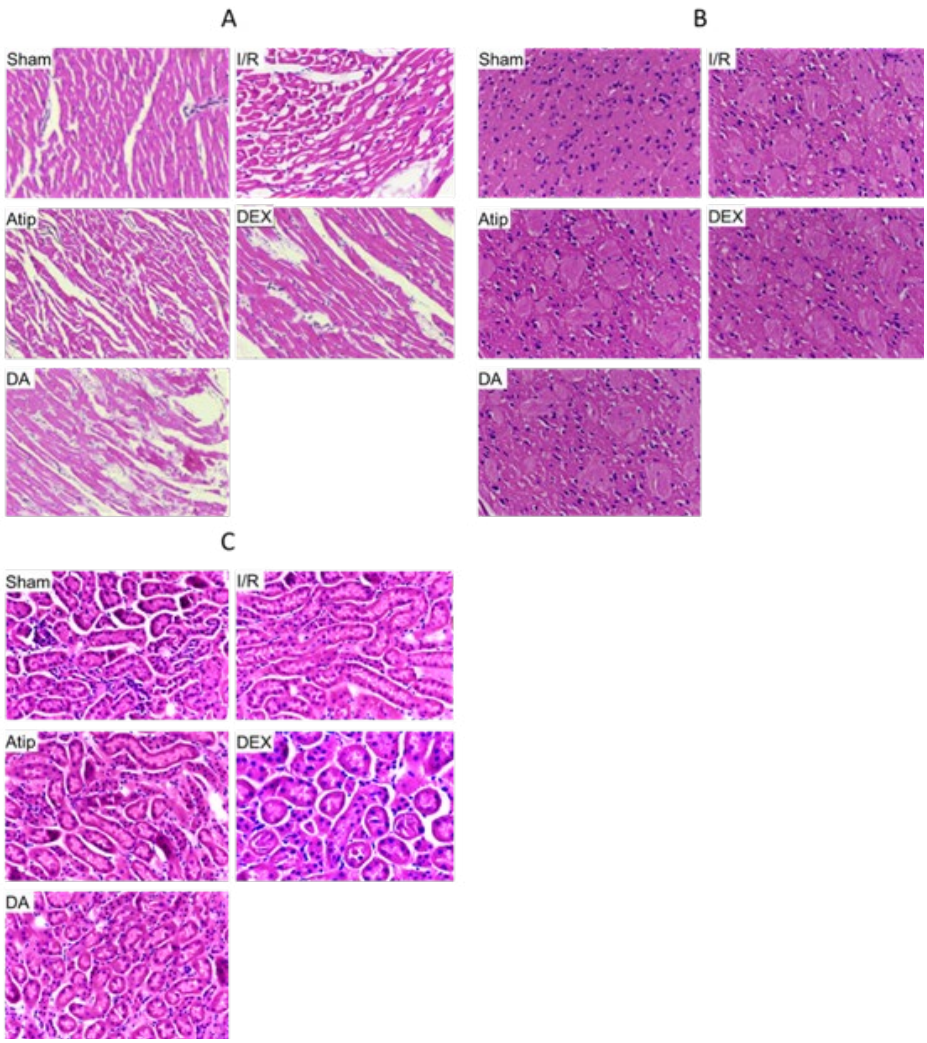

Figure 1: The histological change of myocardial, brain and kidney tissue in each group (HE staining, ×200).

A: myocardial B: cerebral C: renal
Sham: Sham group; I/R: Ischemia reperfusion injury group; Atip: Atipamezole group; DEX: Dexmedetomidine group; DA: Dexmedetomidine + Atipamezole group.

Changes of Serum Creatinine and Urea Nitrogen Concentration in Kidney Tissue

Compared with Group Sham, increment of the content levels of serum creatinine and urea nitrogen were observed in all the other four groups($P<0.05$). Compared with Group I/R, there was no significant change in Group Atip and Group DA($P>0.05$), but the levels of serum creatinine and urea nitrogen content in Group DEX significantly decreased($P<0.05$). Compared with Group Atip, the levels of serum creatinine and urea nitrogen in Group DEX decreased significantly($P<0.05$), but Group DA had no significant difference($P>0.05$). Compared with Group DEX, Group DA increased($P<0.05$).

Table 1:The change of serum creatinine and blood urea nitrogen (Mean±SD, n=10)

Group	Scr(μmol/L)	BUN (mmol/L)
Sham	22.4±3.4	7.6±2.0
I/R	39.8±2.1*	19.0±2.2*
Atip	39.3±1.9*	19.2±2.6*
DEX	28.8±2.3*#△	13.4±1.8*#△
DA	39.1±2.5*▲	19.2±2.4*▲
*P<0.01 vs Sham group; #P<0.01 vs I/R group; △P<0.01 vs Atip group; ▲P <0.01 vs DEX group		

Changes of Serum CK-MB and LDH Activity in Myocardial Tissue

Compared with Group Sham, the serum CK-MB and LDH activity in the other groups were increased(P<0.05); Compared with Group I/R, there was no significant change in Group Atip and Group DA(P>0.05), but the serum CK-MB and LDH activity in Group DEX decreased(P<0.05). Compared with Group Atip, the serum CK-MB and LDH activity in Group DEX decreased significantly(P<0.05), but Group DA had no significant difference(P>0.05). Compared with Group DEX, Group DA increased(P<0.05).

Table 2: Changes of CK-MB and LDH activity in myocardial tissue (Mean±SD, n=10)

Group	CK-MB(U/L)	LDH(U/L)
Sham	527±58	1 542±109
I/R	1 509±116*	6 273±205*
Atip	1 471±50*	6 078±247*
Dex	893±134*#△	4 019±229*#△
DA	1 385±160*▲	6 015±239*▲
*P<0.01 *vs* Sham group; #P<0.01 vs I/R group; △P<0.01 *vs* Atip group; ▲P<0.01 *vs* DEX group		

Changes of Brain Water Content in Brain Tissue

Compared with Group Sham, the brain water content in I/R, Atip and DA group were increased(P<0.05); Compared with Group I/R, there was no significant change in Group Atip and Group DA(P>0.05), but the brain water content in Group DEX decreased(P<0.05). Compared with Group Atip, the brain water content in Group DEX decreased significantly(P<0.05), but Group DA had no significant difference(P>0.05). Compared with Group DEX, Group DA increased(P<0.05).

Table 3: Changes of brain water content in brain tissue (Mean±SD, n=10)

Group	brain water content detection (%)
Sham	73.81±2.08
I/R	82.68±2.46*
Atip	82.30±2.53*
DEX	77.49±1.84#△
DA	82.27±2.47*
*P<0.01 *vs* Sham group; #P<0.01 *vs* I/R group; △P<0.01 *vs* Atip group; ▲P<0.01 *vs* DEX group	

Changes of Apoptosis Index in Brain, Myocardial and Kidney Tissue

Compared with Group Sham, the apoptosis index in the other 4 groups were increased(P<0.05); Compared with Group I/R, there was no significant change in Group Atip and Group DA(P>0.05), but the apoptosis index in Group DEX decreased(P<0.05). Compared with Group Atip, the apoptosis index in Group DEX decreased significantly, but Group DA had no significant difference(P>0.05). Compared with Group DEX, Group DA increased(P<0.05).

Table 4: The apoptosis rate of myocardial, brain and kidney tissue in each group (Mean±SD, n=10)

GROUP	myocardial	brain	kidney
Sham	1.45±0.38	5.45±1.68	8.32±2.81
I/R	8.83±0.57*	32.75±3.54*	60.04±6.84*
Atip	8.75±0.49*	32.50±3.92*	59.47±6.01*
DEX	5.38±0.46*#△	21.08±3.80*#△	39.55±9.76*#△
DA	8.48±0.59*▲	32.79±3.62*▲	60.50±8.24*▲
*P<0.01 *vs* Sham group; #P<0.01 *vs* I/R group; △P<0.01 *vs* Atip group; ▲P<0.01 *vs* DEX group			

Sham: Sham group; I/R: Ischemia reperfusion injury group; Atip: Atipamezole group; DEX: Dexmedetomidine group; DA: Dexmedetomidine + Atipamezole group.

Changes of Caspase3 Activity in Brain, Myocardial and Kidney Tissue

Compared with Group Sham, the caspase3activities in the other 4 groups were increased(P<0.05); Compared with Group I/R, there was no significant change in Group Atip and Group DA(P>0.05), but the caspase3activity in Group DEX decreased(P<0.05). Compared with Group Atip, the caspase3 activity in Group DEX decreased significantly(P<0.05), but Group DA had no significant difference(P>0.05). Compared with Group DEX, Group DA increased(P<0.05).

Table 4: The Caspase3 enzymatic activity of myocardial, brain and kidney tissue in each group (Mean±SD, n=10)

GROUP	myocardial	brain	kidney
Sham	60.41±14.44	38.25±6.63	39.49±5.41
I/R	142.18±27.36*	134.36±5.40*	105.61±7.61*
Atip	146.64±0.49*	135.18±4.62*	106.66±8.25*
DEX	104.24±17.96$^{\#\triangle}$	99.32±8.80$^{*\#\triangle}$	81.75±6.94$^{*\#\triangle}$
DA	151.30±33.70$^{*\blacktriangle}$	134.85±6.57$^{*\blacktriangle}$	109.87±7.97$^{*\blacktriangle}$
*P<0.01 *vs* Sham group; #P<0.01 *vs* I/R group; $^{\triangle}P$<0.01 *vs* Atip group; $^{\blacktriangle}P$<0.01 *vs* DEX group			

Expression of Proteins in Brain, Myocardial and Kidney Tissue

Compared with Group Sham, the protein contents of p-JNK, Caspase12, CHOP and GRP78 in all other groups were significantly higher(P<0.05). Compared with Group I/R, Group Atip and DA had no significant difference. In Group DEX, the protein contents of p-JNK, Caspase12 and CHOP decreased but that of GRP78 increased. Compared with Group Atip, the protein contents of p-JNK, Caspase12 and CHOP in Group DEX decreased but that of GRP78 increased, and Group DA had no significant-difference. Compared with Group DEX, the protein contents of p-JNK, Caspase12 and CHOP in Group DA increased but that of GRP78 had no significant difference.

Table 4: The Caspase3 enzymatic activity of myocardial, brain and kidney tissue in each group (Mean±SD, n=10)

GROUP	p-JNK	caspase-12	CHOP	GRP78
Sham	0.158±0.033	0.271±0.014	0.213±0.026	0.287±0.012
I/R	0.854±0.021$^{*\blacktriangle}$	0.750±0.039$^{*\blacktriangle}$	0.781±0.020$^{*\blacktriangle}$	0.618±0.017$^{*\blacktriangle}$
Atip	0.874±0.066$^{*\blacktriangle}$	0.748±0.039$^{*\blacktriangle}$	0.756±0.063$^{*\blacktriangle}$	0.613±0.060$^{*\blacktriangle}$
DEX	0.488±0.032*	0.563±0.015*	0.472±0.032*	0.820±0.031*
DA	0.874±0.054$^{*\blacktriangle}$	0.784±0.009$^{*\blacktriangle}$	0.824±0.051$^{*\blacktriangle}$	0.727±0.038$^{*\blacktriangle}$
*P<0.01 *vs* Sham group; $^{\blacktriangle}P$<0.01 *vs* DEX group.				

Table 5: The p-JNK, Caspase12, CHOP, GRP78 protein expression level change of brain tissue in each group (Mean±SD, n=10)

GROUP	p-JNK	Caspase12	CHOP	GRP78
Sham	0.16±0.03	0.22±0.03	0.21±0.02	0.22±0.03
I/R	0.73±0.03$^{*\blacktriangle}$	0.67±0.02$^{*\blacktriangle}$	0.72±0.03$^{*\blacktriangle}$	0.59±0.03$^{*\blacktriangle}$
Atip	0.73±0.03$^{*\blacktriangle}$	0.68±0.03$^{*\blacktriangle}$	0.70±0.03$^{*\blacktriangle}$	0.58±0.04$^{*\blacktriangle}$
DEX	0.42±0.03*	0.42±0.03*	0.41±0.03*	0.80±0.03*
DA	0.73±0.03$^{*\blacktriangle}$	0.66±0.03$^{*\blacktriangle}$	0.69±0.03$^{*\blacktriangle}$	0.78±0.05$^{*\blacktriangle}$
*P<0.01 *vs* Sham group; $^{\blacktriangle}P$<0.01 *vs* DEX group.				

Table 6: The p-JNK, Caspase12, CHOP, GRP78 protein expression level change of renal tissue in each group (Mean±SD, n=10)

GROUP	p-JNK	Caspase12	CHOP	GRP78
Sham	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
I/R	0.7±0.0*	0.6±0.0*	0.7±0.0*	0.5±0.0*
Atip	0.7±0.0*	0.6±0.0*	0.7±0.0*	0.5±0.0*
DEX	0.4±0.0*#	0.4±0.0*#	0.4±0.0*#	0.7±0.0*#
DA	0.7±0.0$^{*\blacktriangle}$	0.6±0.0$^{*\blacktriangle}$	0.7±0.0$^{*\blacktriangle}$	0.8±0.0*#
*P<0.01 *vs* Sham group; #P<0.01 *vs* I/R group; $^{\blacktriangle}P$<0.01 *vs* DEX group				

Expression of mRNA in Brain, Myocardial and Kidney Tissue Compared with Group Sham, the mRNA contents of JNK, Caspase12, CHOP and GRP78 in all other groups were significantly higher($P<0.05$). Compared with Group I/R, Group Atip and DA had no significant difference. In Group DEX, the mRNA contents of JNK, Caspase12 and CHOP decreased but that of GRP78 increased. Compared with Group Atip, the mRNA contents of JNK, Caspase12 and CHOP in Group DEX decreased but that of GRP78 increased, and Group DA had no significant difference. Compared with Group DEX, the mRNA contents of JNK, Caspase12 and CHOP in Group DA increased but that of GRP78 had no significant difference.

Table 7: The JNK, Caspase12, CHOP, GRP78 mRNA expression level change of heart tissue in each group (Mean±SD, n=10)

GROUP	JNK mRNA	caspase-12 mRNA	CHOP mRNA	GRP78 mRNA
Sham	0.147±0.021	0.144±0.026	0.138±0.042	0.076±0.022
I/R	0.479±0.053*	0.572±0.018*	0.581±0.020*	0.273±0.019*
Atip	0.477±0.035*	0.566±0.034*	0.595±0.064*	0.293±0.054*
DEX	0.312±0.032*#□	0.396±0.034*#□	0.379±0.014*#□	0.476±0.034*#△
DA	0.445±0.019*▲	0.593±0.053*▲	0.589±0.048*▲	0.467±0.020*

Table 8: The JNK ,Caspase12 ,CHOP ,GRP78 mRNA expression level change of brain tissue in each group (Mean±SD, n=10)

GROUP	JNK	Caspase12	CHOP	GRP78
Sham	0.13±0.02	0.12±0.02	0.14±0.04	0.07±0.03
I/R	0.42±0.03*	0.51±0.03*	0.55±0.03*	0.26±0.03*
Atip	0.43±0.03*	0.50±0.03*	0.53±0.03*	0.27±0.03*
DEX	0.29±0.03*#△	0.33±0.02*#△	0.34±0.03*#△	0.45±0.03*#△
DA	0.42±0.02*▲	0.53±0.01*▲	0.53±0.03*▲	0.44±0.03*▲

Table 9: The JNK, Caspase12, CHOP, GRP78 mRNA expression level change of renal tissue in each group (Mean±SD, n=10)

GROUP	JNK	Caspase12	CHOP	GRP78
Sham	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
I/R	0.4±0.0*	0.5±0.0*	0.5±0.0*	0.3±0.0*
Atip	0.4±0.0*	0.5±0.0*	0.5±0.0*	0.3±0.0*
DEX	0.3±0.0*#△	0.3±0.0*#△	0.3±0.0*#△	0.5±0.0*#△
DA	0.4±0.0*▲	0.5±0.0*▲	0.5±0.0*▲	0.5±0.0*#△
*$P<0.01$ *vs* Sham group; #$P<0.01$ *vs* I/R group; △P<0.01 *vs* Atip group; △P<0.01 *vs* DEX group				

Discussion

DEX is a highly selective α2 adrenergic receptor agonist. It plays a role in anti-inflammatory and organ protection through activating the cholinergic anti-inflammatory passageway [6]. Once acting on the α2 adrenergic receptors in the central nervous system, it stimulates the hyperpolarization of cells, inhibits the discharge of neurons, and then blocks the conduction of the pain signal to the brain, resulting in sedation, analgesia, antianxiety and inhibition of the sympathetic activity and indirectly improving the effect of vagal tone [7, 8]. Other effects of DEX include anti-chill, stop saliva and diuresis. It is commonly used in patients with vascular diseases for perioperative anesthesia and sedative analgesia, providing good effect of perioperative organ protection, and effectively mitigating the organ injury induced by intraoperative ischemia. Studies have shown that DEX has a positive effect on the Isoflurane-induced neuronal apoptosis and cognitive impairment in mice [9]. In 48 hours of cerebral ischemia/reperfusion injury, the use of DEX can effectively prevent the delayed neuronal death in the hippocampus of mice [10].

For organ ischemia/reperfusion, the intracellular signal transduction involves multiple pathways, in which the ERS and JNK signal transduction pathways play an important role in organ ischemia/reperfusion [11]. ATP depletion, ischemia and hypoxia, oxidative stress, and glucose/nutrient deficiency, etc. all may cause ERS, when a large number of unfolded or misfolded proteins were accumulated that beyond the processing capacity of endoplasmic reticulum, it will activated unfolded protein response, Endoplasmic reticulum overload response□Caspase -12 mediated apoptosis signaling pathways, etc. for responding to the changes of conditions and the restoration of favorable environmental of protein folding of endoplasmic reticulum. ERS can induce the expression of endoplasmic reticulum molecular partner, such as glucose regulated protein 78(GRP78) and GRP94, to produce protective effect as well as induce independently cell apoptosis. The moderate ERS will protect the body, but sustained strong ERS will lead to apoptosis.

In this study, the tested proteins, JNK, Caspas12, CHOP and GRP78, were all ERS-related. GRP78 was a calcium-binding molecule chaperone in the endoplasmic reticulum. In case of ERS due to external stimuli, a large number of misfolded or unfolded proteins will be accumulated, and then the GRP78 will be also expressed in large amount to bond with the misfolded and unfolded proteins in endoplasmic reticulum to maintain the stability of the internal environment and protect the organization. Therefore, the rapidly increased of GRP78 is considered as the most sensitive marker of ERS [12, 13]. JNK signal transduction pathway, which is related to the stress-induced apoptosis, is one of the important pathways in MAPK signaling pathway. it can be activated by a variety of extracellular stress, resulting in apoptosis. Studies have shown that JNK is over-activated during hypoxia and hypercapnia induced pulmonary hypertension injury, and that the inhibition of JNK activation can significantly reduce apoptosis and mitigate the pulmonary hypertension injury. Cysteine aspartate specific proteinase-12(Caspase-12) widely presents in various tissue of rat and is one of the major apoptotic signaling molecules of ERS. The disorder of calcium balance in endoplasmic reticulum or excessive accumulation of endoplasmic reticulum protein may lead to the expression of Caspase-12 and result in the occurrence of apoptosis. CHOP, also known as growth arrest and DNA damage-inducible 153(GADD153), is a specific ERS transduction factor and considered as a marker of ERS, an important signaling molecule that promotes apoptosis. Its expression level is very low under normal circumstances, but in case of ERS, its expression will be greatly increased. Apoptosis is activated through the mitochondrial pathway, during which the mitochondrial pro-apoptotic protein and apoptosis-inducing factors will be released to activate the caspase cascade reaction and induce apoptosis, manifested as increased Caspase3 apoptotic protease.

The results indicated that, compared with Group Sham, the activity of CK-MB and LDH, the levels of serum creatinine, urea nitrogen and brain water content, the Caspase3 activity, the apoptosis index and the expression levels of JNK, Caspase12, CHOP and GRP78 in the other four groups had an increasing trend. Under the light microscope, the tissue of myocardium, brain and kidney had different degree of injury, which indicated that the lung ischemia/reperfusion did induce the over-response to ERS in myocardium, brain and kidney, and then ERS increased the expression of ERS-related proteins that induced the apoptosis and led to tissue injury through the mitochondrial pathway. Compared with Group I/R, there is no obvious difference in the above indicators in Group Atip, indicating that aldimazole had no protective effect in myocardium, brain and kidney against the injury induced by lung ischemia/reperfusion. Compared with Group I/R and Atip, the activity of CK-MB and LDH, the levels of serum creatinine, urea nitrogen and brain water content, the Caspase3 activity, the apoptosis rate and the expression levels of JNK, Caspase12 and CHOP in Group DEX all decreased except for the increased expression of GRP78.The mitigated cellular edema and injurious changes in the cells of myocardium, brain and kidney were observed under the light microscope. All these indicated that DEX may reduce the expression of JNK, Caspase12 and CHOP by inhibiting the response to ERS,and thus lower the number of apoptotic cells and mitigate the lung ischemia/reperfusion-induced injury in myocardium, brain and kidney, playing a protective role in organs. After the comparison between Group DA and DEX, we found that the protective effect of DEX on the tissue can be blocked by aldimazole, the selective α2-adrenergic receptor blocker, which was manifested as the increased activity of CK-MB, LDH andthe levels of serum creatinine, urea nitrogen and brain water content, the Caspase3 activity, the apoptosis index and the expression levels of JNK, Caspase12, CHOPin Group DA and the aggravated injury in the tissue of myocardium, brain and kidney as observed under the light microscope. It suggested that the protective effect of DEX on lung ischemia/reperfusion-induced myocardium, brain and kidney injury may be related to the activation of the *α2*-adrenoceptor.

In summary, the administration of DEX prior to lung ischemia/reperfusion can mitigate the lung ischemia/reperfusion-induced injury in myocardium, brain and kidney, and its mechanism may be related to the activation of the α2-adrenoceptor and the inhibition of the over-response to ERS [14-26].

Author Contributions

Wang wan-tie designed the experiments and wrote the paper.

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