Exploration of protective effect of Sevoflurane preconditioning on hypoxia reoxygenation injury of myocardial cells in rats and related molecular mechanisms

Ye-Qiu Li*, Zheng-Lan Zhan, Qin-Fang Li

Department of Anesthesiology, People’s Hospital of Dongxihu District, Wuhan City, Hubei Province, 430040

ARTICLE INFO

Article history:
Received 25 Jan 2016
Received in revised form 15 Feb 2016
Accepted 29 Jan 2016
Available online 28 Feb 2016

Keywords:
Sevoflurane
Hypoxia reoxygenation
Mitochondria
Apoptosis
Oxidative stress

ABSTRACT

Objective: To explore the protective effect of Sevoflurane preconditioning on hypoxia reoxygenation injury of myocardial cells in rats and related molecular mechanisms. Methods: Cardiomyocytes of SD rats 1 to 2 days after birth were cultured and divided into NC group (no special treatment), HR group (hypoxia reoxygenation processing) and S group (hypoxia reoxygenation after sevoflurane preconditioning). Myocardial cell injury indexes, mitochondrial pathway apoptosis indexes and oxidative stress indicators were detected. Result: (1) Myocardial injury indexes: myocardial cell apoptosis rate as well as CK, CK-MB, AST and LDH levels in cell culture medium of HR group were significantly higher than those of NC group, and myocardial cell apoptosis rate as well as CK, CK-MB, AST and LDH levels in cell culture medium of S group were significantly lower than those of HR group; (2) mitochondrial pathway apoptosis indexes: Bcl-2, Cx43 and Omi/HtrA2 contents in mitochondria of HR group were lower than those of NC group, and the content of Bax was higher than that of NC group; the contents of cytochrome C and Omi/HtrA2 in cytoplasm were higher than those of NC group, and the content of Cx43 was lower than that of NC group; the contents of Bcl-2, Cx43 and Omi/HtrA2 in mitochondria of S group were higher than those of HR group, and the content of Bax was lower than that of HR group; the contents of cytochrome C and Omi/HtrA2 in cytoplasm were lower than those of HR group, and the content of Cx43 was higher than that of HR group; (3) oxidative stress indexes: the contents of ROS and MDA in cells of HR group were higher than those of NC group, and the contents of SOD, GSH-Px, VitC and VitE were lower than those of NC group; the contents of ROS and MDA in cells of S group were lower than those of HR group, and the contents of SOD, GSH-Px, VitC and VitE were higher than those of HR group. Conclusion: Sevoflurane preconditioning has protective effect on hypoxia reoxygenation injury of myocardial cells in rats, and its molecular mechanisms include inhibiting mitochondrial pathway apoptosis and relieving oxidative stress.

1. Introduction

Myocardial ischemia-reperfusion (I/R) injury is a common clinical pathological physiological phenomena in Cardiology Department and Cardio-Thoracic Surgery Department, and both reperfusion after thrombolytic or interventional treatment and reperfusion after cardiopulmonary bypass heart surgery in patients with myocardial infarction can cause I/R injury[1,2]. I/R injury will affect the normal myocardial systolic and diastolic function and the prognosis of the disease, and reducing or preventing I/R injury through effective interventions is an important clinical issue. Sevoflurane is a clinical common inhalation anesthetic that has protective effect on myocardial I/R injury, but the specific molecular mechanism is not yet clear[3,4]. In the following research, myocardial cell hypoxia reoxygenation was used as the model to stimulate myocardial I/R injury, and after sevoflurane preconditioning, the molecular mechanism of myocardial protective effect of sevoflurane was discussed.
2. Experimental materials and methods

2.1. Experimental materials

The sevoflurane was from Baxter Company in US, Annexin V/PI double labeling kits were from Shenzhen JingMei Biotechnology Company, the DCFH-DA probe was from Sigma Company, microplate reader was from Bio-rad Company in US, and fluorescence spectrophotometer was from Hitachi Company in Japan.

2.2. Cell culture method

SD rats 1-2 d after birth were taken and put to death, myocardial tissue was collected, washed for three times with D-Hanks solution, then cut up, digested for 5-8 times with 0.06% pancreatic enzyme and 0.04% type II collagenase and centrifuged, cell suspension was obtained and inoculated in culture bottle, and after differential adhesion for 90min, cells that were not adhered were collected and continuously cultured for 24h and used in subsequent experiments and treatment.

2.3. Cell treatment method

Normal control group (NC group) didn’t receive special treatment; hypoxia reoxygenation (HR group) received 2 h of hypoxia and 1h of reoxygenation; sevoflurane (S group) received sevoflurane preconditioning for 20 min at first and then received the same hypoxia reoxygenation as the HR group. The method of hypoxia reoxygenation was as follows: cells were placed in incubator with 95%N2-5%CO2 for 2 h of hypoxia and then put back in culture medium with 95% air-5% CO2 for 1h of reoxygenation; the methods of sevoflurane processing was as follows: cells were put in the sterile airtight container, the breathing circuit of anesthesia machine was connected, the vaporizer was opened and the concentration of sevoflurane was adjusted to 2.5% , and after 20 min, cells were eluted for 10 min in medium with 95% air-5% CO2.

2.4. Flow cytometry measurement of the apoptosis rate and the level of ROX

Myocardial cells after treatment were collected and digested with pancreatic enzyme, and cell suspension was obtained and divided into two. One was stained by Annexin V/PI double labeling kits, and then the proportion of Annexin V and PI double positive apoptotic cells was detected by flow cytometer; the other was stained by DCFH-DA probe, and then the mean fluorescence intensity (MFI) at 488 nm excitation wavelength and 525 nm emission wavelength was detected by flow cytometer.

2.5. Measurement method of molecule contents in the cell culture medium

Myocardial cell culture medium after processing was collected, the contents of CK, CK-MB, AST and LDH were detected by automatic biochemical analyzer, the contents of SOD and GSH-Px were detected by radioimmunoprecipitation, and the contents of MDA, VitC and VitE were detected by Elisa kit.

2.6. Determination method of protein content in cells

Myocardial cells after treatment were collected, the mitochondrial protein samples and cytoplasm protein samples in cells were separated by mitochondrial protein and cell cytoplasm protein extraction kit and then diluted in accordance with the appropriate proportion, and Elisa kit was used to determine the contents of Bcl-2, Bax, Cx43, Omi/HtrA2, cytochrome C.

2.7. Statistical methods

SPSS 15.0 software was used to analyze the data above, analysis among three groups was by variance analysis, pair-wise comparison between two groups was by LSD-t test, and differences were considered to be statistically significant at a level of P<0.05.

3. Results

3.1. Hypoxia reoxygenation injury index of myocardial cells

There was difference in hypoxia reoxygenation injury indexes among three groups of myocardial cells, and the specific analysis was as follows: myocardial cell apoptosis rate as well as CK, CK-MB, AST and LDH levels in cell culture medium of HR group were significantly higher than those of NC group; myocardial cell apoptosis rate as well as CK, CK-MB, AST and LDH levels in cell culture medium of S group were significantly lower than those of HR group.

3.2. Mitochondrial apoptosis pathway indexes of myocardial cells

There was difference in mitochondrial apoptosis pathway indexes among three groups of myocardial cells, and the specific analysis was as follows: Bcl-2, Cx43 and Omi/HtrA2 contents in mitochondria of HR group were lower than those of NC group, and the content of Bax was higher than that of NC group; the contents of cytochrome C and Omi/HtrA2 in cytoplasm were higher than those of NC group, and the content of Cx43 was lower than that of NC group.

3.3. The oxidative stress indicators of myocardial cells

There was difference in the oxidative stress indexes among three groups of myocardial cells, and the specific analysis was as follows: the contents of ROS and MDA in cells of HR group were higher than those of NC group, and the contents of VitC and VitE were lower than those of NC group; the contents of ROS and MDA in cells of S group were lower than those of HR group, and the contents of ROS and MDA in cells of HR group were higher than those of NC group.
Note: compared with the HR group, apoptosis myocardial cells from ischemia reperfusion injury and reduce and sevoflurane preconditioning or post-conditioning can protect protective role in ischemia-reperfusion injury of myocardial cells, recent years, some studies have shown that sevoflurane plays a widely used in combined intravenous-inhalation anesthesia. In Sevoflurane is a clinical common inhalation anesthetic, and it reoxygenation conditions could cause myocardial cell injury. Hypoxia reoxygenation processing of myocardial cells cultured in vitro can cause injury to myocardial cells, and it’s also the common model to simulate the myocardial ischemia reperfusion in the in vivo environment. After ischemia reperfusion injury in myocardial cells, it is expressed as increase of apoptosis cells and large release of creatine kinase (CK) and creatine kinase isoenzyme (CK-MB) as well as aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in cytoplasm, and detection of the cell apoptosis rate as well as CK, CK-MB, AST and LDH levels in cell culture medium can reflect the degree of myocardial cell injury. After hypoxia reoxygenation processing, it was found in the research that myocardial cell apoptosis rate as well as CK, CK-MB, AST and LDH in cell culture medium can reflect the degree of myocardial cell injury. After hypoxia reoxygenation processing, it was found in the research that myocardial cell apoptosis rate as well as CK, CK-MB, AST and LDH levels in cell culture medium of HR group were significantly higher than those of NC group. This meant that sevoflurane could protect myocardial cells from damage caused by hypoxia reoxygenation and inhibit the cell apoptosis.

Mitochondrial pathway is the important link of myocardial cell apoptosis caused by hypoxia reoxygenation. Under the physiological conditions, the cytochrome C is main located in the respiratory chain of mitochondria; when hypoxia reoxygenation conditions cause myocardial respiratory chain dysfunction, the expression levels of Bax and Bcl-2 on mitochondrial membrane will change, and the Bcl-2/Bax ratio is reduced and causes cell apoptosis caused by hypoxia reoxygenation.

4. Discussion

Hypoxia reoxygenation processing of myocardial cells cultured in vitro can cause injury to myocardial cells, and it’s also the common model to simulate the myocardial ischemia reperfusion in the in vivo environment. After ischemia reperfusion injury in myocardial cells, it is expressed as increase of apoptosis cells and large release of creatine kinase (CK) and creatine kinase isoenzyme (CK-MB) as well as aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in cytoplasm, and detection of the cell apoptosis rate as well as CK, CK-MB, AST and LDH levels in cell culture medium can reflect the degree of myocardial cell injury. After hypoxia reoxygenation processing, it was found in the research that myocardial cell apoptosis rate as well as CK, CK-MB, AST and LDH in cell culture medium can reflect the degree of myocardial cell injury. After hypoxia reoxygenation processing, it was found in the research that myocardial cell apoptosis rate as well as CK, CK-MB, AST and LDH levels in cell culture medium of HR group were significantly higher than those of NC group. This meant that hypoxia reoxygenation conditions could cause myocardial cell injury.

Sevoflurane is a clinical common inhalation anesthetic, and it is widely used in combined intravenous-inhalation anesthesia. In recent years, some studies have shown that sevoflurane plays a protective role in ischemia-reperfusion injury of myocardial cells, and sevoflurane preconditioning or post-conditioning can protect myocardial cells from ischemia reperfusion injury and reduce apoptosis. After model of myocardial cell hypoxia reoxygenation injury was established in this study, preconditioning way was used for sevoflurane intervention, and the intervention condition was 2.5% sevoflurane for 20 min. Myocardial cells went through hypoxia reoxygenation conditions after sevoflurane preconditioning, then the cell damage was detected, and the results showed that myocardial cell apoptosis rate as well as CK, CK-MB, AST and LDH levels in cell culture medium of S group were significantly lower than those of HR group. This meant that sevoflurane could protect myocardial cells from damage caused by hypoxia reoxygenation and inhibit the cell apoptosis.

Mitochondrial pathway is the important link of myocardial cell apoptosis caused by hypoxia reoxygenation. Under the physiological conditions, the cytochrome C is main located in the respiratory chain of mitochondria; when hypoxia reoxygenation conditions cause myocardial respiratory chain dysfunction, the expression levels of Bax and Bcl-2 on mitochondrial membrane will change, and the Bcl-2/Bax ratio is reduced and causes cytochrome C release from mitochondria into the cell cytoplasm, then activating apoptosis mediated by downstream caspase. The process of apoptosis caused by mitochondrial pathway is accompanied by the change of contents a variety of molecules in the mitochondria. Omi/HtrA2 is the protein found in the mitochondrial intermembrane space, and in cases of mitochondrial injury, it is translocated from the mitochondria into cell cytoplasm, and then initiates cell apoptosis through combination with XIAP.
43 (Cx43) is the protein existing in both myocardial cell membrane and mitochondrial membrane, and it has protective effect on myocardial cells. Analysis of mitochondrial apoptosis pathway indexes in myocardial cells with hypoxia reoxygenation in the research showed that the contents of Bcl-2, Cx43 and Omi/HtrA2 in mitochondria of S group were higher than those of HR group, and the content of Bax was lower than that of HR group; the contents of cytochrome C and Omi/HtrA2 in cytoplasm were lower than those of HR group, and the content of Cx43 was higher than that of HR group. This meant that sevoflurane could reduce mitochondrial pathway apoptosis of ischemic-hypoxic myocardial cells.

Research in recent years has confirmed that in the hypoxia reoxygenation injury process of cardiac muscle cells, the intracellular oxidative stress and endoplasmic reticulum stress are significantly activated. Oxidative stress is mainly mediated by reactive oxygen species (ROS), and it can cause excessive consumption of antioxidants such as SOD, GSH-Px, VitC and VitE in cells, thus causing oxidation of lipid composition in biological membrane and production of malondialdehyde (MDA). Analysis of oxidative stress indicators in cells in the research showed that the contents of ROS and MDA in cells of HR group were higher than those of NC group, and the contents of SOD, GSH-Px, VitC and VitE were lower than those of NC group; the contents of ROS and MDA in cells of S group were lower than those of HR group, and the contents of SOD, GSH-Px, VitC and VitE were higher than those of HR group. This meant that hypoxia reoxygenation could cause oxidative stress injury in myocardial cells, which is manifested as the production of large numbers of ROS and consumption of a large number of antioxidants; sevoflurane preconditioning could relieve oxidative stress damage and reduce the production of ROS and consumption of antioxidants. To sum up, it can be concluded as follows: sevoflurane preconditioning has protective effect on hypoxia reoxygenation injury of myocardial cells in rats, and its molecular mechanisms include inhibiting mitochondrial pathway apoptosis and relieving oxidative stress.

Reference


