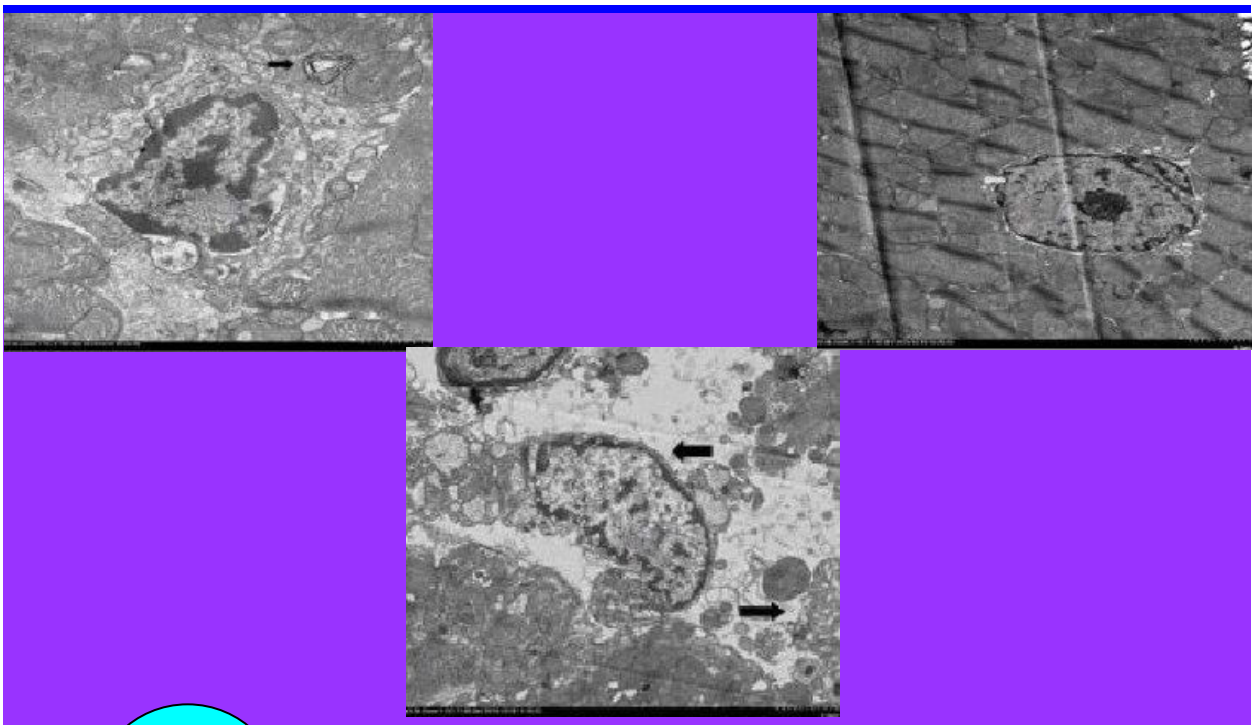

BJP

Bangladesh Journal of Pharmacology

Research Article

Effect of sevoflurane postconditioning on myocardial oncosis and autophagy in ischemia–reperfusion injury in isolated rat heart

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
Bangladesh J Pharmacol 2018; 13: 255-263
ISSN: 1991-0088

Effect of sevoflurane postconditioning on myocardial oncosis and autophagy in ischemia-reperfusion injury in isolated rat heart

Qi Feng Tang[1],Yang Xie[2], Hong Xie[3], Wen Qiang Jiang[2] and Zhi Yuan Fang[2]

[1]Department of Anesthesiology, Suzhou BenQ Medical Center, Nanjing Medical University, Suzhou 215008, Jiangsu, China; [2]Department of Anesthesiology, Suzhou Hospital Affiliated to Nanjing Medical University, Suzhou Municipal Hospital, Suzhou 215006, Jiangsu, China; [3]Department of Anesthesiology and Critical Care, The Second Affiliate Hospital of Soochow University, Suzhou 215008, Jiangsu, China.

Article Info

Received: 19 April 2018
Accepted: 22 August 2018
Available Online: 28 August 2018

DOI: 10.3329/bjp.v13i3.36437

Cite this article:
Tang QF, Xie Y, Xie H, Jiang WQ, Fang ZY. The effect of sevoflurane postconditioning on myocardial oncosis and autophagy in ischemia-reperfusion injury in isolated rat heart. Bangladesh J Pharmacol. 2018; 13: 255-63.

Abstract

The effects of sevoflurane postconditioning on myocardial oncosis and autophagy were studied in 32 isolated rat heart of ischemia-reperfusion injury. The hearts were perfused by: a) Sham surgery group, b) simple sevoflurane group, c) simple ischemia-reperfusion group (I/R) group, and d) sevoflurane postconditioning group. The ratios between the gray density values of the target bands to the gray density value of GADPH were used to reflect the Beclin-1, LC3II/I, and porimin expression levels. The LC3II/I level in the sevoflurane postconditioning group was lower than the level in the I/R group. The porimin level in the sevoflurane postconditioning group was lower than the level in the I/R group. The myocardial infarction range in the sevoflurane postconditioning group (33 ± 5%) was significantly diminished compared with the range in the I/R group (53 ± 6%) ($p<0.05$). Sevoflurane decreased the occurrence of oncosis and alleviated myocardial ischemia-reperfusion injury by inhibiting MPTP opening.

Introduction

In acute myocardial infarction animal model, myocardial reperfusion injury accounts for more than half of the final myocardial infarction injury. Therefore, prevention of myocardial ischemia-reperfusion injury is one of the major measures for the prevention of cardiac and vascular adverse events in patient with cardiac surgery and vascular surgery. Currently, many studies focus on the prevention of ischemic-reperfusion injury, including preconditioning and postconditioning (Rachmat et al., 2014; Camarco et al., 2014). The postconditioning measures include ischemia postconditioning, volatile anesthetic postconditioning, sufentanil postconditioning, and propofol postconditioning (Csonka et al., 2014; Huang et al., 2011). The concept of ischemic-postconditioning to provide many rounds of transient interruption of perfusion/reperfusion treatment after myocardial ischemia but prior to reperfusion (Zhao et al., 2003). This technique has cardiac protection functions similar to ischemic preconditioning, including a reduction of myocardial infarct sizes, protection of endothelial functions of the coronary artery, and reduction of the ischemic myocardial inflammatory reaction.

Volatile anesthetic postconditioning can simulate ischemic postconditioning, administration at the early stage of ischemia-reperfusion can also reduce myocardial ischemia-reperfusion injury and has important clinical significance. However, the mechanism of myocardial protection in volatile anesthetic postconditioning is not completely clear. This study primarily investigated the effect of sevoflurane postconditioning on myocardial oncosis and autophagy in ischemia-reperfusion injury

in isolated rat heart.

Materials and Methods

Experimental animals

A total of 32 clean-grade adult male Sprague Dawley rats with body weights 270-250 g were provided by the Experimental Animal Center of the Medical College of Soochow University, China.

Preparation of a perfusion model for isolated hearts

Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate at 3 mL/kg. After injection of 1,000 U/kg of heparin sodium through the tail vein, the chest was opened for heart collection. The heart was immediately placed in a pre-cooled (4°C) K-H buffer containing (mmol/L) NaCl (118.5), $NaHCO_3$ (25.0), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $CaCl_2$ (1.4), and glucose (11.0) (pH 7.4). The aortae were catheterized to perform Langendorff retrograde perfusion using 95% O_2-5% CO_2-saturated K-H buffer. The heart was immobilized using silk thread (5-0). The perfusion tubing was installed with a 3-way switch to control the K-H buffer. A small opening was made at the left atrial appendage and a pressure transducer with an YP200 cuff was inserted into the left ventricle. The U/4C501H Med Lab biological signal acquisition and processing system was connected to perform real-time monitoring of the left heart function parameters, including the heart rate (HR), left ventricular systolic pressure, and left ventricular end-diastolic pressure. Hearts with HRs >200 beats/min and left ventricular systolic pressure >80 mmHg (1 mmHg = 0.133 kPa) after balanced perfusion for 15-20 min were used in the experiments.

Administration of sevoflurane

Mixed gas (95% O_2-5% CO_2) was passed through a specified sevoflurane vaporizer (lot number: 2217; Maruishi Pharmaceutical, Japan) and then continuously passed into the K-H buffer. During this period, the sevoflurane concentration in the K-H buffer was monitored using an anesthetic gas monitor (Datex, Finland) to ensure the concentration of sevoflurane in the K-H buffer was maintained at 2.5% (Liou et al., 2010). In the pre-experiment, our group pre-filled K-H buffer with sevoflurane and the K-H buffer was measured every 3 min. The results indicated that continuously pre-filling the K-H buffer with sevoflurane for 15 min resulted in a concentration of 0.4 ± 0.1 mmol/L. Thus, the sevoflurane concentration in the K-H buffer could be maintained at 2.5%.

Experimental groupings

A total of 32 hearts in the successfully prepared *in vitro* model were divided into 4 groups as follows (n=8) (Figure 1): Sham surgery group (SHAM group), simple sevoflurane group (S group), simple ischemia-reperfusion group (I/R) group), and sevoflurane post-conditioning group (SEVOP group). (1) SHAM group: Continuous K-H buffer perfusion for 180 min; (2) S group: Continuous K-H buffer perfusion for 60 min followed by perfusion of K-H buffer containing 2.5% sevoflurane (lot number: 2217; Maruishi Pharmaceutical, Japan) at 60 min for 15 min and then perfusion of K-H buffer for 105 min; (3) I/R group: Balance for 30 min, ischemia for 30 min, and reperfusion for 120 min; and (4) SEVOP group: Perfusion of K-H containing 2.5% sevoflurane for 15 min during the early stage of reperfusion.

Preparation of protein samples and Western blotting procedures

The gels were scanned and the net optical density

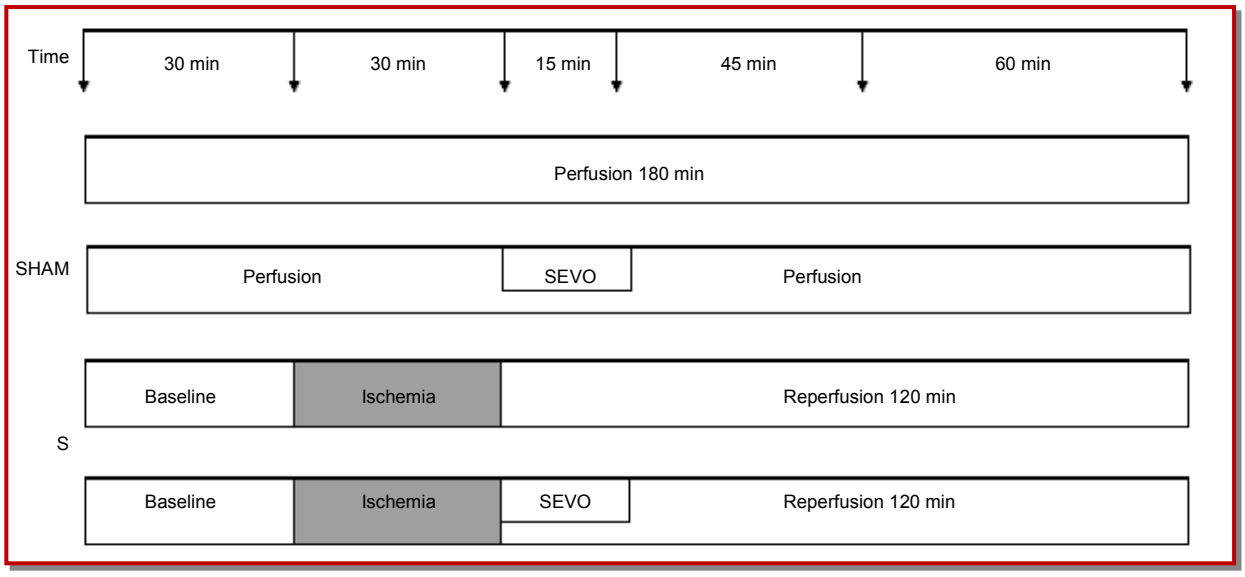

Figure 1: Experimental design of perfusion in different groups

values of the bands were measured using the Image J software. The ratios between the gray density values of the target bands to the gray density value of GADPH were used to reflect the Beclin-1, LC3II/I, and Porimin expression levels.

Electron microscopy procedures

Collection of materials and pre-fixation: Two sample blocks approximately 0.5-1.0 mm^3 in size were rapidly excised from each rat. The tissue samples were rapidly immersed in 5% glutaraldehyde solution within 1 min. After washing, post-fixation, washing again, dehydration, penetration, embedding, ultra-thin sectioning, and positive staining, the samples were observed under a transmission electron microscope.

Electron microscopy observation: The numbers of autophagic vacuoles in the 100 μm^2 cross-sections of the cytoplasm and the number of oncotic cells were counted and statistically analyzed using the double-blind method. Ten fields of each specimen were observed for 5 specimens per group. Each group contained 5 rats, and 10 fields in each specimen were continuously selected for the statistical analysis.

Determination of the myocardial infarction ranges

At the end of the 2 hours reperfusion, the rat heart in the successfully isolated Langendorff perfusion model was randomly selected and rapidly removed. After freezing at -80°C for 5 min, the heart was transversely cut into 6 tissue blocks with a 2 mm thickness using a heart cutter. The samples were placed in 1 2, 3, 5% triphenyltetrazolium chloride triazole (Sigma, USA) for staining. The samples were continuously vortexed during the staining process to allow even staining. The samples were incubated in the dark at a constant temperature for 20 min, washed with PBS buffer, and fixed in 10% formalin for 24 hours. Comparison method 1: The infarct region was stained a white-gray color and the non-infarct region was stained a brick red color. The Alpha View gel image analytical software (Alpha EaseFC, USA) was used to analyze the percentage of the infarcted myocardia to the total myocardial range. Comparison method 2: The left ventricle (LV) was divided into the normal region, non-ischemic infarct region, and ischemic infarct region under a dissection microscope and weighed. The percentage of myocardial weight of the ischemic region (myocardial weight of the ischemic non-infarct region + myocardial weight of the infarct region) in the LV myo-cardial weight, the percentage of myocardial weight of the ischemic infarct region in the LV myocardial weight, and the percentage of myocardial weight of the ischemic infarct region to the myocardial weight of the ischemic region were calculated. The myocardial infarction range was presented as the percentage of myocardial weight in the infarct region to the myocardial weight of the ischemic region. The Alpha View gel image analytical software was used for the analysis and comparison.

Statistical analysis

The analyses were performed using the GraphPad Prism 5.00 software. Measurement data were presented as the mean ± standard deviation (SD). Comparisons among groups were performed using one-way analysis of variance. Comparisons within a group were performed using repeated measures analysis of variance. $p<0.05$ indicated that the difference had statistical significance.

Results

Production of autophagic vacuoles and oncotic cells

The numbers of autophagic vacuoles and oncotic cells were 1.2 ± 0.7 and 3.2 ± 1.5 in the Sham group and 1.0 ± 0.6 and 3.0 ± 1.3 in the S group, respectively (Figure 2). The difference between these groups was not significant, indicating that administration of sevoflurane alone did not influence the number of autophagic vacuoles and oncotic cells. In the ischemia-reperfusion group, the numbers of autophagic vacuoles and oncotic cells were 10.4 ± 2.4 and 15.5 ± 4.2 in the I/R group and 6.8 ± 1.8 and 11.7 ± 3.5 in the SEVOP group, respectively. These results indicated that the number of autophagic vacuoles and oncotic cells significantly increased after ischemia-reperfusion and that sevoflurane postconditioning significantly reduced the numbers of autophagic vacuoles and oncotic cells.

Expression and quantitative analysis of the autophagy-related molecules LC3I, LC3II, and Beclin-1 and the oncosis-related porimin protein

The Beclin-1 level in the I/R group was higher than the level in the Sham group, indicating that myocardial ischemia-reperfusion injury could induce the up-regulation of Beclin-1 expression ($p<0.05$) or cause autophagic flux impairment to reduce Beclin-1 clearance. The Beclin-1 level in the SEVOP group was lower than the level in the I/R group, indicating that sevoflurane postconditioning in the ischemia-reperfusion group down-regulated Beclin-1 protein expression ($p<0.05$). Alternatively, sevoflurane postconditioning may have promoted autophagy functions to increase Beclin-1 clearance, thereby reducing the Beclin-1 protein expression level (Figure 3A and C).

The LC3II/I level in the I/R group was higher than the level in the Sham group, indicating that myocardial ischemia-reperfusion injury increased the up-regulation of LC3II/I expression or caused autophagic flux impairment to reduce LC3II/I clearance ($p<0.05$). The LC3II/I level in the SEVOP group was lower than the level in the I/R group, indicating that sevoflurane postconditioning after ischemia-reperfusion down-regulated LC3II/I expression. Alternatively, sevoflurane postconditioning may have promoted autophagy functions to increase LC3II/I clearance, thereby redu-

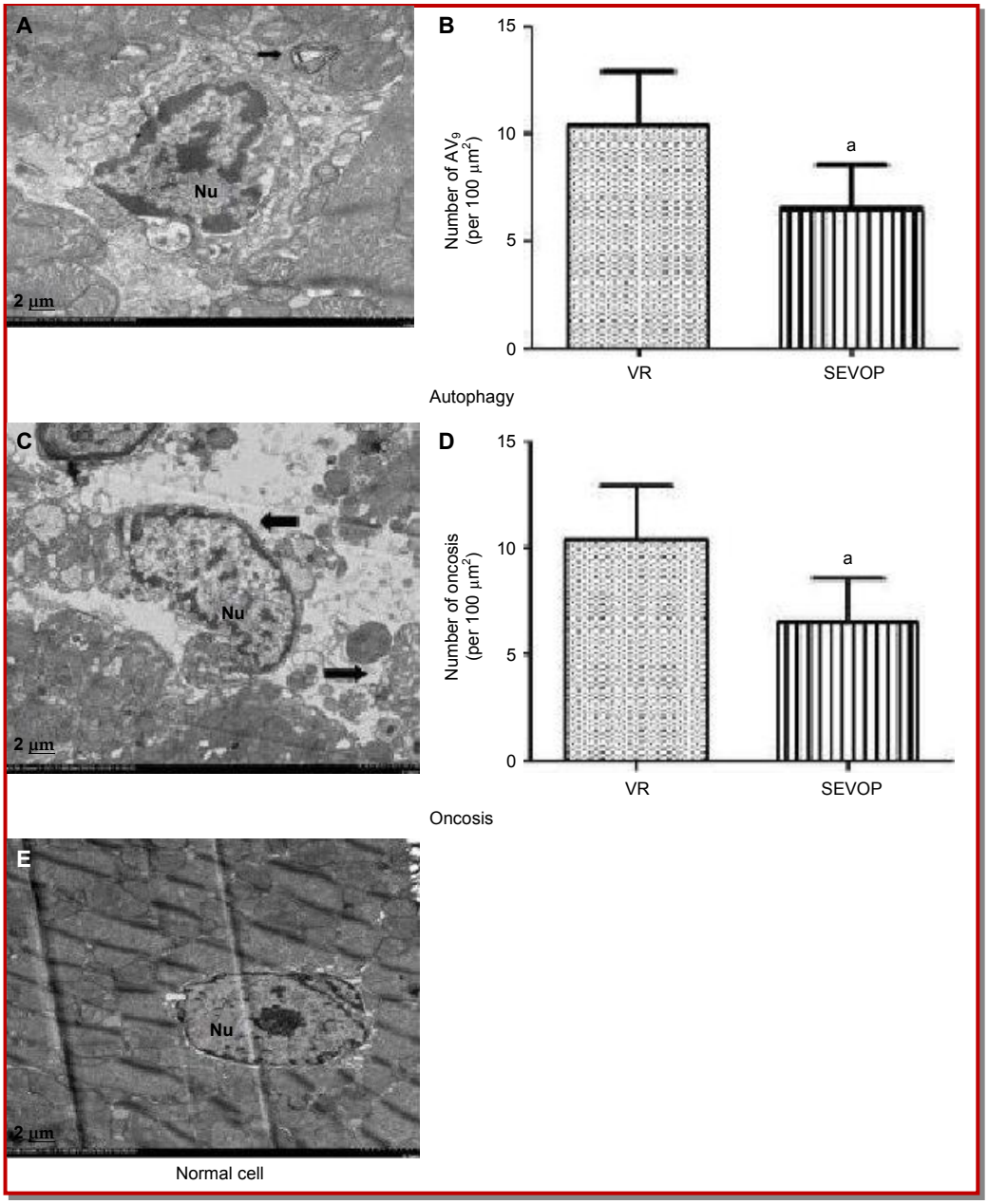

Figure 2: Autophagic vacuoles, oncotic cells, and normal cells in rat heart in the I/R group under a transmission electron microscope

(A) Autophagic vacuoles are a vesicular structure with a double-layer or single-layer membrane, cytoplasm, and organelle components (×2000 magnification, bar=2 am, shown by the arrow). (B) Comparison of the numbers of autophagic vacuoles between the two groups. (C) Oncosis results in cell swelling, increased volume, cytoplasmic vacuoles, endoplasmic reticulum swelling, significant reduction of cristae, and formation of electronic transparent areas. (D) Comparison of the numbers of oncotic cells. (E) Cells in the sevoflurane preconditioning group. Autophagic vacuoles and oncotic cells in all groups were quantitatively analyzed. After one-way analysis of variance, the Student-Newman-Keels method was used to compare the mean values among multiple samples (n = 5).

The results were presented as the mean ± standard deviation. $^{a}p<0.05$ indicated statistical significance.

Sham: Sham surgery group; IR group: Ischemia-reperfusion group; SEVOP group: Sevoflurane postconditioning in the myocardial ischemia-reperfusion group. AVs, autophagic vacuoles; Oncosis: Oncotic cells

cing the LC3II/I protein expression level ($p<0.05$) (Figure 3B and D).

The porimin expression level in the I/R group was higher than the level in the Sham group, indicating that myocardial ischemia-reperfusion injury promoted the occurrence of oncosis and induced the up-regulation of Porimin expression ($p<0.05$). The porimin level in the SEVOP group was lower than the level in the I/R group, indicating that sevoflurane postconditioning in the ischemia-reperfusion group inhibited the occurrence of oncosis and down-regulated porimin protein expression ($p<0.05$) (Figure 3E and F).

Sevoflurane postconditioning diminished the myocardial infarction range

No myocardial infarction occurred in the Sham and S groups. The myocardial infarction range was expressed in weight as the percentage of the myocardial weight of the infarct region to the myocardial weight of the ischemic region. The myocardial infarction range in the SEVOP group (33 ± 5%) was significantly diminished compared with the range in the I/R group (53 ± 6%) ($p<0.05$) (Table I). The results showed that the difference was significant ($p<0.05$) (Figure 4A and B).

Discussion

Cell autophagy is one type of survival mechanism under starvation conditions. Under nutritional deficiency, autophagy activity increases to degrade non-key

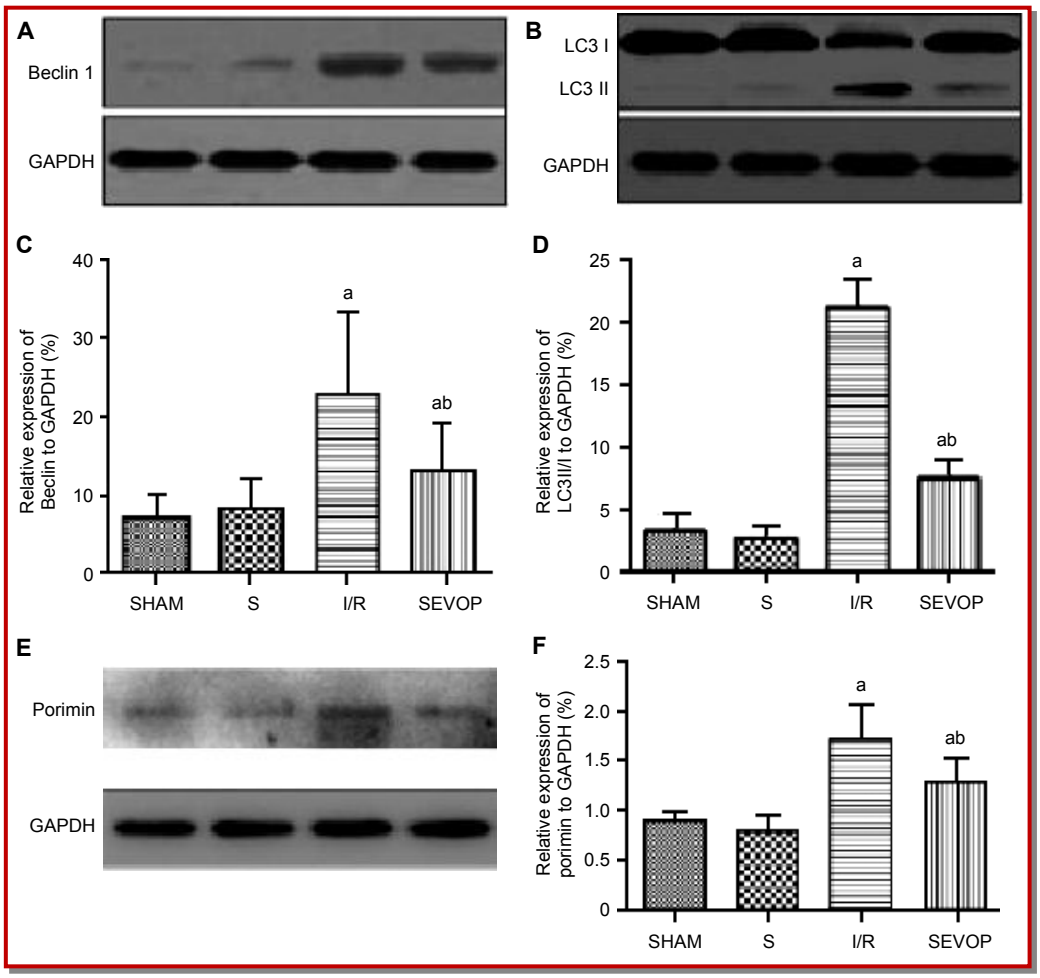

Figure 3: Western blotting results and quantitative analysis of Beclin-1, LC3II/I, and Porimin protein expression in rat hearts in the Sham, S, I/R, and SEVOP groups

(A) Western blotting results of Beclin-1 protein expression in rat hearts of the 4 groups. (B) Western blotting results of LC3II/I protein expression in rat hearts of the 4 groups. (C) Quantitative analysis of the Western blotting results of Beclin-1 protein expression in rat hearts of the 4 groups.
(D) Quantitative analysis of the Western blotting results of LC3II/I protein expression in rat hearts of the 4 groups. After one-way analysis of variance, the Student-Newman-Keels method was used to compare the mean values among multiple samples.
The results were presented as the mean ± standard deviation.
Compared to the Sham group, $^{a}p<0.05$; compared to the I/R group, $^{b}p<0.05$. $p<0.05$ indicated statistical significance.
Sham: Sham surgery group; S: Sevoflurane group; I/R: Ischemia-reperfusion group; SEVOP: Sevoflurane postconditioning in the myocardial ischemia-reperfusion group

Table I

Comparison of myocardial infarction areas in rats between the I/R and SEVOP groups ($n = 6$, $x \pm s$)

Group	Left ventricular weight (mg)	Ischemic region weight (mg)	Infarct region weight (mg)	Ischemic region/ left ventricle weight (%)	Infarct region/ left ventricle weight (%)	Infarct region/ ischemic region weight (%)
I/R	592 ± 16	215 ± 18	112 ± 16	37 ± 3	19 ± 5	53 ± 7
SEVOP	560 ± 66	190 ± 28	65 ± 12[a]	35 ± 2	12 ± 4[a]	34 ± 5[a]

Compared to the I/R group, [a]p<0.05

components in cells. During this process, nutrients are released to provide materials for biosynthesis to ensure

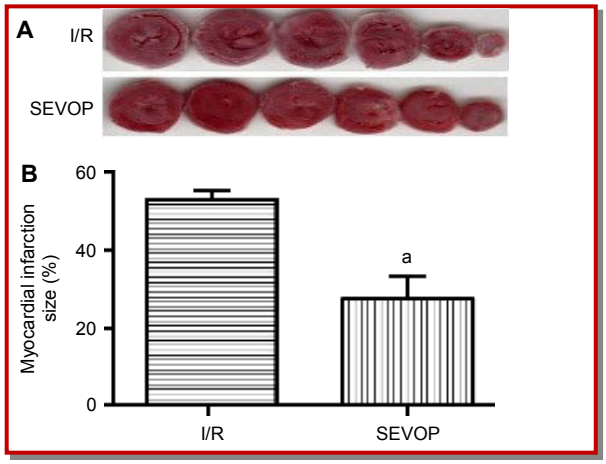

Figure 4: Comparison of myocardial infarction ranges after 2 hours of reperfusion between the two groups

Myocardial I/R caused myocardial infarction. Sevoflurane postconditioning reduced the infarction range. (A) Comparison between the I/R and SEVOP groups. After TTC staining, the results were analyzed using the Alpha View gel image analytical software. (B) The difference compared with the I/R group was significant ([a]p<0.05). I/R: Myocardial ischemia-reperfusion group; SEVOP: Sevoflurane postconditioning in the myocardial ischemia-reperfusion group

the continuation of key life processes. The typical process has 4 steps: Induction, autophagosome formation (including a double-membrane structure of substances in injured cells), autophagic lysosomal fusion, and degradation and recycling of degraded substances (Przyklenk et al., 2012). The whole process is also called the autophagic flux. LC3-II is the most commonly used molecular marker for the detection of autophagy levels in cells. LC3 [the microtubule-associated protein 1 light chain 3 (LC3)] is a ubiquitin-like molecule and the mammalian homolog of the yeast autophagy-related protein (Atg)-encoded product. After translation, the unmodified LC3 precursor (proLC3) is cleaved by the proteolytic enzyme At94 to form LC3-I, which exposes the C-terminal glycine. During autophagy induction, LC3-I is conjugated to the highly lipophilic phosphatidylethanolamine (PE) group through the binding of the exposed glycine to At97 (E1 activity), At93 (E2-like binding activity) and the Atgl2-At95-Atgl6L (E3-like conjugating activity) polymer to form LC3-II. The PE group enhances the integration of LC3-II into the membrane lipids of the autophagic pore and autophagic vacuoles. In autophagosomes, LC3-II not only functions in the selective degradation of substrates (the interaction between LC3-II and p63/SQSTM1 targets related protein aggregates in self-renewal) but also has membrane aggregation and fusion promotion functions in *in vitro* studies. Additionally, LC3-II supports fusion between autophagosomes and other membrane components, such as endosomes or even mitochondria. Currently, LC3-II is the only characteristic protein that is specifically localized to the autophagy apparatus from the autophagic lysosomal degradation process (Mehrpour et al., 2010).

The increase in LC3-II levels can be due to an increase in the production of autophagic vacuoles or the impairment of autophagosome clearance. The impairment of autophagic vacuole clearance may occur due to the impairment of translocation into lysosomes, the impairment of fusion between the autophagic vacuoles and lysosomes, and the impairment of proteolytic enzyme activity in the lysosomes. In basic experiments, LC3II/I was typically used as a method to evaluate autophagy activity at the tissue level. It is used to detect the number of autophagosomes and the LC3II:LC3I (LC3II/I) ratio under conditions with or without the application of the lysosome inhibitors bafilomycin A1 and chloroquine. Each analysis requires two matched samples for comparison and confirmation of the occurrence of an autophagic flux. For example, two groups of test samples are treated with or without chloroquine. These conditions allow the determination of whether the increase in autophagosomes is due to the increased production of autophagosomes or the impairment of the clearance function of autophagosomes due to the impairment of autophagic-lysosomal fusion, thereby causing the increase in the number of autophagosomes (Ma et al., 2012). This method provides an estimation of the property and the degree of the autophagic flux.

In this study, the IC3II/I level in the I/R group was higher than the level in the Sham group, indicating that myocardial ischemia-reperfusion injury could induce the up-regulation of LC3II/I expression. Alternatively, the ischemia-reperfusion injury may have caused autophagic flux impairment, thereby reducing LC3II/I clearance ($p<0.05$). The LC3II/I level in the SEVOP group was lower than the level in the I/R group,

indicating that sevoflurane postconditioning could down-regulate LC3II/I expression after ischemia-reperfusion or that sevoflurane postconditioning could promote autophagy functions to increase LC3II/I clearance, thereby reducing the LC3II/I protein expression level ($p<0.05$). In our study, the gray density value of LC3 in the I/R group was high and the gray density value of LC3II/I in the SEVOP group was decreased, which was consistent with the aforementioned experimental results.

Other autophagy-related proteins also cannot be used alone as the molecular markers for the measurement of autophagy. For example, Beclin-1 expression and the activity of the cysteine protease At94 are not only associated with the increase of autophagy. However, we measured Beclin-1 expression in this study due to the important function of Beclin-1-mediated autophagy in the reperfusion stage. Our results confirmed that the Beclin-1 expression level was significantly increased after reperfusion and that sevoflurane postconditioning reduced the Beclin-1 expression level.

Autophagy in cells is a dynamic, multi-step, and complex process. Detection of autophagy markers using western blotting or the observation of autophagic vacuole formation by electron microscopy can only observe static autophagy levels. However, because the number of autophagic vacuoles at a given time point may be due to an increase in the production of autophagic vacuoles or the impairment of autophagic vacuole clearance, autophagy studies cannot be limited to static observations. Autophagic flux reflects the whole process from autophagic vacuole production to autophagic lysosomal degradation. Because autophagic flux represents the whole process of autophagy, autophagic flux changes can reflect the autophagy activity (Zhang et al., 2013).

Currently, the methods available for autophagic flux studies are very limited. However, autophagic flux in cells can be detected using a combination of a variety of methods. Electron microscopy is the most direct and classic method to observe the number and morphology of autophagy vacuoles, autophagic lysosomes, and lysosomes in cells. The limitation of electron microscopy is that it can only observe static autophagy (Klionsky et al., 2012). This study combined the application of the autophagy inhibitor chloroquine and the observation of autophagic vacuoles using microscopy in the presence or absence of chloroquine to observe changes in the number of autophagic vacuoles. Therefore, we could confirm the increase of autophagic vacuole clearance by sevoflurane postconditioning through the promotion of autophagic flux instead of simply attributing the decrease in the number of autophagic vacuoles to the inhibition of autophagy activity.

Swelling of oncotic cells primarily occurs due to the functional failure of ion pumps on the cell membrane, which causes water and sodium retention in the cells. A recent report showed that Na^+ activated non-selective anion channels through oxidative stress to enter cells, which is a new mechanism for the induction of cell swelling (Ma et al., 2001). Upon the occurrence of oncosis, mitochondria release apoptosis-inducing factor (AIF). AIF is a type of non-caspase-dependent chromatin condensation factor. After entering the cell nucleus, it can cause preliminary chromatin condensation and the degradation of large DNA fragments (Zhang et al., 2013).

Under normal conditions, AIF is localized in the mitochondrial membrane space. Mature AIF is a monomer with a relative molecular weight of 57,000 that can bind to the flavin adenine dinucleotide (FAD) group and has oxidoreductase activity for the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH). Its oxidoreductase activity depends on the presence of the FAD group. Recent studies showed that AIF translocated from the mitochondria to the cell nucleus to cause nuclear condensation and DNA breakage under the action of chemotherapeutic drugs, such as cytarabine and doxorubicin, in acute monocytic leukemia cells. This effect could not be blocked by cysteinyl aspartate-specific proteinase (caspase) inhibitors. During the occurrence of oncosis, the change in cell membrane permeability is an early event and is the key determinant of this type of cell death. Some studies have proposed a hypothetical cell death mechanism promoted by membrane injury in which the occurrence of oncosis is a program of changes in the cell membrane and the functions of physiological or pathological factors on the cell membrane promote cell oncosis (Barros et al., 2001; Sapunar et al., 2001). Moinfar et al successfully cloned a membrane protein named pro-oncosis receptor inducing membrane injury (Porimin) and preliminarily confirmed that it was specifically expressed on the surface of cells that were about to undergo oncosis. Porimin is a type of membrane damage receptor protein that belongs to the transmembrane mucin family and contains 118 amino acids. Its interaction with its ligand can induce an increase in membrane permeability to cause cell swelling and death (Carter et al., 2003). Application of its monoclonal antibody into Jurkat cells directly induced cell oncosis. However, the specific molecular link, signal transduction pathways, characteristic enzymatic changes, and gene expression changes of Porimin are not clear. Caspases and the substrate poly(ADP-ribose) polymerase (PARP) play important roles in cell apoptosis and oncosis. Upon DNA damage, PARP transfers the ADP of the oxidized form of nicotinamide adenine dinucleotide (NAD) to key enzymes involved in DNA repair. When PARP is over-expressed, a large amount of NAD and ATP are consumed and the cells undergo oncosis. Therefore, administration of PARP inhibitors to inhibit oncosis has important significance for the suppression of secondary inflammatory reac-

tions, alleviation of cell and tissue injury, and promotion of repair. The functions of caspases and the substrate PARP in oncosis have received attention. Upon DNA damage, PARP transfers the ADP of NAD to key enzymes involved in DNA repair, whereas the new synthesis of NAD requires the consumption of a large amount of ATP. Therefore, some scholars considered that a certain amount of ATP and NAD in cells was a prerequisite for the development of apoptosis (Moinfar et al., 2000).

The purpose of PARP cleavage by caspases is to block ATP consumption during NAD synthesis and to preserve ATP energy for the apoptosis process, which also consumes energy (Sapunar et al., 2001). When PARP is over-expressed, a large amount of NAD and ATP are consumed. Without proper amounts of ATP and NAD, cells cannot start the apoptosis process and will undergo oncosis. Cells turn to oncosis after pretreatment with the caspase inhibitor carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (zVAD-fmK) (anti-Fas-induced apoptosis is inhibited). Therefore, cells undergo apoptosis after caspase activation. Conversely, when caspase activities are inhibited by the ATP level, B-cell lymphoma 2 (BCL-2) family, and caspase inhibitors, the cells turn to oncosis.

Experiments showed that oncosis was associated with the production of the mitochondrial permeability transition pore (MPTP) (Ryoke et al., 2002). The opening of MPTP can cause the release of a large amount of cytochrome C (CytC) and a reduction of the ATP levels. Without energy support, caspase cannot be activated in time; therefore, the cells rapidly undergo oncosis. Reactive oxygen species (ROS) and their derivatives produced in the mitochondria can induce MPTP opening, inhibit electron transfer in the respiratory chain, reduce ATP production, and participate in the occurrence of oncosis through oxidative injury to membrane proteins. The development of cell oncosis can also be inhibited by treating the redox state in cells with anti-oxidants. Oncosis and apoptosis are two types of cell death. The concepts for the differentiation between oncosis and apoptosis are described below. (1) Organisms depend on apoptosis to accurately control the number of cells in tissues to maintain normal structure and morphology. Organisms use oncosis to remove excessive tissues and cells on a large scale; for example, the disappearance of middle fingers and toe webs during embryonic development is achieved through cell oncosis (Kern et al., 2002). (2) The characteristic presentation of apoptosis is cytoplasmic shrinkage and nuclear condensation, whereas the characteristic presentation of oncosis is cytoplasmic swelling and karyolysis. Additionally, an inflammatory reaction is generated surrounding oncotic cells due to the increase in cell membrane permeability (Shinoura et al., 1999). (3) Apoptosis is active death that consumes ATP and is developed under close regulation of genes from start to finish. Oncosis in a process of passive death when cells have an ATP deficiency; the association of its development and gene regulation are not clear. (4) Apoptosis is associated with caspase activation. After caspase activities are inhibited by the ATP levels and caspase inhibitors, cells primarily die of oncosis. As a method of cell death, oncosis is sudden (i.e., I/R) cell death and is associated with a depletion of energy in the cells. Studies showed that cell apoptosis and necrosis under the same stimulation conditions are determined by the presence of a specific amount of ATP. If energy is not satisfactory, the apoptosis process cannot be completed and the cells will turn to oncosis; otherwise, the cells will turn to apoptosis.

Cell oncosis and apoptosis can be present together and exhibit a certain distribution feature. Moreover, they are interchangeable under certain conditions. The center region of ischemia is primarily characterized by oncosis, whereas the surrounding region primarily exhibits apoptosis. Although cell apoptosis and oncosis can be caused by the same type of stimulation, they are associated with the blood supply of tissues, stimulation strength, and action time. Cells with an abundant blood supply, weaker stimulation strength, and shorter action time primarily undergo apoptosis; otherwise, they tend to develop oncosis (Hein et al., 2003).

This study detected and quantitatively analyzed Beclin-1 and LC3II/I protein expression in the sham, S, I/R, and SEVOP groups. The results showed that the LC3II/I level in the I/R group was higher than that in the sham group, indicating that myocardial ischemia-reperfusion injury could induce the up-regulation of Beclin-1 and LC3II/I expression or that ischemia-reperfusion injury caused autophagic flux impairment to reduce Beclin-1 and LC3II/I clearance ($p<0.05$). Beclin-1 and LCII/I protein expression was lower in the SEVOP group than in the I/R group, indicating that sevoflurane postconditioning could down-regulate Beclin-1 and LC3II/I expression after ischemia-reperfusion or that sevoflurane postconditioning could promote autophagy functions to increase Beclin-1 and LC3II/I clearance, thereby causing the reduction in the Beclin-1 and LC3II/L protein expression levels.

In this study, porimin protein expression in the I/R group was higher than in the sham group and porimin protein expression in the SEVOP group was lower than expression in the I/R group. The study results indicated that myocardial ischemia-reperfusion caused a rising trend of oncosis in cardiomyocytes, whereas sevoflurane postconditioning after myocardial ischemia-reperfusion inhibited porimin protein expression and decreased the occurrence of oncosis to exert its protective function for myocardial ischemia-reperfusion injury. When ischemia-reperfusion injury is more severe, the level of MPTP opening is larger, the ampli-

tude of the reduction of the intracellular ATP level is larger, and more cells undergo oncosis. Sevoflurane decreased the occurrence of oncosis and alleviated myocardial ischemia-reperfusion injury by inhibiting MPTP opening.

Conclusion

The administration of sevoflurane alone did not affect the number of autophagic vacuoles and oncotic cells. After ischemia-reperfusion, the number of autophagic vacuoles and oncotic cells significantly increased, whereas sevoflurane postconditioning significantly decreased the number of autophagic vacuoles and oncotic cells.

References

Barros LF, Stutzin A, Calixto A, Catalan M, Castro J, Hetz C, Hermosilla T. Nonselective cation channels as effectors of free radical-induced rat liver cell necrosis. Hepatology 2001; 33: 114-22.

Carter BZ, Kornblau SM, Tsao T, Wang RY, Schober WD, Milella M, Sung HG, Reed JC, Andreeff M. Caspase-independent cell death in AML: Caspase inhibition *in vitro* with pan-caspase inhibitors or *in vivo* by XIAP or Survivin does not affect cell survival or prognosis. Blood 2003; 102: 4179-86.

Camarco JP, Morais LK, Moreno JD, Rassi MC, Camarco MN, Guimaraes PF, Quireze Junior C. The feasibility of an experimental model of donors after cardiac death in remote ischemic preconditioning studies for renal transplantation in porcine. Acta Cir Bras. 2014; 29: 82-86.

Csonka C, Szucs G, Varga-Orvos Z, Bencsik P, Csont T, Zvara A, Puskas LG, Ferdinandy P. Ischemic postconditioning alters the gene expression pattern of the ischemic heart. Exp Biol Med (Maywood). 2014; 239: 141-50.

Hein S, Arnon E, Kostin S, Schonburg M, Elsasser A, Polyakova V, Bauer EP, Klovekorn WP, Schaper J. Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: Structural deterioration and compensatory mechanisms. Circulation 2003; 107: 984-91.

Huang Z, Zhong X, Irwin MG, Ji S, Wong GT, Liu Y, Xia ZY, Finegan BA, Xia Z. Synergy of isoflurane preconditioning and propofol post-conditioning reduces myocardial reperfusion injury in patients. Clin Sci (Lond). 2011; 121: 57-69.

Kern JC, Kehrer JP. Acrolein-induced cell death: A caspase-influenced decision between apoptosis and oncosis/necrosis. Chem Biol Interact. 2002; 139: 79-95.

Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo -Arozena A, Adeli K, Agholme L, Agnello M, Agostinis P, Aguirre-Ghiso JA, Ahn HJ. Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy 2012; 8: 445-544.

Liou CM, Yang AL, Kuo CH, Tin H, Huang CY, Lee SD. Effects of 17beta-estradiol on cardiac apoptosis in ovariectomized rats. Cell Biochem Funct. 2010; 28: 521-28.

Ma F, Zhang C, Prasad KV, Freeman GJ, Schlossman SF. Molecular cloning of porimin, a novel cell surface receptor mediating oncotic cell death. Proc Natl Acad Sci USA. 2001; 98: 9778-83.

Ma X, Liu H, Foyil SR, Godar RJ, Weinheimer CJ, Hill JA, Diwan A. Impaired autophagosome clearance contributes to cardiomyocyte death in ischemia/reperfusion injury. Circulation 2012; 125: 3170-81.

Mehrpour M, Esclatine A, Beau I, Codogno P. Overview of macroautophagy regulation in mammalian cells. Cell Res. 2010; 20: 748-62.

Moinfar F, Mannion C, Man YG, Tavassoli FA. Mammary "comedo"-DCIS: Apoptosis, oncosis, and necrosis: An electron microscopic examination of 8 cases. Ultrastruct Pathol. 2000; 24: 135-44.

Przyklenk K, Dong Y, Undyala VV, Whittaker P. Autophagy as a therapeutic target for ischaemia/reperfusion injury? Concepts, controversies, and challenges. Cardiovasc Res. 2012; 94: 197-205.

Rachmat J, Sastroasmoro S, Suyatna FD, Soejono G. Ischemic preconditioning reduces apoptosis in open heart surgery. Asian Cardiovasc Thorac Ann. 2014; 22: 276-83.

Ryoke T, Gu Y, Ikeda Y, Martone ME, Oh SS, Jeon ES, Knowlton KU, Ross JJr. Apoptosis and oncosis in the early progression of left ventricular dysfunction in the ardiomyopathic hamster. Basic Res Cardiol. 2002; 97: 65-75.

Sapunar D, Vilovic K, England M, Saraga-Babic M. Morphological diversity of dying cells during regression of the human tail. Ann Anat. 2001; 183: 217-22.

Shinoura N, Yoshida Y, Nishimura M, Muramatsu Y, Asai A, Kirino T, Hamada H. Expression level of Bcl-2 determines anti- or proapoptotic function. Cancer Res. 1999; 59: 4119-28.

Zhang XJ, Chen S, Huang KX, Le WD. Why should autophagic flux be assessed? Acta Pharmacol Sin. 2013; 34: 595-99.

Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: Comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol. 2003; 285: H579-88.

Author Info
Yang Xie (Principal contact)
e-mail: xieydoctor@163.com