High basal level of autophagy in high-altitude residents attenuates myocardial ischemia–reperfusion injury

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Objective: Hypoxia can induce autophagy, which plays an important role in cardioprotection. The present study tested the hypothesis that patients with congenital heart disease living at a high altitude could resist ischemia–reperfusion injury better than those at a low altitude, through elevated basal autophagy by chronic hypoxia.

Methods: Twelve Tibetan patients residing at a high altitude of >3000 m and 12 Han patients residing at a low altitude of <500 m with simple atrial or ventricular septal defects were prospectively recruited. All patients underwent cardiopulmonary bypass, maintaining a flow rate of approximately 2.4 to 2.8 L/min/m^2 and mean arterial pressure of ≥40 to 60 mm Hg. Myocardial ischemia–reperfusion injury between the 2 groups was compared using cardiac troponin I, brain natriuretic peptide, hematoxylin eosin staining, and the terminal deoxynucleotidyl transferase dUTP nick end labeling test. Autophagy-related proteins microtubule-associated protein 1 light chain 3 II (LC3II), Beclin1, and lysosomal-associated membrane protein 2 (LAMP2) and their upstream protein BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (Bnip3) were evaluated with Western blotting.

Results: The maximal cardiac troponin I concentration and increasing x-fold of brain natriuretic peptide in the high-altitude group were obviously lower than those in the low-altitude group (3.10 ± 0.77 vs 7.10 ± 2.28 ng/mL and 2.51 ± 0.94 vs 14.66 ± 6.83, respectively). The preoperative and postoperative levels of LC3II, LAMP2, and upstream Bnip3 in the high-altitude group were obviously greater. No difference was found in the Beclin1 level between the 2 groups at baseline or ischemia–reperfusion.

Conclusions: Patients living at a high altitude with congenital heart disease resisted ischemia–reperfusion injury during cardiac surgery better than those at a low altitude, possibly through elevated basal autophagy induced by chronic hypoxia. (J Thorac Cardiovasc Surg 2014;148:1674-80)

It has been previously reported that the prevalence of congenital heart disease (CHD) in residents living at a high altitude is about 1.37%, 2 to 3 times that of those living at a low altitude.[1] According to the Chinese census in 2005 (China's Fifth National Census), the total Tibetan ethnic population was 5,416,021; most of whom live at altitudes >3000 m. Hence, many Tibetans will require cardiac intervention. With the improvement in healthcare and transportation, many Tibetans with CHD in the Qinghai-Tibet Plateaus will have the opportunity to undergo surgery at the heart center in the lowlands. However, only limited data are available on myocardial ischemia–reperfusion injury during cardiac surgery in high-altitude residents.

The average arterial partial oxygen pressure of habitants in the Qinghai-Tibet Plateaus has been about 57 mm Hg.[2,3] It is well established that chronic hypoxia improved Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (Bnip3) expression, which could increase the autophagy level through inhibition of the mammalian target of rapamycin (mTOR).[4] Autophagy is an intracellular bulk degradation process, in which cytosolic, long-lived proteins and organelles are degraded and recycled. Autophagy occurs at basal levels but can be further induced by stress, such as nutrient depletion and hypoxia.[5] It confers resistance against hypoxia, which could make the native population of the Qinghai-Tibet Plateaus to acclimatize to the high altitude and tolerate hypoxia well.

Evidence from in vitro and in vivo animal studies has suggested that autophagy can play a significant role in myocardial ischemia–reperfusion injury.[6-10] In particular, basal levels of autophagy are important for maintaining cellular homeostasis and protecting cells against damaged or dysfunctional organelles. Enhancing autophagy can protect against ischemia–reperfusion injury in cardiac myocytes.[11] However, the availability of clinical data is limited.

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Abbreviations and Acronyms

AMPK = adenosine 5′-monophosphate-activated protein kinase
Bnip3 = BCL2/adenovirus E1B 19-kDa protein-interacting protein 3
BNP = brain natriuretic peptide
CHD = congenital heart disease
cTnI = cardiac troponin I
LAMP2 = lysosomal-associated membrane protein 2
LC3 = microtubule-associated protein 1 light chain 3
mTOR = mammalian target of rapamycin
TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling

Therefore, we hypothesized that high-altitude patients with CHD could resist subsequent surgical ischemia–reperfusion injury during cardiac surgery better than those living at a low altitude by the elevated basal autophagy induced by chronic hypoxia. To assess this hypothesis, the incidence of myocardial ischemia–reperfusion injury of Tibetan patients with CHD was compared with that of patients living at a low altitude. We also investigated the initial autophagy status, its response to myocardial ischemia–reperfusion, and the upstreaming of hypoxia-related Bnip3.

METHODS

The Clinical Research Ethics Committee of Daping Hospital, Third Military Medical University (Chongqing, China) approved the present clinical study and the use of human tissue. All participants provided written informed consent before study enrollment. To accurately evaluate the effect of basal autophagy on myocardial ischemia–reperfusion injury, the patients who met the following criteria were included: (1) simple atrial or ventricular septal defect, which could be sewn closed directly within a short and similar aortic clamping time; (2) age 6 to 18 years, lessening age's effect on autophagy; and (3) without organ dysfunction or special preoperative medical history.

A total of 12 Tibetan patients who were undergoing elective open heart surgery with cardiopulmonary bypass were prospectively recruited for the high-altitude group (residence altitude, >3000 m). A total of 12 Han patients were matched as a control group, the low-altitude group (residence altitude, <500 m).

All surgeries were performed with the patient under general anesthesia and with cardiopulmonary bypass, maintaining a flow rate of approximately 2.4 to 2.8 L/min/m^2 and a mean arterial pressure of ≥40 to 60 mm Hg using y a centrifugal pump (MaQuet Rotaflow, Mahwah, NJ). del Nido cardioplegia was given as a single 15-mL/kg dose antegrade. With the cardioplegia passing through a cooling coil in ice, the delivery temperature was 8°C to 12°C. The aortic root pressure was not monitored, but the surgeon monitored aortic root distension closely during delivery to prevent capillary damage from high shear forces with too rapid delivery. Right atrial tissue (about 200 mg) was harvested before the establishment of cardiopulmonary bypass and 10 minutes after opening the crossclamp. One half of the right atrial tissue was immediately frozen in liquid nitrogen and processed for Western blotting, and one half was fixed in 10% neutral formalin for immunohistochemistry. Cardiac troponin I (cTnI), a marker of myocardial injury, was tested using the Alere Triage fluorescence immunoassay (Alere, Inc, Waltham, Mass). Brain natriuretic peptide (BNP) was measured using a chemiluminescence immunoassay from a blood sample withdrawn for other routine blood tests. Blood samples were collected for these laboratory tests preoperatively and 6, 24, and 72 hours postoperatively.

Histologic Analysis

The fixed atrial tissue in 10% neutral formalin was processed for histologic examination using standard techniques. It was then embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin for histologic evaluation of tissue damage. For a semiquantitative estimation of the tissue damage, the method described by Zingarelli and colleagues[12] was used. According to this scoring system, the following criteria were used: score 0, no damage; score 1 (mild), interstitial edema and focal necrosis; score 2 (moderate), diffuse myocardial cell swelling and necrosis; score 3 (severe), necrosis with the presence of contraction bands and neutrophil infiltration; and score 4 (highly severe), widespread necrosis with the presence of contraction bands, leukocyte infiltration, and hemorrhage.

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling Assay

The myocardial tissues embedded in paraffin were sectioned (2 μm thickness) for the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay with a commercial in situ cell death detection kit (Roche, Mannheim, Germany). According to the manufacturer's protocol, the deoxyribonucleic acid nick ends were visualized with diaminobenzidine. The numbers of 4′,6-diamidino-2-phenylindole–positive nuclei and TUNEL-positive nuclei were quantified from the average of 3 randomly selected fields/section using Image Pro Plus, version 7.0 (Media Cybernetics, Rockville, Md), performed by a technician who was unaware of the samples' group. The number of TUNEL-positive cells is expressed as a percentage of the total number of cells.

Western Blot Analysis

Total proteins were isolated from the right atrial tissue samples. The sample was then size fractionated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to Immobilon-P membranes (EMD Millipore, Billerica, Mass). The blotted membranes were incubated with antibodies against microtubule-associated protein 1 light chain 3 II (LC3II), lysosomal-associated membrane protein 2 (LAMP2), Beclin1, and Bnip3 (all at a 1:600 dilution; Abcam, Cambridge, UK). After incubation with the appropriate horseradish peroxidase-associated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, Calif), the signals were visualized using an enhanced chemiluminescence detection system (Amersham Bioscience, Piscataway, NJ). Quantification of the Western blot data was performed by measuring the intensity of the hybridization signals using ImageJ, version 1.42, software (National Institutes of Health, Wayne Rasband, available at: http://rsb.info.nih.gov/ij).

Statistical Analysis

The data are expressed as the mean ± the standard error of the mean. The difference in the mean between the 2 groups was evaluated using the t test when sample size was appropriate and the population was normally distributed; otherwise, the Mann-Whitney U test was used. Statistical analyses were performed using IBM SPSS Statistics, version 19.0 software (SPSS, Inc, Armonk, NY).

RESULTS

Perioperative Patient Variables

No difference was found in age, gender, preoperative hemoglobin, hematocrit, aortic clamping time, mechanical

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