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Effect of L-carnitine on Cardiomyocyte Apoptosis and Cardiac Function in Patients Undergoing Heart Valve Replacement Operation

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Summary: The effects of L-carnitine, as an ingredient of cardioplegia solution, on cardiac function and cardiomyocyte apoptosis in patients undergoing heart valve replacement operation were investigated. Twenty-three cases undergoing heart valve replacement with cardiopulmonary bypass (CPB) were randomly allocated into two groups: L-carnitine group ($n=12$, 12 g/L L-carnitine was put in the ST. Thomas cardioplegia) and control group ($n=11$, identical to the L-carnitine group except that normal saline was administered instead of L-carnitine). Serum cardial troponin I (cTnI) levels, the left ventricular ejection fraction (LVEF), and cardiac index (CI) were measured perioperatively. A bit of myocardial tissue obtained from right atria was taken before CPB and by the end of intracardiac procedure to undergo electron microscopy examination and estimate apoptosis by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL). From the end of CPB to 3 days after operation, the serum levels of cTnI in the L-carnitine group was significantly lower than that in the control group ($P<0.05$). Heart color ultrasonogram showed that the CI index and LVEF at 7th day postoperatively in the L-carnitine group were significantly higher than in the control group ($P<0.05$). Compared to the control group, L-carnitine significantly alleviated the morphologic changes of cardiac muscle cells (electron microscopy examination) and decreased the amounts of apoptotic cardiac muscle cells (TUNEL). Furthermore, the dosage of vasoactive drugs used after operation was significantly less in the L-carnitine group ($P<0.01$). It was concluded that L-carnitine cardioplegia solution could improve cardiac function in patients undergoing heart valve replacement operation and alleviate CPB-mediated apoptosis of cardiac muscle cells.

Key words: heart valve replacement operation; cardioplegia solution; cardiac function; apoptosis; L-carnitine

Carnitine is an essential cofactor for fatty acid (FA) metabolism and the predominant source of ATP in the normal aerobic heart. During myocardial ischemia, FA metabolism is impaired and tissue carnitine levels are depleted. Since the heart cannot synthesize carnitine, plasma carnitine can play an important role in maintaining myocardial carnitine levels during reperfusion[1]. Carnitine deficiency can lead to abnormalities in myocardial function. These abnormalities are masked by endogenous glycogen, but not accompanied by structural alterations of the myocardium or by altered activities of important mitochondrial enzymes[2]. In contrast, some scholars believed that L-carnitine had no obvious effects on the clinical parameters after CPB, but can protect the myocardial ultrastructures in the treated subjects[3]. The purpose of this study was to investigate the influence of L-carnitine cardioplegia solution on cardiac function and cardiomyocyte apoptosis when it was used as an ingredient of cardioplegia solution in the process of heart valve replacement operation.

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1 PATIENTS AND METHODS

The study protocol was approved by our institutional experimental and scientific committee and all patients were given an informed consent. The patient's ages, sex, body weight, CPB time, cross-clamping time, left ventricular ejection fraction (LVEF) before operation, New York Heart Association functional class and the sorts of surgical operation showed no significant difference between two groups (table 1). The patients with myocardial infarction and diabetes previously were excluded.

A standard anesthetic technique was used with sufentanil, midzolam and pancuronium. Heart rate, mean arterial pressure, central venous pressure, and artery blood saturation of oxygen (SaO_2) were monitored during operation. CPB with non-pulsatile perfusion flow [(2.2 to 2.4 L/(min. m^2)] was conducted by using membrane oxygenator with catheter filtration. Mild hypothermia (27 ℃ to 30 ℃) was maintained. In antegrade delivery, cardioplegia solution was administered at a pressure of 80 mmHg (1 mmHg = 0.133 kPa), with a minimum flow of 200 mL/min at 4 ℃. Twenty-three cases undergoing heart valve replacement (ST. Jude Medical mechanical valves) with CPB were divided into L-carnitine group ($n=12$, 12 g/L L-carnitine

was put in the ST. Thomas cold crystal cardiac arresting liquid) and control group ($n=11$, identical to the L-carnitine group except that normal saline was administered instead of L-carnitine).

Table 1 Preoperative data and perioperative course

Parameters	L-carnitine group	Control group
Age (years)	48.2±9.7	51.4±11.3
Sex (Female/Male)	5/7	4/7
Body weight (kg)	66.2±8.9	64.3±9.2
Operation methods		
Mitral valve replacement (MVR)	7	7
Aorta valve replacement (AVR)	3	3
Double valve replacement (DVR)	2	1
New York Heart Association class	3.1±0.1	3.2±0.2
LVEF (%)	58.6±6.7	61.4±7.9
Cross-clamping time (min)	77.3±12.3	68.6±15.5
CPB time (min)	109.2±25.3	117.8±31.2

1.1 Sample Collection and Parameters Measurements

1.1.1 Measurement of Serum Cardial Troponin I (cTnI) Venous blood was drawn for determination of cTnI at the following time points: before operation, 20 min after the beginning of CPB, completion of CPB, and 8 h, one day, 3 days, and 7 days after operation. cTnI levels were measured with a Chiron ACS180^{R} analyzer (Chiron Dianostics Corp., East Walpole, MA) using a direct chemiluminescence method.

1.1.2 Evaluation of the Cardiomyocyte Apoptosis Cardiomyocyte apoptosis was detected with terminal deoxynucleotidyl transferase-mediated dUTP-biotin in nick end-labeling (TUNEL) assay according to manufacturer's instruction (Boehringer Mannheim Inc., Germany) and the method described by Cavrieli *et al*[4]. The number of positive apoptotic cardiomyocytes was counted in tissue sections under light microscopy (magnification, × 400) and the positive expression rate was calculated.

1.1.3 Electron Microscopic Examination Myocardial tissues were obtained from right atria before CPB and by the end of intracardiac procedure. Samples were routinely fixed for electron microscopy with Karnovsky fixative and embedded in epoxy resin[5].

1.1.4 Heart Color Ultrasonogram LVEF was measured with echocardiogram at 1 day before operation and 7 day after operation, and CI was calculated by using standard formulas. The amounts of dopamine and dobutamine used postoperatively, and the postoperative cardiac auto-rebeating rate were recorded.

1.2 Statistical Analysis

Data were presented as $\bar{x}\pm s$. Statistical analyses were performed using the SPSS10.0 statistical program. Two-sample Student's t test (two-tailed) was used for continuous data, and Pearson's χ^2 test or Fischer's exact test was used for categorical data when comparing variables between the two groups. Repeated measures analysis of variance was used to test repeated observation variables after the operation. A P value less than 0.05 was considered statistically significant.

2 RESULTS

2.1 Effects of L-carnitine on Serum Concentration of cTnI

Since the end of CPB to 3 days after operation, the serum levels of cTnI in the L-carnitine group were significantly lower than in the control group (5.71±1.14 ng/mL vs 7.87±1.89 ng/mL on 1 day after operation respectively, $P<0.05$; and 5.01±0.89 ng/mL vs 7.53±1.43 ng/mL on 3 day after operation respectively, $P<0.05$).

2.2 Electron Microscopic Examination

Electron microscopy revealed that only slight swelling of mitochondria in the cardia muscle cells was found and the myocardial fiber was intact by the end of operation in L-carnitine group, however, in the control group swelling of mitochondria with vesicle formation, fissure of part of mitochondria ridges, and disappearance of glycogen particles were found (fig. 1A, B).

2.2 Effects of L-carnitine on Cardiomycyte Apoptosis

The TUNEL-positive nuclei appeared condensed. 38 % of the patients in L-carnitine group and 78 % of the patients in control group were positive for myocardial apoptosis. L-carnitine could significantly reduce the TUNEL-positive rate in comparison to control group (fig. 2A, B). Before CPB, there was no significant difference in the percentage of cardiomycyte apoptosis between the two groups. Compared to the values before CPB, the percentage of apoptotic cardiomycytes in both groups was significantly increased at the end of

CPB.

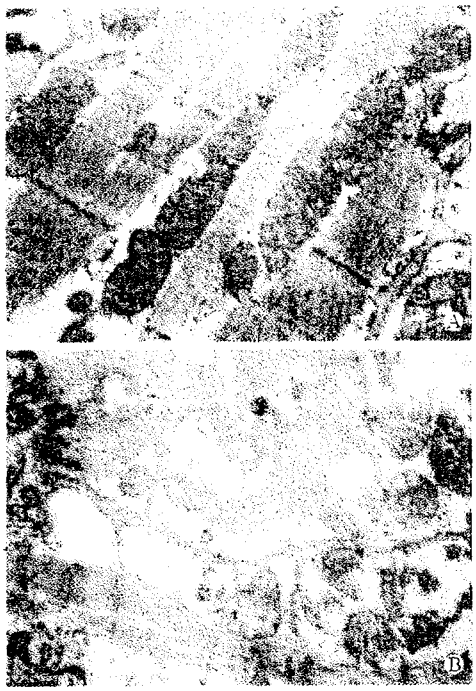

Fig. 1 Electron microscopic examination A: L-carnitine group (×8000); B: Control group (×8000)

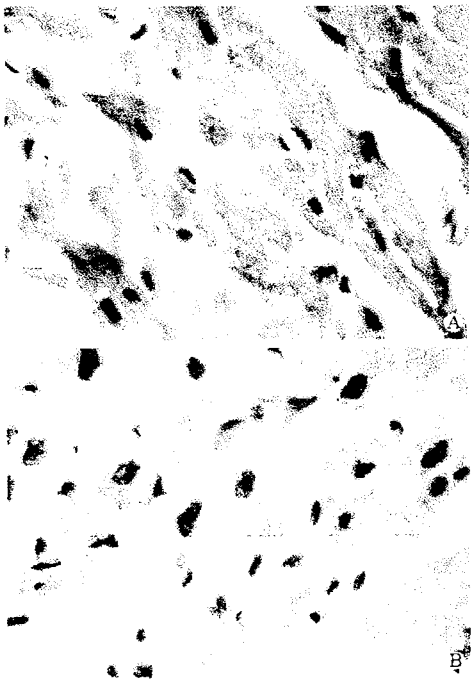

Fig. 2 Apoptotic myocyte nuclei in RA A: L-carnitine group (TUNEL × 400); B: Control group (TUNEL × 400)

2.3 Heart Color Ultrasonogram

At 7 day postoperation, heart color ultrasonogram demonstrated that the CI and LVEF were significantly higher in the L-carnitine group than in the control group (2.86±0.55 *vs* 2.11±0.35; 64.3±8.6 *vs* 51.7±4.9, respectively, $P<0.05$).

2.4 Vaso-active Drugs and Auto-Rebeating Rate

The usage of dopamine and dobutamine (the dose ratio was 1 : 1) in the L-carnitine group was significantly less than in the control group (329±54 mg *vs* 679±147 mg, $P<0.01$) at 24 h postoperation. Furthermore, the postoperative cardiac auto-rebeating rate was higher in the L-carnitine group than in the control group (87.9 % *vs* 45.7 %, $P<0.01$).

3 DISCUSSION

During cardiac operations, myocardial preservation has remained the major concern of cardiac surgeons, because cardiac dysfunction after CPB is a common clinical problem. Hyperkalemic crystalloid cardioplegic solutions have been widely used for myocardial protection in cardiac surgery, and were effective in inducing electromechanical arrest, but they were only partially cardioprotective, and ventricular dysfunction has been observed[5,6]. Thus, we designed this study to investigate the myocardial protective effect of L-carnitine, as an ingredient of cardioplegia solution, in patients undergoing heart valve replacement operation.

Myocardial function depends on ATP supplied by oxidation of several substrates. In the adult heart, this energy is obtained primarily from fatty acid oxidation through oxidative phosphorylation. Alterations in glucose oxidation and transportion developed in diabetic heart may compromise myocardial performance under conditions in which ATP provided by glycolysis is relevant, such as in ischemia and reperfusion[7]. Carnitine is an ammo acid derivative found in high energy demanding tissues (skeletal muscles, myocardium, the liver and the suprarenal glands). It is essential for the intermediary metabolism of fatty acids. Carnitine is indispensable for beta-oxidation of long-chain fatty acids in the mitochondria but also regulates CoA concentration and removal of the produced acyl groups. AcylCoAs act as restraining factor for several enzymes participating in intermediary metabolism. Transformation of AcylCoA into acylcarnitine is an important system for removing the toxic acyl groups. Although primary deficiency is unusual, depletion due to secondary causes, such as a disease or a medication side effect, can occur[8]. Shortage of some co-factors such as L-carnitine also leads to energy depletion[7]. However, recent studies showed that total and free carnitine levels were significantly reduced immediately after CPB and remained a low level until 2 h after CPB. These depressed free carnitine levels might affect cardiac metabolism in the heart after open heart surgery[9]. Ischemia and reperfusion may induce

apoptosis in myocardial[9,10]. During cardiac surgery, cardiac global ischemia and reperfusion of the heart, potentially leading to apoptosis, may play a role in tissue damage and ventricular dysfunction. This process may be a precursor of heart failure[11].

In this study, we demonstrated that there were marked increases in the number of TUNEL-positive cardiomyocytes in postischemic samples compared with samples obtained preischemia. Furthermore, 38 % of the patients in L-carnitine group and 78 % of the patients in control group were positive for myocardial apoptosis. Our results supported the view that cardiomyocyte apoptosis can be induced in myocytes of the patients undergoing heart valve replacement with CPB, and L-carnitine cardioplegia solution can reduce cardiomyocyte apoptosis. In fact, L-carnitine has been tested for its ability to inhibit Fas-activated apoptosis of human Jurkatt T-cell line[12].

The present study was also designed to compare the effects of L-carnitine cardioplegia solution and the ST. Thomas cold crystal cardiac arresting liquid on cardiac function. It was found that from the end of CPB to 3 days after operation, the serum levels of cTnI were significantly lower, and the postoperative cardiac auto-rebeating rate, CI and LVEF were higher in L-carnitine group than that in the control group, but the usage of vaso-active drugs after operation in the L-carnitine group were significantly reduced. Furthermore, the electron microscopy revealed swelling of mitochondria with vesicle formation, fissure of part of mitochondria ridges, and disappearance of glycogen particles in the cardiac specimens obtained from the control group. In contrast, in L-carnitine group there was only slight swelling of mitochondria in the cardiac muscle cells, and the myocardial fiber was intact. All of the above findings showed that L-carnitine cardioplegia solution exerts good protective effect on myocardium in cardiac surgery.

In summary, this study suggests that L-carnitine is a relevant target to improve cardiac function and reduce apoptosis of cardiomyocytes during cardioplegic myocardial ischemia in patients undergoing heart valve replacement operation.

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