Effects of Carnitine on Cardiac Function After Cardioplegic Ischemia in Neonatal Rabbit Hearts

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Background. Ischemia immediately impairs myocardial fatty acid metabolism and reduces the concentration of carnitine which is an essential cofactor for fatty acid metabolism in the mitochondria. The purpose of this study was to investigate the effects of carnitine administration on recovery of cardiac function after cardioplegic ischemia in the neonatal heart where fatty acid metabolism is not a predominant source of adenosine triphosphate.

Methods. Isolated blood-perfused neonatal rabbit hearts underwent 3 hours of cold cardioplegic ischemia. The control group (n = 10) was reperfused with unmodified diluted blood. The carnitine group (n = 10) was reperfused with the blood containing 5 mM/L of carnitine. Before ischemia (base line) and after 15 and 30 minutes reperfusion, left ventricular (LV) function and LV compliance were measured using a intraventricular conductance catheter combined with an isovolumic balloon. Coronary blood flow was measured and myocardial oxygen consumption was calculated.

Results. Carnitine significantly improved not only LV systolic function but also LV diastolic function ($p < 0.05$) as well as LV compliance after ischemia. Coronary blood flow and myocardial oxygen consumption were significantly improved after ischemia in the carnitine group compared with the control group ($p < 0.05$).

Conclusions. These results suggest that carnitine strikingly improves LV functional recovery and aerobic metabolism after cold cardioplegic arrest, and may improve cardiac performance in neonates after open heart surgery.

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β-oxidation of long-chain fatty acids (FA), which occurs in the mitochondria, is the most important and efficacious aerobic source of adenosine triphosphate (ATP) in the normal adult heart [1]. Carnitine, which is a naturally produced amino acid, has important roles in the FA metabolism as well as glucose oxidation, including 1) facilitation of β-oxidation by transporting activated FA into the mitochondria [2, 3], 2) enhancement of the metabolic flux in the tricarboxylic acid cycle by sparing free CoA [1], 3) activation of the transport of adenine nucleotides across the inner mitochondrial membrane by preventing adenylate translocase inhibition by long-chain acyl-CoA [4], and 4) stimulation of activity of pyruvate dehydrogenase by decreasing the mitochondrial acetyl-CoA/CoA ratio, thus enhancing the oxidative utilization of glucose [5].

During normothermic no-flow ischemia, this highly aerobic fatty acid oxidation is inhibited; FA and carnitine intermediates, such as acyl-CoA, acyl-carnitine, and FA itself, readily accumulate in the ischemic tissue [6]. These intermediates increase ischemic injury by adenylate translocase and pyruvate dehydrogenase inhibition [7, 8] and by modifying the structure and function of membranes [9]. However, cellular levels of free carnitine, free CoA, acetyl-CoA, and acetyl-carnitine decrease [6]. Although previous studies have successfully demonstrated the effects of carnitine on cardiac functions in experimental isolated adult mammal hearts [8, 11, 12] and clinical practice [12], the effects in experimental ischemia-reperfusion models have been conflicting [13].

Between 1 and 7 days of life, the newborn rabbit heart shifts dramatically from predominantly using carbohydrates (glycolysis and glucose oxidation) to predominantly using fatty acids as an energy substrate [9, 14, 15]. However, contributions of carnitine to the developing metabolism and the ischemic damaged metabolism in the neonatal heart are still unclear, especially after cold cardioplegic arrest and blood reperfusion.

The purpose of the current study was to investigate the effects of carnitine administration on the recovery of cardiac function and aerobic metabolism after cold cardioplegic arrest in neonatal rabbit hearts.

Material and Methods

Experimental Preparation

An isolated blood-perfused Langendorff model was used to study 20 hearts from 7-day-old neonatal rabbits (Japanese white rabbit), which are commonly used as a model of the newborn heart [16]. Rabbits were anesthetized with an intraperitoneal injection of 60 mg/Kg sodium pentobarbital and 100 IU heparin. After achieving an appropriate plane of anesthesia, the thoracic cavity was opened and the heart was quickly excised and placed in ice-cold normal saline. The aorta was cannulated retrogradely for isolated perfusion. The heart was perfused at 40 cm H_2O aortic pressure

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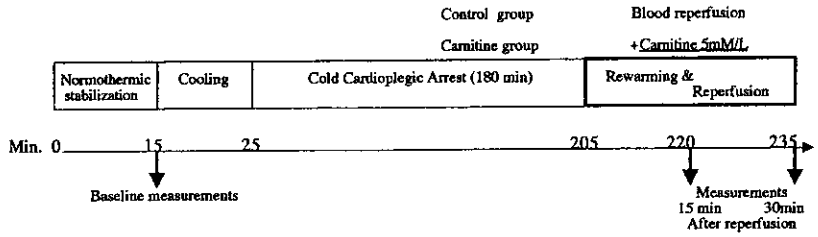

Fig 1. Study protocol.

by gravity. Heparinized fresh homologous whole blood diluted with modified Krebs-Henseleit bicarbonate buffer (NaCl 118 mM/L, $NaHCO_3$, 25 mM/L, KH_2PO_4 1.2 mM/L, KCl 4 mM/L, $CaCl_2$ 1.8 mM/L, and Glucose 11.1 mM/L) at hematoclit 15% was used as perfusate and oxygenated with a mixture of 95% oxygen and 5% carbon dioxide. The perfusate and water bath were controlled at 37°C by heater-circulator except during the hypothermic phase, which was produced by circulating ice water. A latex balloon containing a microtip pressure transducer (SPC-350, Millar Instruments Inc, Houston, TX) was placed into the left ventricule (LV) through the left atrium to measure LV function.

Measurements

LV function was measured during isovolumic contraction by inflating the intraventricular balloon by stepwise increment (0.01 ml). LV developed pressure (DP) and its first derivative (±dP/dt) were recorded at each volume. The systolic function was evaluated by measuring the maximum DP, positive maximum dP/dt. The diastolic function was evaluated by measuring the negative maximum dP/dt and assessing modified compliance at 30 minutes of reperfusion, which was the slope of a line obtained from end-diastolic pressure (EDP) volume points at EDPs of 10 mm Hg (V10) and 20 mm Hg (V20).

Coronary blood efflux was allowed to drip through the open pulmonary artery onto a glass cylinder, and coronary blood flow (CBF) was measured. Arterial and venous hemoglobin concentration, oxygen content, and saturation were measured. Myocardial oxygen consumption (MVO_2) was calculated from the equation:

$$MVO_2\,(ml/min/g) = (\text{arterial oxygen content} - \text{coronary venous oxygen content}) \times \text{CBF/heart weight (g)}.$$

$$\text{Oxygen content (ml/dl)} = 1.39 \times \text{hemoglobin concentration (g/dl)} \times \text{oxygen saturation (\%)}/100 + 0.0031 \times \text{partial oxygen pressure (mm Hg)}.$$

Experimental Protocol

Baseline measurements were made after a 15-minute stabilization period. Then, the perfusate and water bath were cooled to 20°C. At 10 minutes after the start of cooling, when both temperatures reached 20°C, the heart was subjected to cold cardioplegic arrest by infusion of St. Thomas cardioplegic solution every 30 minutes (3 ml initial dose and 1.5 ml following dose) and topical cooling. The composition of the cardioplegic solution was NaCl 110 mM/L, $NaHCO_3$ 10 mM/L, KCl 16 mM/L, $MgCl_2$ 16 mM/L, and $CaCl_2$ 1.2 mM/L. After 180 minutes of cold ischemia, reperfusion was begun with the perfusate with or without carnitine at 20°C followed by rewarming to normothermia. LV function and metabolism were assessed at 15 and 30 minutes of reperfusion.

Experimental groups (Fig 1): The hearts were divided into two groups; (1) in the control group (n = 10), the hearts were perfused with the same unmodified blood during reperfusion; (2) in the carnitine group (n = 10), the hearts were perfused with the blood containing 5 mM/L of L-carnitine (Sigma, St. Louis, MO).

All the animals in this study received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Institutes of Health (NIH Publication No. 80-23, revised 1978).

Statistics

All values were expressed as mean ± standard error (SE) and analyzed by a statistical analysis system (Stat View version 4.5, Abacus Concepts Inc, Berkeley, CA). Repeated measures of analysis of variance (ANOVA) was used to compare the differences in recovery between groups. Data were further compared by Student–Newman–Keuls test if ANOVA was significant. A p value less than 0.05 was considered to be significant.

Results

There were no significant differences among the 2 groups at baseline (Table 1). Therefore, further results are given as percentage of the baseline values.

Figure 2 demonstrates percent of change of maximum LVDP and positive maximum dP/dt. The carnitine group had a significantly greater recovery of both parameters compared with the control group. The carnitine group also showed a significantly greater recovery in maximum −dP/dt than the control group, as shown Figure 3.

Figure 4 demonstrates modified LV compliance in end-diastole. In the control group, the line obtained from end-diastolic pressures-volume points at two different preloads had shifted leftward, and the slope of the line

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Table 1. Baseline Measurements[a]

Variable	Control Group	Carnitine Group
DP (max) (mm Hg)	50.4 ± 2.4	47.3 ± 2.4
dP/dt (max) (mm Hg/s)	101.8 ± 8.4	111.3 ± 7.8
−dP/dt (max) (mm Hg/s)	−91.5 ± 8.4	−99.0 ± 8.2
V10 (ml)	0.099 ± 0.012	0.085 ± 0.003
V20 (ml)	0.12 ± 0.02	0.11 ± 0.01
CBF (ml/min)	1.8 ± 0.3	2.5 ± 0.5
MVO_2 (ml/min/g heart weight)	4.6 ± 0.6	3.7 ± 0.4

[a] Values are mean ± SE. There are no significant differences between the 2 groups by ANOVA.

CBF = coronary blood flow; DP = developed pressure; DP (max) = maximum peak LVDP; dP/dt (max) = maximum of peak positive LV dP/dt; −dP/dt (max) = maximum of peak negative LV dP/dt; EDP = end-diastolic pressure; LV = left ventricle; MVO2 = myocardial oxygen consumption; V10 and V20 = volume to produce an EDP of 10 mm Hg and 20 mm Hg, respectively.

after reperfusion was steeper than at baseline. Thus, compliance was decreased in the control hearts after the reperfusion. Contrarily, in the carnitine group, the line sifted rightward. However, the slope did not change significantly after reperfusion. These compliance data are not based on statistical significance but are meant to represent qualitatively the concepts discussed in the Comments section.

Figure 5 demonstrates percent of change of CBF and MVO_2. The carnitine group had significantly higher CBF and MVO_2 than the control group.

Comment

There were three major findings of this study of neonatal hearts. First, carnitine significantly improved not only LV systolic function but also the LV diastolic function after the cold cardioplegic ischemia. These effects of carnitine on cardiac function agree with previous studies in the adult rat [10, 11]. In addition, the current study shows strikingly that carnitine improves not only −dP/dt but also the modified compliance. Second, carnitine significantly improved CBF after the cold ischemia compared with the control group, suggesting that carnitine has beneficial effects on coronary endothelial integrity. Third, carnitine significantly improved recovery of the MVO_2, representing aerobic metabolism, as well as the CBF and the cardiac function. These findings show that carnitine has beneficial effects on recovery of cardiac performance and aerobic metabolism.

Although previous studies have successfully demonstrated the effects of carnitine on cardiac functions in experimental isolated adult mammmal hearts [8, 10, 11] and in clinical practice [12], the effects in the experimental ischemia-reperfusion model have been conflicting. It is reported that carnitine supplementation in cardioplegia impaired cardiac function after reperfusion in crystalloid perfused rat model [15]. Differences in hemodynamic outcome may stem from differences in the experimental designs employing crystalloid perfusion different from the clinical setting. In addition, zero-flow ischemia was instituted without cardioplegic protection in most of the previous studies. To better reflect the clinical setting during open heart surgery, we established a diluted-blood-perfused model with cold cardioplegic arrest. We believe that blood contains many beneficial substances, such as proteins, leukocytes, diluted fatty acid, and carnitine, which have a positive role during reperfusion compared with crystalloid which does not have these protective elements, thus potentially worsening mild ischemia-reperfusion injury.

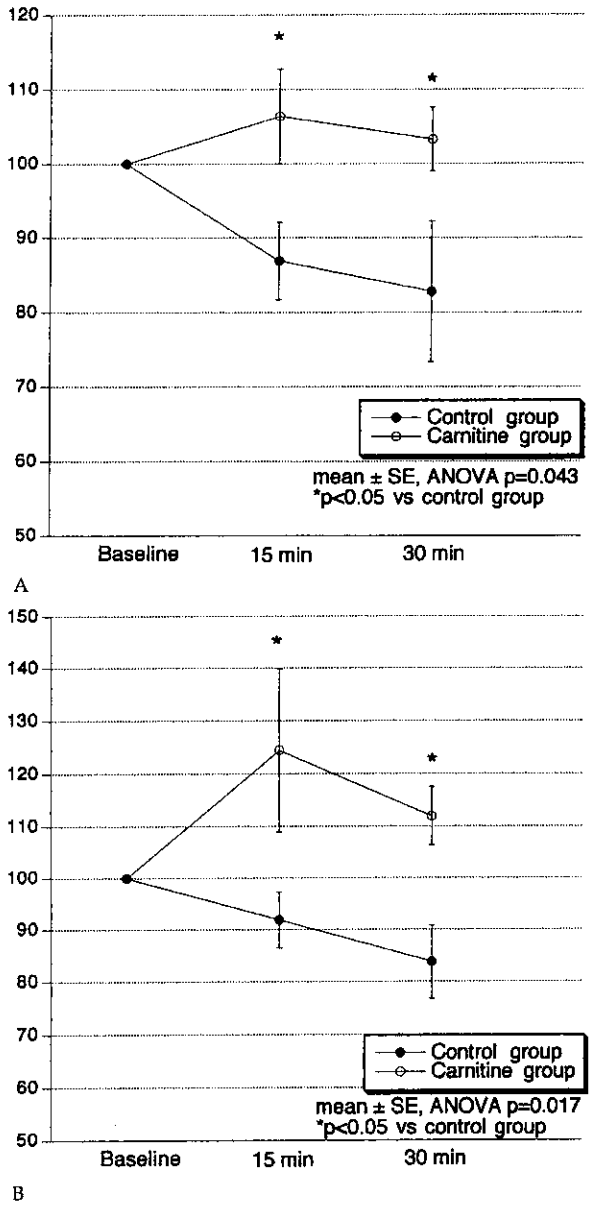

Fig 2. (A) Percent of change of maximum left ventricular (LV) developed pressure (DP) and (B) of positive maximum dP/dt are shown. The carnitine group had significantly greater recovery of both parameters compared with the control group.

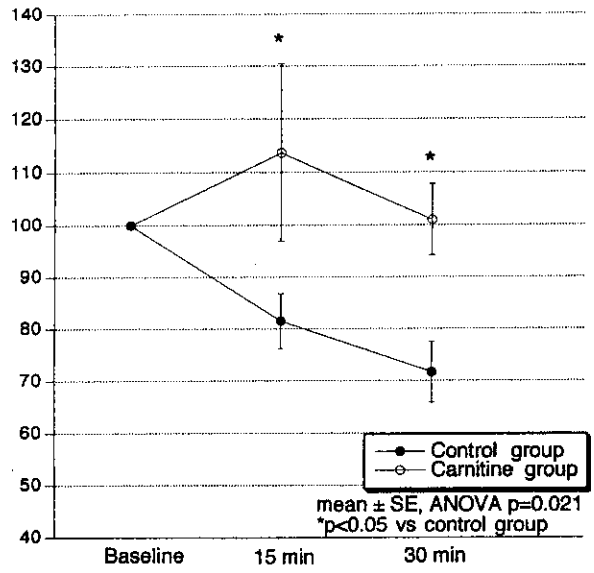

Fig 3. Percent of change of maximum negative dP/dt are shown. The carnitine group showed a significantly greater recovery than the control group.

Previous studies have demonstrated the beneficial effects of carnitine given throughout the procedure, on LV functional recovery after ischemia in the adult rat isolated heart [10, 11]. In the current study, carnitine was supplemented only in the perfusate after cardioplegic arrest in the neonatal heart and still carnitine had very beneficial effects on LV functional and metabolic recovery. Moreover, since cold cardioplegic arrest is used to maintain cardiac metabolism as low as possible, one could argue that carnitine should not be supplemented in the cold cardioplegia. In addition, it was reported that adenosine triphosphate (ATP) level during cold cardioplegic arrest had no correlation with the LV functional recovery after reperfusion in newborn piglet heart [17].

Thus, while carnitine pretreatment before ischemia has been shown to have a beneficial effect on LV functional recovery in adult diabetic animals [10], our study shows the effects on the neonatal heart, which might differ from the adult [9, 14, 15] during reperfusion.

It is well known that LV diastolic function deteriorates after cold cardioplegic arrest in adult mammals [18], in neonatal hearts [19, 20] and following open heart surgery [21], and its treatment is still a challenging clinical problem. In this study, carnitine dramatically improved the recovery of $-dP/dt$ and of LV compliance. The $-dP/dt$ represents the LV isovolumic "active" relaxation where actomyosin crossbridge is uncoupled with sufficient ATP [22]. On the other hand, compliance is a fundamental mechanical property which represents the LV chamber stiffness and influences both the Starling curve and the pressure-volume loop, as well as the early diastolic filling rate of the LV [24].

Compliance provides insight into the extent of functional and structural defects of the myocardium such as myocardial edema, interstitial fibrosis, hypertrophy, and acute volume overload [23]. While compliance is usually thought to represent passive chamber property, it is also effected by ischemia and must therefore have an active compound. Hence, carnitine might play a role in maintaining sufficient ATP in the cardiac myocyte after the reperfusion to improve compliance. Previous studies have successfully demonstrated that L-carnitine suppressed the generation of free-radical oxygen radicals, which is one of the most important causes of ischemia-reperfusion injuries [24], and preserved phospholipids [25] against ischemia-reperfusion injury. These beneficial effects of carnitine could maintain the membrane stability in coronary epithelial cells, as well as cardiac myocytes, which in turn lead to improved the CBF and the LV structural integrity.

In this study, we did not examine fatty acid and carbohydrate metabolism and the concentration of ATP and carnitine derivatives. Although the precise mechanisms of the effects of carnitine still remain unclear, we believe that carnitine could have several beneficial effects on metabolism after cardioplegic arrest in the neonatal heart. One of the possible mechanisms of its beneficial effects might be stimulation of carbohydrate utilization [5]. This is especially important in the neonatal heart where carbohydrates are utilized as predominant energy substrates [9, 14, 15]. On the other hand, it is reported the increased glucose oxidation by dichloroacetate may not be enough to alter the recovery of mechanical function in the neonatal ischemia-reperfusion rabbit heart [16]. In this case, carnitine also might normalize the impaired fatty acid metabolism during reperfusion, because it is reported that free fatty acids are still the preferred substrates in the early reperfusion period [11], and an

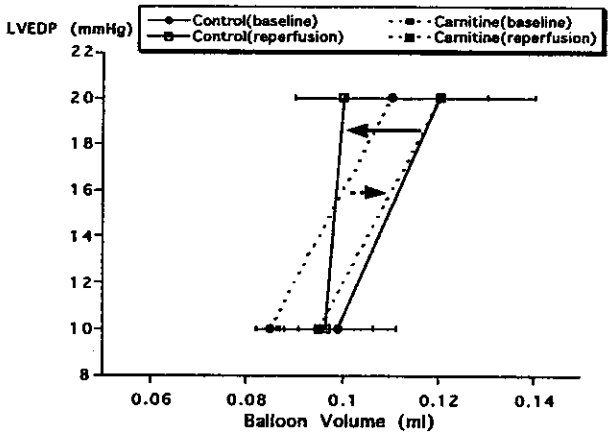

Fig 4. The intraventricular balloon volumes required to produce end-diastolic pressure (EDP) of 10 mm Hg and 20 mm Hg were plotted in a line against each EDP. The line represents modified left ventricular (LV) compliance in end-diastole. In the control group, the line shifted leftward, and the slope of the line after reperfusion was steeper than at baseline. Thus compliance was decreased in the control hearts after the reperfusion. Contrarily, in the carnitine group, the line sifted rightward. However, the slope did not change significantly after reperfusion.

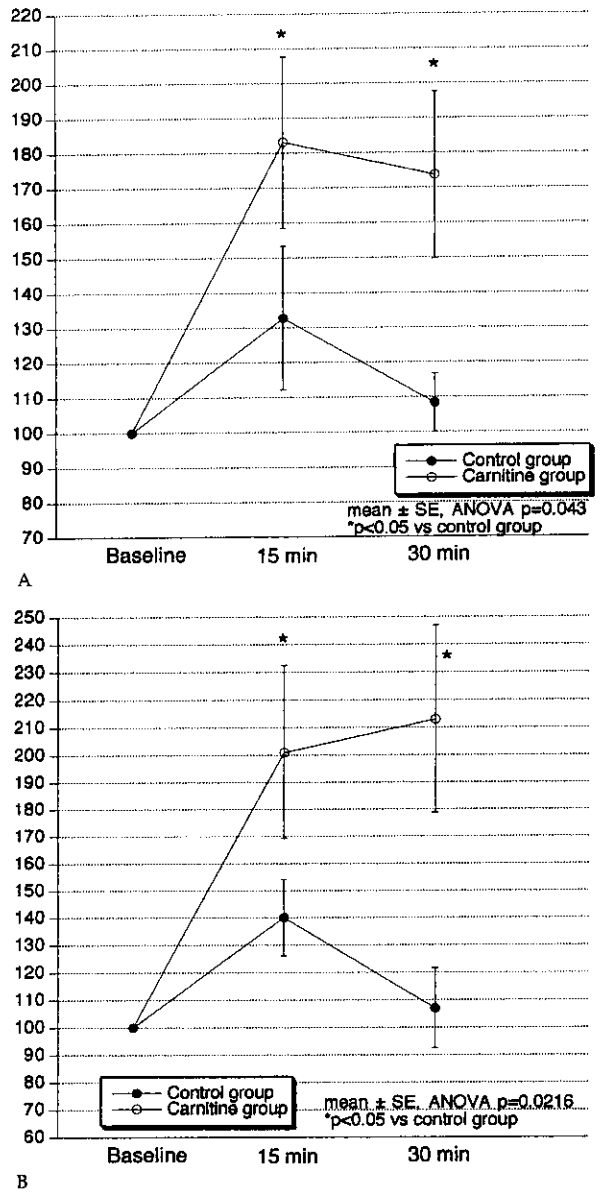

Fig 5. (A) Percent of change of coronary blood flow (CBF) and (B) of myocardial oxygen consumption (MVO_2) are shown. The carnitine group had significantly higher CBF and MVO_2 than the control group.

abnormally stimulated high rate of fatty acid oxidation is found during reperfusion in adult rat heart [26].

In this study, we introduced cold cardioplegic arrest to help preserve fatty acid metabolism better than the simple normothermic no-flow conditions which are frequently used in this kind of experiment. Therefore, the fatty acid metabolism, which is well controlled by sufficient carnitine, could be one of the major sourses of ATP after cardioplegic arrest, even in the neonatal heart where the fatty acid metabolism is not mature but developing.

We conclude that carnitine supplementation improved the LV functional recovery and aerobic metabolism after cold cardioplegic ischemia in the blood-perfused neonatal rabbit heart. It is suggested that a carnitine supplement might improve cardiac performance in neonates after open heart surgery.

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