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Original article

Effects of Acetyl-L-Carnitine on Cardiac Arrhythmias and Infarct Size in Ischemic-Reperfused Isolated Rat Heart

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Abstract

Objective(s)

This study aimed to examine whether acetyl-L-carnitine (ALC) was able to reduce cardiac arrhythmias and infarct size in the ischemic-reperfused isolated rat heart.

Materials and Methods

The isolated hearts were mounted on a Langendorff apparatus then perfused by a modified Krebs-Henseleit solution during 30 min regional ischemia and 120 min reperfusion (control) or by enriched Krebs solution with 0.375, 0.75, 1.5 and 3 mM of ALC (treatment groups). The ECGs were recorded and analyzed to determine cardiac arrhythmias. The infarct size was determined by using a computerized planimetry package.

Results

During ischemia, all used concentrations of ALC decreased number and duration of ventricular tachycardia (VT), total number of ventricular ectopic beats (VEBs) ($P<0.01$), incidence of total ventricular fibrillation (VF) and the time spent for reversible VF ($P<0.05$). At the reperfusion phase, duration of VT, incidence of total VF and reversible VF were significantly lowered by ALC ($P<0.05$). In addition, infarct size significantly was decreased in all treated groups. In the control group, the infarct size was 23±3.1%, however, ALC (0.375, 0.75 and 3 mM) reduced it to 8.7±2.3, 5.3±1.4, and 8±2.9%, respectively ($P<0.01$).

Conclusion

Considering the results, it may be concluded that ALC has protective effects against cardiac ischemia-reperfusion (I/R) injuries by reduction of infarct size and arrhythmias in isolated rat heart. Among the potential cardioprotective mechanisms for ALC, increase in glucose oxidation and resulting reduced lactate production, reduction of toxic fatty acid metabolites and removing free radicals from the myocytes are more relevant.

Keywords: Acetyl-L-carnitine, Arrhythmia, Ischemia, Myocardial infarction, Rat, Reperfusion

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Introduction

Acetyl-L-carnitine (ALC) is an ester of the trimethylated amino acid, L-carnitine, and is synthesized in the human brain, liver, and kidney by the enzyme ALC transferase. ALC facilitates the uptake of acetyl CoA into the mitochondria during fatty acid oxidation, enhances acetylcholine production, and stimulates protein and membrane phospholipids synthesis (1). ALC plays a major role in normal mitochondrial function, being a transport molecule for free fatty acids and an important acetyl-group donor in high-energy metabolism and free fatty acid beta-oxidation (2, 3). Its main body stores are in skeletal and cardiac muscle. It is found along with free plasma L-carnitine and other acyl-esters of varying chain length (4). The formation of ALC originates with cytoplasmic thiokinase which forms acyl-coenzyme A from free fatty acids, ATP and coenzyme A (CoA) (5). This substance is combined with carnitine to form acetyl-carnitine *via* carnitine palmitoyltransferase I. (5, 6). The enzymatic formation of ALC in the mitochondrial matrix is reversible, releasing free CoA and acetyl-CoA which can readily be exchanged across membranes, thus providing metabolic energy to intracellular organelles.

It is claimed that ALC provides several benefits in certain pathologies. There may be some benefit in cases of end stage renal disease or peripheral arterial disease (7). Lipoic acid when supplemented alongside ALC appears to reverse some of the damage to mitochondria associated with aging (8). ALC supplementation has been shown to be neuroprotective in instances of cerebral ischemia (9), peripheral nerve injury (10) and in the treatment of Parkinson's disease in animals (11). ALC supplementation has also been shown to reverse symptoms associated with mental decline in the elderly (12). ALC is being researched in the treatment of Alzheimer's disease (13). There is some evidence that L-carnitine (parent compound of ALC) supplementation may exert a cardioprotective role in cardiomyopathy, prevention of arrhythmias in myocardial infarction and increasing exercise tolerance in angina (14). Arsenian *et al* (1996) demonstrated a decrease in mortality and incidence of circulatory failure in a group of patients with acute myocardial infarction who were administered 3 g of L-carnitine along with solution of glucose, insulin, potassium and magnesium (15). In a study in 2003, effects of short time perfusion of L-carnitine and ALC on the incidence of reperfusion-induced arrhythmias and infarct size were investigated in isolated rat heart in the setting of global ischemia. Results of the above study showed that perfusion of 0.05, 0.5 and 5 mM of L-carnitine and ALC for 10 min before induction of global ischemia failed to reduce the incidence of ventricular fibrillation (VF). In addition, infarct size was reduced only by high concentration (5 mM) of the agents (16).

Despite the mentioned protective effects of ALC, its cardioprotective effects on myocardial infarction size and cardiac arrhythmias in the setting of regional ischemia (not global ischemia) are not completely understood. In the present study, effects of ALC perfusion for the whole period of 30 min regional ischemia followed by 120 min reperfusion (I/R) on myocardial infarction size and arrhythmias were investigated in isolated rat heart.

Materials and Methods

The following chemicals were purchased: ALC (Sigma Tau company), NaCl, $NaHCO_3$, KCl, KH_2PO_4, $MgSO_4$, $CaCl_2$, D-glucose (Merck company), Sodium pentobarbital (Kela company, Belgium) and Heparin (Daru-pakhsh company, Iran).

Animals and surgical procedure

Male Wistar rats weighing 270-330 g were used in this study. The rats were pretreated with intra-peritoneal (ip) injection of 1000 IU/kg heparin then anaesthetized by sodium pentobarbital (60 mg/kg, ip) (17). The hearts were excised rapidly and mounted on a non-recirculating langendorff apparatus under 100 mmHg pressure at 37 °C and perfused throughout the experiments with modified Krebs-Henseleit (K/H) solution which was previously equilibrated with 95% O_2-5% CO_2. A fluid filled balloon was introduced into the

left ventricle and inflated to give a preload of 8–10 mmHg (17, 18). After 20 min stabilization, the hearts were subjected to 30 min regional ischemia followed by 120 min reperfusion. In the control group (n=8), the hearts were perfused only by normal K/H solution throughout the experiment, while in the treatment groups (4 groups, n=8 in each group), they were perfused with enriched K/H solution with 0.375, 0.75, 1.5 and 3 mM of ALC respectively during I/R. Induction of regional ischemia was achieved by temporary occlusion of left anterior descending (LAD) coronary artery followed by 120 min reperfusion (19, 20). An epicardial ECG was recorded by a polygraph during the experiment. Based on the Lambeth conventions, the ECGs were analyzed to determine the total number of ventricular ectopic beats (VEBs), the number of beats occurring as ventricular tachycardia (VT), and the incidence and duration of VT and VF during ischemia and the first 30 min of reperfusion time (20, 21). Animal procedure was approved by local ethics committee.

Measurement of myocardial infarct size

To determine the infarct size, at the end of 120 min reperfusion period, the ligature around the LAD artery was re-tied and the heart was slowly perfused with 2-3 ml of saline solution containing 0.25% Evans blue dye (w/v) via the side arm of the aortic cannula (21). The hearts were frozen, and then the ventricles of the frozen hearts were sliced transversely in a plane perpendicular to the apico–basal axis into 2 mm thick sections. The slices were incubated with 1% (w/v) triphenyltetrazolium chloride (TTC) solution in phosphate buffer for 15 min at 37 °C to dye the non–infarcted region (21, 22). This procedure resulted in the normally perfused tissue being stained blue, non-infarcted, non-perfused tissue stained brick red, and infarcted tissue remaining unstained and appeared pale (23).

Statistical analysis

Except for the incidence of VT and VF which are expressed as percentage, all the other results are expressed as mean±SEM. To compare the number of VT, VEBs and duration of VT and VF between groups, the Mann-Whitney non-parametric U-test was employed. For analyzing the incidence of VT and VF, Fisher Irwin test (Chi-square with Yates correction) was used. The percentage of infarct size was analyzed using one way ANOVA and then considerable differences examined by LSD post hoc range test (19, 21). Differences between groups were considered significant at a level of $P< 0.05$.

Results

The effects of ALC on ischemic and reperfusion arrhythmias are summarized in Table 1 and 2. During ischemia, all used concentrations of ALC decreased number and duration of ischemic VT and number of ventricular ectopic beats (VEBs) versus the control group ($P<0.01$). ALC reduced the incidence of total ventricular fibrillation (VF) and the time spent for reversible VF ($P<0.05$).

Table 1. Effects of ALC (0.375, 0.75, 1.5 and 3 mM) on cardiac arrhythmias during 30 min ischemia in isolated rat heart.

Groups	Ischemia time						
	VEBs number	VT number	VT duration (sec)	Rev VF duration (sec)	Rev VF incidence (%)	Total VF incidence (%)	VT incidence (%)
Control	941±224	473±166	74±31	65±42	50	60	90
ALC (0.375 mM)	289±61**	100±49*	16±8	0*	0*	0*	71
ALC (0.75 mM)	113±23**	23±10**	4±2**	0*	0*	0*	57
ALC (1.5 mM)	154±37**	37±12**	6±2*	0*	0*	0*	86
ALC (3 mM)	69±15**	23±14**	4±2**	44±23	14	14	43*

*$P<0.05$, **$P<0.01$ versus the control group. N=8 rats in each group. VT: Ventricular Tachycardia, VEBs: Ventricular Ectopic Beats (Single+Salvos+VT), Rev VF: Reversible Ventricular Fibrillation, Irrev VF: Irreversible Ventricular Fibrillation.

Table 2. Effects of ALC (0.375, 0.75, 1.5 and 3 mM) on cardiac arrhythmias during 30 min reperfusion in isolated rat heart.

Groups	Reperfusion time						
	VEBs number	VT number	VT duration (sec)	Rev VF duration (sec)	Rev VF incidence (%)	Total VF incidence (%)	VT incidence (%)
Control	330±105	176±42	30±7	60±29	80	90	80
ALC (0.375 mM)	70±28*	24±17	4±2**	171±171*	14	29*	57
ALC (0.75 mM)	187±77	110±75	18±12	105±72	43	57	71
ALC (1.5 mM)	139±69	49±38	9±7*	0**	0**	29*	43
ALC (3 mM)	254±154	179±125	31±20	13±8*	29	29*	57

* P<0.05, **P<0.01 versus the control group. N=8 rats in each group. VT; Ventricular Tachycardia, VEBs; Ventricular Ectopic Beats (Single+Salvos+VT), Rev VF; Reversible Ventricular Fibrillation, Irrev VF; Irreversible Ventricular Fibrillation.

In addition, incidence of VT was significantly reduced only by 3 mM concentration (P<0.05). During reperfusion phase, number of VEBs were reduced significantly mainly by lower concentrations (P<0.05). Also, reversible VF duration was decreased from 61±29 sec in the control group to 0 sec by 1.5 mM of ALC (P<0.01). As depicted in Table 2, duration of VT showed significant reduction by 0.375 and 1.5 mM of ALC (P<0.01 and P<0.05, respectively). Moreover, as shown in Figure 1, ALC (0.375, 1.5 and 3 mM) reduced the incidence of total VF from 90% (control) to 29% (P<0.05). The same concentrations of ALC also reduced the incidence of reversible VF from 80% to 14% (P<0.05), 0% (P<0.01) and 29% (P<0.05), respectively (Figure 1).

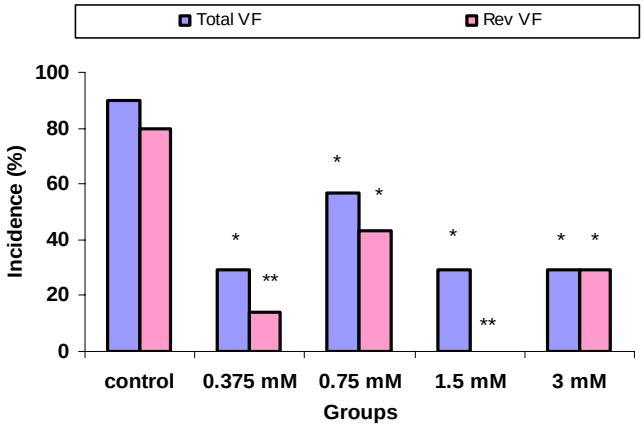

Figure 1. Incidence of total ventricular fibrillation (Total VF) and reversible VF (Rev VF) in the control and isolated rat hearts receiving Acetyl-L-carnitine during 30 min reperfusion. *P< 0.05, **P< 0.01, versus the control group. n=8 rats in each group.

In the control group, infarct size was 23±3.1%, however, ALC (0.375, 0.75 and 3 mM) reduced infarct size to 8.7±2.3, 5.3±1.4, and 8±2.9%, respectively (P<0.01) and ALC (1.5 mM**)** reduced infarct size to 12±4 (P<0.05) (Figure 2).

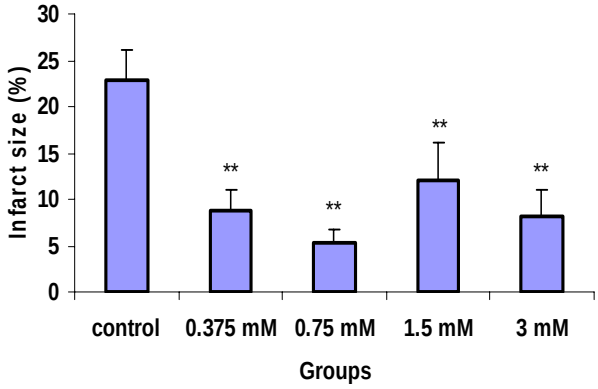

Figure 2. Myocardial infarct size in the control and isolated rat hearts receiving 0.375, 0.75, 1.5 and 3 mM of Acetyl-L-carnitine during 30 min ischemia followed by 120 min reperfusion. Data are represented as mean±SEM. **P< 0.01 versus control group. n=8 rats in each group.

Discussion

The most important cause of mortality in the course of cardiac surgery and myocardial infarction are ventricular arrhythmias such as VT and VF (24). The present study focused on the pharmacological effects of ALC on I/R-induced cardiac arrhythmias and infarct size in isolated rat heart.

For the first time in the medical literature, the results of present study showed that ALC

produces antiarrhythmic effects against I/R-induced arrhythmias such as VT, VEBs and VF. Perfusion of ALC produced significant reduction in the number and duration of ischemic VT, number of VEBs, duration and incidence of reversible VF and total VF in ischemia time. At the reperfusion phase, duration of VT, incidence of total VF and reversible VF were significantly lowered by ALC when it is used during 30 min regional ischemia and 30 min reperfusion.

Our findings also demonstrated that ALC caused marked and potent protective activity against I/R injuries as reduction of infarct size in this model of study.

ALC plays a fundamental role in the production and dissemination of cellular energy as well as in the regulation of metabolic pathways since it is a co-factor required for transport of long-chain fatty acids through the mitochondrial membrane (25). ALC also acts as a scavenger of free radicals in many cells (25). Cardioprotective effects of ALC on myocardial infarction size and cardiac arrhythmias in the setting of regional ischemia are not completely understood. Only in one *in vitro* study, Cui *et al* in 2003 investigated the effects of ALC on incidence of reperfusion-induced VF and infarct size after 30 min global ischemia in isolated rat heart. Their results showed that perfusion of 0.5 and 5 mM of ALC for 10 min before the induction of global ischemia (not regional ischemia) failed to reduce the incidence of VF (16). Their results also demonstrated significant reduction in infarct size only by the concentration of 5 mM ALC (16). Our results are consistent with the results of Cui *et al* in the case of infarct size reduction quality only. However, in contrast to their results, all the used concentrations of ALC in our model significantly reduced infarct size even the lowest concentration (0.375 mM). In addition, our results showed that ALC not only lowered VF incidence in reperfusion time but also lowered the number and duration of VT, number of VEBs, duration and incidence of reversible VF and total VF in both ischemia and reperfusion time when it was used throughout I/R. In our opinion some methodological differences between the above studies (ie type of ischemia and duration of ALC perfusion into the heart) caused different results by low concentrations of ALC.

In addition, our group and other researchers previously reported the protective effects of L-carnitine (the parent compound of ALC) on I/R- induced cardiac arrhythmias and infarct size in the setting of regional ischemia in isolated rat heart (15, 17, 19). It seems that the potential cardioprotective mechanisms of ALC action are very similar to L-carnitine. ALC has important roles in fatty acids metabolism as well as glucose oxidation including (i) facilitation of beta-oxidation by transporting fatty acids into the mitochondria (25), (ii) enhancement of the metabolic flux in the tricarboxylic acid cycle by sparing free CoA (26), (iii) activation of the transport of adenine nucleotides across the inner mitochondrial membrane by preventing adenylate translocase inhibition by long chain acyl-CoA (27), and (iv) stimulation of activity of pyruvate dehydrogenase (PDH) by decreasing the mitochondrial acetyl-CoA/CoA ratio, thus increasing the oxidative utilization of glucose (28). It is important to note the vital role of fatty acid transport into mitochondria and its potential importance in regulation of Ca^{2+} release from sarcoplasmic reticulum, because long chain acylcarnitines play a key role in arrhythmogenesis (29). It has been shown that long-chain acylcarnitines accumulate in ischemic tissue (30) and incorporate into cytosolic membrane compartments (31). Thus, long-chain acylcarnitines increase intracellular Ca^{2+} (32) and intracellular Na^{+} (33) by inducing cell-to-cell electrical uncoupling (34), and may thereby lead electrophysiologic and contractile dysfunction (35, 36) in the myocardium. Therefore, the accumulation of long-chain acylcarnitines under pathophysiologic conditions has deleterious consequences which are likely exacerbated by the influences on Ca^{2+} regulatory proteins (30). Taken together and regarding the different suggested mechanisms, it seems that ALC protects isolated heart against I/R induced injuries such as arrhythmia and infarction via different mechanisms. Maybe, stimulation of

glucose oxidation and and resulting redued lactate and fatty acid metabolites production in the myocytes and scavenging of free radicals by ALC may have important roles in this condition.

Conclusion

By considering the results, it may be concluded that ALC has protective effects against I/R injuries by reduction of infarct size and arrhythmias in isolated rat heart when it was used for the whole period of 30 min regional ischemia followed by 120 min reperfusion. Future studies are required to determine the exact cardioprotective mechanism(s) of action of this agent.

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