

## ANTIARRHYTHMIC AND MYOCARDIAL METABOLIC EFFECTS OF VERAPAMIL DURING CORONARY ARTERY REPERFUSION

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The accumulation of metabolic intermediates subsequent to impaired  $\beta$ -oxidation of free fatty acids has been suggested to be a cause of cellular damage and ventricular arrhythmias in the ischemic heart.

The effects of verapamil on ventricular arrhythmias and free fatty acids metabolism during coronary artery reperfusion in experimental dogs were evaluated over a period of 40 minutes and followed by reperfusion for 15 minutes. One tenth mg/kg/min of verapamil was administered for 5 minutes before occlusion and followed by an infusion of 0.01 mg/kg/min to the end of the experiment. Myocardial samples were obtained from both the non-ischemic and ischemic areas after coronary artery reperfusion and then ATP, free carnitine, long chain acyl carnitine and long chain acyl CoA were measured. In the control group, 3 dogs (27%) had ventricular fibrillation and 2 dogs (18%) had ventricular tachycardia during coronary occlusion. In addition, 2 dogs (25%) developed ventricular fibrillation after reperfusion. On the other hand, all 6 dogs treated with verapamil had neither ventricular fibrillation nor tachycardia during both coronary artery occlusion and reperfusion. ATP and free carnitine levels in the ischemic area were significantly higher in the verapamil group than in the control group (ATP:  $p < 0.01$ , free carnitine:  $p < 0.05$ ), while long chain acyl carnitine levels in the ischemic area were significantly lower in the verapamil group than in the control group ( $p < 0.01$ ). However, there was no significant change in long chain acyl CoA levels between the control and verapamil groups.

This study shows that verapamil protects against ventricular arrhythmias and has beneficial effects on the metabolization of free fatty acids.

In conclusion, it is suggested that one of the antiarrhythmic mechanisms of verapamil in ventricular tachyarrhythmias may be due to the prevention of the accumulation of metabolic intermediates of free fatty acids.

### Key Words:

Coronary reperfusion  
Fatty acid metabolism  
Long chain acyl carnitine  
Verapamil  
Ventricular arrhythmias

THE purpose of this study is to evaluate the effects of verapamil on ventricular arrhythmias, myocardial levels of free carnitine, long chain acyl carnitine, long chain acyl CoA and adenosine triphosphate (ATP) following coronary artery reperfusion.

Calcium antagonistic drugs have been used

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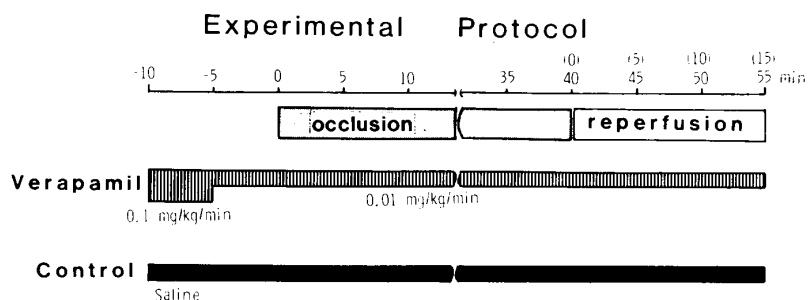


Fig.1. Experimental protocol.

effectively in the treatment of angina pectoris, especially coronary artery spasm and hypertension, for some time and a recent study suggests that they hold considerable promise for the treatment of ventricular arrhythmias. Verapamil has been most extensively studied and Kaumann and Aramendia<sup>1</sup> found that it provided a high level of protection against ventricular fibrillation in dogs subjected to coronary artery occlusion. Ventricular fibrillation occurs during coronary artery reperfusion; verapamil markedly reduces the incidence of such fibrillation.<sup>2</sup> However the antiarrhythmic mechanism of verapamil is not well known.<sup>3</sup> Diltiazem has been shown to decrease fatty acid levels in the ischemic heart<sup>4</sup> and Masters et al.<sup>5</sup> recently demonstrated that verapamil also reduced free fatty acid (FFA) uptake in the ischemic myocardium.

FFA is substrate for oxidative metabolism in the normal heart, but an increase in plasma FFA, which occurs frequently in patients with acute myocardial infarction<sup>6</sup> has been postulated to precipitate life threatening ventricular arrhythmias.<sup>7</sup> During myocardial ischemia,  $\beta$ -oxidation of FFA is inhibited by the limited supply of oxygen and so fatty acid derivatives, such as long chain acyl carnitine and long chain acyl CoA, increase in the cytosol.<sup>8,9</sup> This accumulation has been suggested as a cause of cellular damage and ventricular arrhythmias in the ischemic heart because of inhibitory effects on intracellular enzyme activities and on the cell membrane transport systems.<sup>10-12</sup> Therefore, it may be beneficial to reduce these metabolites in the cytosol of ischemic myocardium.

Accordingly, this study was performed to ascertain whether verapamil has any effects on FFA metabolic intermediates in the reperfused myocardium. This study suggests that one of the antiarrhythmic mechanisms of verapamil in ventricular tachyarrhythmias may be due to the prevention of the accumulation of metabolic

intermediates of FFA.

## MATERIALS AND METHODS

Seventeen mongrel dogs of both sexes, weighing from 10 to 14 kg, were used in the study. Each was anesthetized with a single intravenous injection of sodium pentobarbital (30 mg/kg). The trachea was intubated and respired with room air using a Harvard respirator. After attachment of limb leads for electrocardiographic recording, a thoracotomy was performed in the left fifth intercostal space. The pericardium was opened and the heart suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was dissected free from surrounding tissue just distal to origin of the first diagonal branch and a snare of 1-0 silk suture placed around the LAD. The end of the silk suture was pulled and the LAD occluded with an intracranial arterial clamp for 40 minutes. Soon after the onset of occlusion the ischemic area was clearly apparent as a sharply delineated cyanotic area. Dogs which did not demonstrate the appearance of epicardial cyanosis over the left ventricle were excluded from this study. In all dogs the coronary artery occlusion, chosen on the basis of a previous study,<sup>3,4</sup> was maintained for 40 minutes and then terminated by releasing the coronary artery clamp. Electrocardiograms were recorded continuously at a speed of 5 mm/sec throughout the experiment and the dogs were divided into control and verapamil groups. The dogs in the control ( $n = 11$ ) received saline only, while those in the verapamil group ( $n = 6$ ) were given 0.1 mg/kg/min of verapamil intravenously for 5 minutes starting 10 minutes prior to coronary artery occlusion. This was followed by an infusion of 0.01 mg/kg/min to the end of the experiment (Fig. 1). The dose of verapamil was chosen on the basis of its efficacy in other



Fig.2. The extraction of carnitine and its acyl derivatives. Acid soluble carnitine = free carnitine + short chain acyl carnitine; acid insoluble carnitine = long chain acyl carnitine; Short chain acyl carnitine includes acetylcarnitine. Total carnitine = acid soluble carnitine + acid insoluble carnitine.

studies<sup>2,13,14</sup> and was provided by Eisai Pharmaceutical Factory Inc. (Japan). The beating hearts were immediately removed from the dogs in both groups and transmural samples representing the ischemic area (from the occluded LAD) and nonischemic area (from the circumflex coronary artery) were rapidly excised 15 minutes after onset of reperfusion. Tissue samples were immediately frozen with a Wollenberger clamp

cooled to the temperature of liquid nitrogen to determine the metabolites. These procedures, from removal of the heart until freezing the tissues, were done within 30 seconds and the frozen samples stored at  $-80^{\circ}\text{C}$ . The assay methods for free carnitine and its acyl derivatives are shown in Fig. 2. One gram of the frozen tissue was homogenized in 5 ml of cold 600 mM perchloric acid (PCA) with a polytron homoge-

TABLE I SCORING SYSTEM FOR VENTRICULAR ARRHYTHMIAS

Grade	Arrhythmias
0	No VPB
1	Isolated unifocal VPB < 5/min
2	Isolated unifocal VPB > 5/min
3	Multifocal VPBs
4	Couplets or salvos VPBs
5	Ventricular tachycardia (VT)*
6	Ventricular fibrillation (Vf)

VPB = ventricular premature beat; \* = More than 5 consecutive VPBs were considered VT.

nizer and centrifuged at 4000 rpm for 20 minutes at 4°C. The supernatant 3.5 ml was adjusted to pH 6.5 to 7.0 with 1 N KOH and kept in ice water for 1 hour. After additional centrifugation at 4000 rpm for 20 minutes at 4°C, the supernatant was used to determine free carnitine. Short chain (C3-C10) and long chain acyl (C12 and upward) carnitine were assayed as free carnitine after alkaline hydrolysis at pH 13 for 1 hour at 40°C and 2 hours at 55°C respectively.<sup>15</sup> Free carnitine acetyl transferase (CAT) was accomplished by the Marquis and Fritz's method.<sup>16</sup> The basic reaction mixture contained, in a volume of 1.0 ml, 200  $\mu$ mole Tris-HCl buffer at pH 7.8, 0.2  $\mu$ mole 5,5-dithiobis-2-nitrobenzoic acid, 0.3  $\mu$ mole acetyl CoA and 2.5  $\mu$ mole EDTA.  $\ell$ -carnitine standards (10 to 80 nM) were included with each assay. Reactions were initiated by addition of 0.1 ml of basic reaction mixture to a test tube containing 1.0 ml of the sample. Before and 5 minutes after the addition of 10  $\mu$ l CAT solution (1 mg protein/ml, pH 7.5), absorption of 5,5-dithiobis-2-nitrobenzoic acid with sulfhydryl was measured at 412 nm and the carnitine level calculated from the absorbency changes before and after the addition of CAT. Long chain acyl CoA was assayed as free CoA after alkaline hydrolysis at pH 11.5 to 12.0 for 15 minutes at 55°C in the presence of 10 mM dithiothreitol, while free CoA was determined by the enzymatic cycling method using citrate synthase, CAT and malate dehydrogenase.<sup>17</sup> ATP was determined by enzyme assay using hexokinase and glucose-6-phosphate dehydrogenase according to Lamprecht and Trautshold's

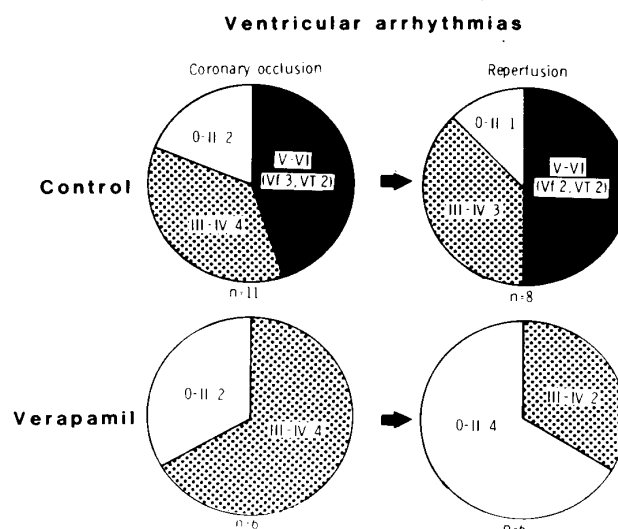


Fig. 3. Incidence of reperfusion ventricular arrhythmias. Vf = ventricular fibrillation; VT = ventricular tachycardia

Changes in the mean score for ventricular arrhythmias

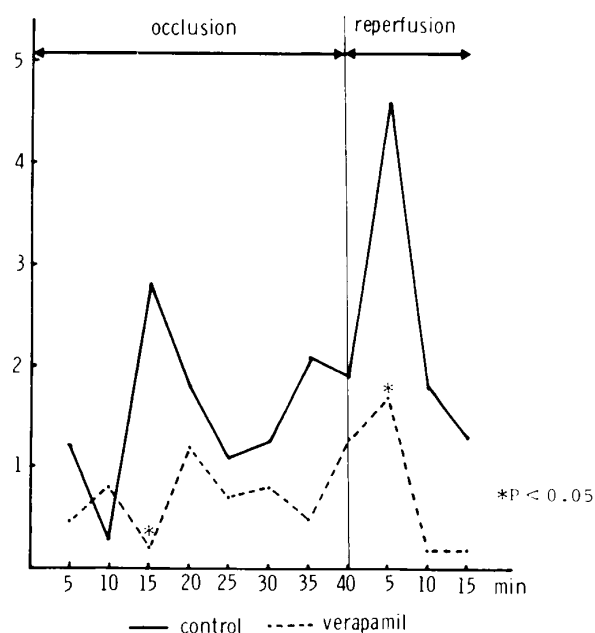


Fig. 4. Changes in the mean score for ventricular arrhythmias. Asterisks represent the significance of changes between the control group and the verapamil group ( $p < 0.05$ ).

method.<sup>18</sup> Lead II of the electrocardiogram was recorded continuously during the experiment in order to analyse the frequency and severity of ventricular arrhythmias which were quantified by an arbitrary scoring system that was produced by a modification of Lown and Wolf's ventricular

TABLE II EFFECTS OF VERAPAMIL ON TISSUE LEVELS OF VARIOUS METABOLITES

	Free carnitine (nmole/g)	Long chain acyl carnitine (nmole/g)	Long chain acyl CoA (nmole/g)	ATP (nmole/g)
<i>Nonischemic area</i>				
Control (n = 6)	910 ± 386	368 ± 100	16.0 ± 5.3	5.8 ± 0.6
Verapamil treated (n = 6)	1179 ± 388	211 ± 86 <sup>#</sup>	17.8 ± 6.3	5.6 ± 0.5
<i>Ischemic area</i>				
Control (n = 6)	633 ± 192 <sup>**</sup>	468 ± 190 <sup>+</sup>	19.2 ± 6.0 <sup>*</sup>	2.9 ± 0.5 <sup>***</sup>
Verapamil treated (n = 6)	1044 ± 379 <sup>**</sup>	199 ± 67 <sup>##</sup>	18.1 ± 4.6	4.4 ± 0.3 <sup>***##</sup>

Values are expressed per gram wet tissue weight and represented as mean ± SD. Dogs which developed ventricular fibrillation were excluded. The significance of the changes is represented as follows: non-ischemic area vs ischemic area (paired t-test); \*\*\* =  $p < 0.001$ , \*\* =  $p < 0.01$ , \* =  $p < 0.05$  and + =  $p < 0.1$ : the control group vs verapamil treated group (non-paired t-test); ## =  $p < 0.01$ , # =  $p < 0.05$ .

arrhythmias grading system<sup>19</sup> (Table I). The maximum score was recorded in individual dogs every 5 minutes and those which developed ventricular fibrillation were sacrificed. Values of the metabolites were expressed as mean ± SD and statistical analysis was made using the Student's t-test. Statistical analysis of ventricular arrhythmias used the chisquare method and the Wilcoxon signed rank test. P values of less than 0.05 were considered statistically significant changes.

## RESULTS

### 1. Ventricular arrhythmias

The preocclusion heart rate was similar in the control and verapamil groups ( $165 \pm 26$  vs  $148 \pm 19$ ). However, verapamil significantly decreased the heart rate from a preocclusion level of  $148 \pm 19$  to  $120 \pm 15$  beats/min after 30 minutes of occlusion ( $p < 0.01$ ). On the other hand, the control group did not significantly alter the heart rate from preocclusion to 30 minutes after occlusion ( $165 \pm 26$  to  $160 \pm 32$ ). The incidence of ventricular arrhythmias due to coronary artery occlusion and reperfusion is shown in Fig. 3. In the control group, 3 dogs (27%) had ventricular fibrillation and 2 dogs (18%) had ventricular tachycardia during coronary artery occlusion. The dogs which developed ventricular fibrillation were excluded from the study and so reperfusion was only performed on 8 dogs which survived 40 minutes of coronary artery occlusion in the control group. Two dogs (25%) developed ventricular tachycardia during coronary artery reperfusion. On the other hand, all 6 dogs treated with verapamil had neither

ventricular fibrillation nor ventricular tachycardia during both coronary artery occlusion and reperfusion. The changes in the mean score of ventricular arrhythmias which were produced by a modification of Lown and Wolf's grading system appeared within a few minutes after coronary artery occlusion and the mean score increased gradually during coronary artery occlusion in both groups. Maximum score was at 15–20 minutes after coronary artery occlusion and then decreased gradually.

The mean score at 15 minutes after coronary artery occlusion was significantly lower in the verapamil group than in the control group ( $p < 0.05$ ). In the control group, reperfusion arrhythmias occurred in the early phase of reperfusion and were apt to develop into ventricular fibrillation and ventricular tachycardia. However, in the verapamil group, reperfusion provoked frequent, repetitive and multifocal ventricular arrhythmias within 5 minutes of reperfusion, but did not develop into either ventricular fibrillation or ventricular tachycardia. The mean score of the verapamil group was significantly lower than that of the control group at 5 minutes after reperfusion ( $p < 0.05$ ) (Fig. 4).

### 2. Tissue levels of carnitine derivatives, long chain acyl CoA and ATP

Effects of reperfusion and verapamil treatment on tissue levels of various metabolites are shown in Table II. The dogs which developed ventricular fibrillation during coronary artery occlusion and reperfusion were excluded from this metabolic study because such procedures may affect cardiac metabolism. Therefore, the study was performed on all 6 dogs in both groups. ATP

levels in the ischemic area decreased significantly in both the control (from  $5.8 \pm 0.6$  to  $2.9 \pm 0.5$   $\mu\text{mole/g}$ ,  $p < 0.001$ ) and the verapamil groups (from  $5.6 \pm 0.5$  to  $4.4 \pm 0.3$   $\mu\text{mole/g}$ ,  $p < 0.001$ ). However, ATP levels in the ischemic area were kept at higher levels in the verapamil group than in the control group ( $4.4 \pm 0.3$  vs  $2.9 \pm 0.5$   $\mu\text{mole/g}$ ,  $p < 0.01$ ), meaning that verapamil retards the decline in ATP levels in the ischemic area.

Free carnitine levels in the control group decreased significantly in the ischemic area compared with the nonischemic area ( $633 \pm 192$  vs  $910 \pm 386$   $\text{nmole/g}$ ,  $p < 0.01$ ). On the other hand, in the verapamil group, free carnitine levels in the ischemic area were  $1044 \pm 379$   $\text{nmole/g}$  and were significantly higher than in the control group ( $633 \pm 192$ ,  $p < 0.05$ ). In the control group, long chain acyl carnitine levels in the ischemic area increased compared with the nonischemic area, but there was no significant differences between them ( $468 \pm 190$  vs  $368 \pm 100$   $\text{nmole/g}$ ,  $p < 0.1$ ). On the other hand, long chain acyl carnitine levels in the ischemic area decreased significantly in the verapamil group compared with the control group ( $199 \pm 67$  vs  $468 \pm 190$   $\text{nmole/g}$ ,  $p < 0.01$ ), indicating that verapamil prevents the accumulation of long chain acyl carnitine in the ischemic area. In the control group, long chain acyl CoA levels in the ischemic area increased significantly compared with the nonischemic area ( $19.2 \pm 6.0$  vs  $16.0 \pm 5.3$   $\text{nmole/g}$ ,  $p < 0.05$ ). In the verapamil group, on the other hand, long chain acyl CoA levels showed no significant changes between the ischemic and nonischemic areas ( $18.1 \pm 4.6$  vs  $17.8 \pm 6.3$   $\text{nmole/g}$ ), i.e., there was no significant difference in the ischemic area between the verapamil and control groups ( $18.1 \pm 4.6$  vs  $19.2 \pm 6.0$   $\text{nmole/g}$ ).

## DISCUSSION

Reperfusion arrhythmias may play an important role in the cause of sudden cardiac death, since relief of coronary artery spasm occurs spontaneously in a variant of angina pectoris.<sup>20</sup> A striking and important characteristic of ischemia is its macroscopic and microscopic heterogeneity and the damage is likely to be a very important factor in the genesis of ventricular arrhythmias. The dispersion of refractoriness in the various areas may be of ultimate importance in the induction of unidirectional

blocks and the maintenance of re-entrant pathways within the ischemic myocardium. Recent studies indicate that reperfusion arrhythmias are distinctly different from arrhythmias due to coronary artery occlusion alone.<sup>22</sup> Reperfusion arrhythmias have been shown to be consequent to enhanced ventricular automaticity in contrast to those arising as a result of ischemia, which are generally due to a re-entrant mechanism.<sup>23</sup> On the other hand, Levits et al.<sup>21</sup> have reported that sudden prolongation in refractory periods following reperfusion leads to an overshoot resulting in a dispersion of refractoriness temporally related to the onset of ventricular arrhythmias, while re-entry appears to be the mechanism for reperfusion arrhythmias.<sup>24</sup> The probability of recovery of non-homogeneity being restored in the first seconds after reperfusion is greater than during coronary artery occlusion. Not all areas within the ischemic area will be equally perfused when a coronary artery occlusion is suddenly released, and therefore the return of electrical activity does not occur at the same speed at all sites. The changes, which during ischemia take several minutes to develop, occur in reverse order in a matter of seconds during reperfusion. Thus re-entry will be facilitated by both the return electrical activity and the greater degree of non-homogeneity.

Calcium antagonist drugs have been used successfully in treating angina pectoris and hypertension, but their ability to prevent arrhythmias is only now being investigated. Verapamil has been the most extensively studied drug in regard to arrhythmias. Kaumann and Aramendia<sup>1</sup> found that verapamil provided nearly complete protection against ventricular fibrillation in dogs subjected to coronary artery occlusion. Brooks et al.<sup>2</sup> found that verapamil raised the vulnerable period threshold and reduced the incidence of spontaneous ventricular fibrillation both after coronary artery occlusion and reperfusion. Likewise, our results indicated that verapamil prevented the occurrence of ventricular fibrillation and tachycardia both after coronary artery occlusion and reperfusion. However, Naito et al.<sup>25</sup> reported that verapamil in a concentration of 0.2 mg/kg did not significantly decrease the incidence of ventricular fibrillation after reperfusion. Perhaps differences in dosage and timing of verapamil administration and the extent of ischemia may be responsible for the differences in results. Although the

mechanisms of verapamil's effect on cardiac action are not entirely clear, it is possible that it<sup>3</sup>; 1) acts as an antagonist of myocardial calcium influx through the slow channel, 2) lessens the metabolic energy requirement, 3) decreases cardiac afterload secondary to its hypotensive effects. 4) results in coronary artery dilatation with improved collateral, and/or 5) reduces the intracellular sequestration of the metabolic byproducts of ischemia. Verapamil causes a dose-dependent prolongation of the functional refractory period of the atrioventricular node and, depresses atrial excitability and prolongation of atrioventricular conduction. Furthermore, verapamil's protective effect with regard to reperfusion arrhythmias may result from a reduction in the accumulation of the products of ischemia such as potassium, calcium, lactic acid, cyclic nucleotides and hydrogen ions. However, Ribeiro et al<sup>27</sup> could not confirm this hypothesis because nifedipine has been shown to exert similar protective effects against ischemia and reperfusion, but has considerably less protective effect against reperfusion arrhythmias. Weishaar et al<sup>4</sup> found that fatty acid levels in ischemic tissue were lower in the diltiazem-treated dogs than in the control dogs. Furthermore, Masters et al<sup>5</sup> have found that verapamil reduces free fatty acid uptake by the heart and concomitantly increases glucose uptake. These results suggest a possible effect on the fatty acid metabolism of calcium antagonist drugs. High levels of circulating FFA have been observed in patients with acute myocardial infarction and correlated with the appearance of ventricular arrhythmias<sup>6,7</sup>. Fatty acid metabolism, which normally represents the major source of high energy phosphates in the aerobic heart, is regulated by both the uptake and oxidation of the fatty acids. In the ischemic heart, the increased NADH:NAD ratio and the inability of the myocardium to reoxidize FADH<sub>2</sub> significantly inhibit  $\beta$ -oxidation. Inhibition of  $\beta$ -oxidation prevents the stepwise degradation of long chain fatty acyl derivatives such as long chain acyl carnitine and long chain acyl CoA<sup>8,9</sup>. These derivatives have inhibitory effects on intracellular enzyme activities and on the membrane transport systems<sup>10-12</sup>. Long chain acyl carnitine is amphiphile and possesses many structural similarities to lysophosphatidylcholine<sup>28</sup>. Amphiphilic substances can induce major changes in membrane function through the insertion of free amphiphile molecules into the

lipid bilayer of the membrane, using a detergent-like effect, and by exchange with membrane phospholipids. The incorporation of a variety of amphiphiles into biological membranes can change the physical properties of the lipid bilayer<sup>29</sup>. The loss of the ability of myocardial membranes to act as a permeability barrier to calcium plays a significant role in the pathogenesis of cell damage in the ischemic heart. Reperfusion of the myocardium after prolonged ischemia has been recognized as leading to extensive myocardial necrosis that is accompanied by ventricular arrhythmias. Long chain acyl CoA inhibits the activity of adenine nucleotide translocase, an important enzyme located in the inner mitochondrial membrane which transfers ATP synthesized in the mitochondria to the cytosol<sup>10</sup>. Higher levels of fatty acids and their derivatives can disrupt cell membranes and therefore are likely to have a beneficial effect in that they reduce both fatty acids and their derivatives levels in ischemic tissue. Because such fatty acyl derivatives can not penetrate the inner mitochondrial membrane, carnitine is essential fatty acyl derivatives are to be transported to the site of oxidation in the mitochondria<sup>30</sup>. Addition of carnitine to mitochondrial preparations can lessen the inhibitory effect of long chain acyl CoA on adenosine nucleotide translocase<sup>31</sup> and L-carnitine can reduce the accumulation of long chain acyl carnitine in the ischemic heart<sup>32</sup> and in the excess FFA supplemented canine heart<sup>33</sup>. Carnitine has also been reported to alleviate ventricular arrhythmias<sup>34</sup> and ST-segment elevation in the ischemic heart<sup>35</sup>.

In this study, free carnitine and ATP levels in the ischemic area were higher in the verapamil group than in the control group. On the other hand, long chain acyl carnitine levels in the ischemic area were significantly lower in the verapamil group than in the control group, but there was no significant change in long chain acyl CoA between both groups. Furthermore, verapamil prevented the occurrence of ventricular fibrillation and tachycardia during both coronary artery occlusion and reperfusion.

This study shows that verapamil has a protective effect on ventricular arrhythmias and a beneficial effect on FFA metabolism. This suggests that one of the antiarrhythmic mechanisms of verapamil in ventricular tachyarrhythmias may be due to by preventing the accumulation of FFA derivatives in the ischemic myocardium.

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