

EFFECT OF CYANIDE IN CARDIOPLEGIC SOLUTION ON ISOLATED RAT HEART FUNCTION

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ABSTRACT

It has been known that cardiomyocytes possess a remarkable ability to down-regulate their energy expenditure on restricted O₂ supply. However it has also been speculated that an ability to suppress aerobic respiration and ATP utilization could be a protective response to prolonged hypoxia.

Having the role of the cytochrome oxidase enzyme in mind, one can ask if its inhibition by using cyanide has a role in improving the heart's function after the cardioplegic period. In order to answer this question, the present study was carried out on two groups of rats. The test and control groups included 10 male rats. Each animal in both test and control groups received cyanide (I.P., 1.6 mg/kg/day) and saline (1 mL/day) respectively, and each heart experienced three stages: 1) normal activity, 2) cardioplegia, and 3) reperfusion stage. St-Thomas II cardioplegic solution was used while it contained 0.5 mmol/L cyanide in the test group. The results indicated that recovery percentage in the reperfusion stage, for the De.L.V.P. and dp/dt parameters were significantly higher in the test [(145.1%±11.97) and (130.97%±9.619)] than the control group [(91.62%±4.59) and (96.1%±4.91)] [(*p*<0.01) and (*p*<0.005)]. H.R. parameters, the rate of coronary solution flow, the variations of left ventricular diastolic pressure in the reperfusion period and tissue pathologic damages did not show significant differences between the two groups.

These results indicated that the application of cyanide in cardioplegic solution induces an improvement of cardiac function in relation to the control group which is likely to be due to the impact of cyanide on cytochrome oxidase.

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INTRODUCTION

A fundamental defense strategy of cells against hypoxia is a coordinated suppression of energy demand as a mean to balance ATP production and utilization. Cardiomyocytes possess a remarkable ability to down-regulate their energy expenditure on restricted O₂ supply.¹ The mechanism underlying metabolic down-regulation in cardiomyocytes is not clear yet. Changes in the efficiency of mitochondrial function, changes in cellular calcium handling, or a more direct pO₂-dependent influence on contractile proteins may be in-

involved.¹ Therefore these could be the important protective mechanisms functioning in the heart.

Ischemic preconditioning (IPC) is considered to be another protective mechanism against cardiac ischemia, which was first described by Murry et al. in 1986. It has been shown that IPC results in 1) reduction in infarct size and 2) improvement of post-ischemic contractile function.² The IPC protective effect may involve alteration in the rate of ischemia-induced depletion of ATP.³ In addition, it was shown that ATP content was better preserved in IPC hearts. The improved coupling of glycolysis and glucose oxidation by

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IPC may contribute to protecting the heart by reducing proton production.²

Cardioplegia is another method for cardiac protection. The use of hypothermic potassium-induced arrest during cardiac surgery has been shown to partially ameliorate ischemic injury by maintaining the heart in a depolarized state, significantly decreasing the energy demand of the myocardium. However, basal metabolic energy requirements are sustained under potassium-induced arrest and thus still constitute a significant energy expenditure.⁴ In spite of modern cardioplegic techniques, myocardial stunning is frequently observed by cardiac surgeons. The degree of stunning can be variable, from minimal myocardial depression to profound cardiac failure.^{5,6} However, it is speculated that one fertile area for research, so far largely ignored, is the preconditioning of impaired myocardium to tolerate surgical insult, as this will preoperatively enhance the tolerance of marginal myocardium to ischemic reperfusion injury.⁷

In addition to these notes, regarding the mechanisms related to protection under hypoxic conditions, it has been suggested that the cytochrome oxidase enzyme plays an important role. Concerning this matter, it has been known that electron transport in the mitochondrion is suppressed during exposure to prolonged hypoxia, through a regulatory effect of molecular oxygen on the catalytic function of the cytochrome-C oxidase enzyme system. It is speculated that an ability to suppress aerobic respiration and ATP utilization could be a protective response to prolonged hypoxia.⁸ It is known that cyanide binds with Fe^{3+} in cytochrome-C oxidase and inhibits this enzyme.^{9,10} Considering the effect of cyanide in one of the most important biochemical pathways regarding adaptation to hypoxia, it seems that this substance is considered to be a suitable agent for inducing cardiac protection. The aim of the present study was to find out whether utilization of this agent before and during cardioplegia can enhance cardiac resistance to ischemia.

MATERIAL AND METHODS

Animals

This study was carried out on male adults NMARI rats obtained from Razi Institute weighing 250-300g. First the animals were divided into two groups (n=10). One group received 1.6 mg/kg/day of KCN (Merck potassium cyanide) with a concentration of 4 g/L through I.P. injection for 30 consecutive days. The control group received a similar volume of normal saline instead of KCN under the same condition.

Isolated heart preparation

Each rat was anesthetized with ether and oxygen. When deep anesthesia was achieved and determined by the absence of a foot reflex, the heart was rapidly excised and placed in

iced Lock's solution. Later the arrested heart was rapidly retrogradely perfused through the aorta according to Langendorff's method with Lock's solution (containing in g: 0.9 NaCl, 0.042 KCl, 0.024 $CaCl_2$, 0.015 $NaHCO_3$, glucose 0.1) which was titrated to pH 7.35-7.45 and filtered by Whatman filter paper No. 541.

Measurement of cardiac parameters

To measure the left ventricular pressure (L.V.P.) and with respect to the animal's weight a small balloon (0.04-0.05 mL) was inserted into the left ventricle through the left atrium. A pressure transducer connected this balloon to a strain gauge coupler which in turn was linked to the physiograph, to measure the left ventricular pressure. Furthermore by using L.V.P. and a differentiator coupler, the derivative of developed pressure (dp/dt) was measured. For ECG recording, surface electrodes were placed on the cardiac surface so that they would be in direct contact with wet cardiac surface but not interfere with its mechanical function. These electrodes were connected to the physiograph through a Hi-gain coupler and recorded the electrocardiogram.

Furthermore by using a drop counter which was placed in a proper underlying cardiac surface and connected to a physiograph through a transducer coupler, the amount of coronary solution flow (C.S.F.) was measured. To record the above mentioned cardiac parameters a Narco Bio-System MK III-P physiograph was used.

Cardioplegic solution

St-Thomas II cardioplegic solution (containing in mM: 110 NaCl, 10 $NaHCO_3$, 16 KCl, 16 $MgCl_2$, 1.2 $CaCl_2$) was used for the control group while in the test group 0.5 mmol/L KCN (Merck-Potassium cyanide) was added to this solution.

Experimental protocols

After heart isolation, each heart in the test and control groups underwent three stages as follows: A- The first stage: in this stage, normal cardiac function was recorded for 20 min. (pre-cardioplegic stage); B- The second stage: in this stage the hearts in the control group were paralyzed by application of St-Thomas II solution, but in the test group, KCN was added to St-Thomas II solution (as mentioned before). The hearts were kept at 22-24°C for 30 min. (cardioplegia stage); C- The third stage: in this stage by reperfusion, cardiac function recovered again and its function was recorded for 30 min. (reperfusion stage).

Analytical methods

The amount of different cardiac parameters such as heart rate (H.R.), developed left ventricular pressure (De.L.V.P.), dp/dt and coronary solution flow (C.S.F.) were measured for each animal in the first stage. Then the mean average of

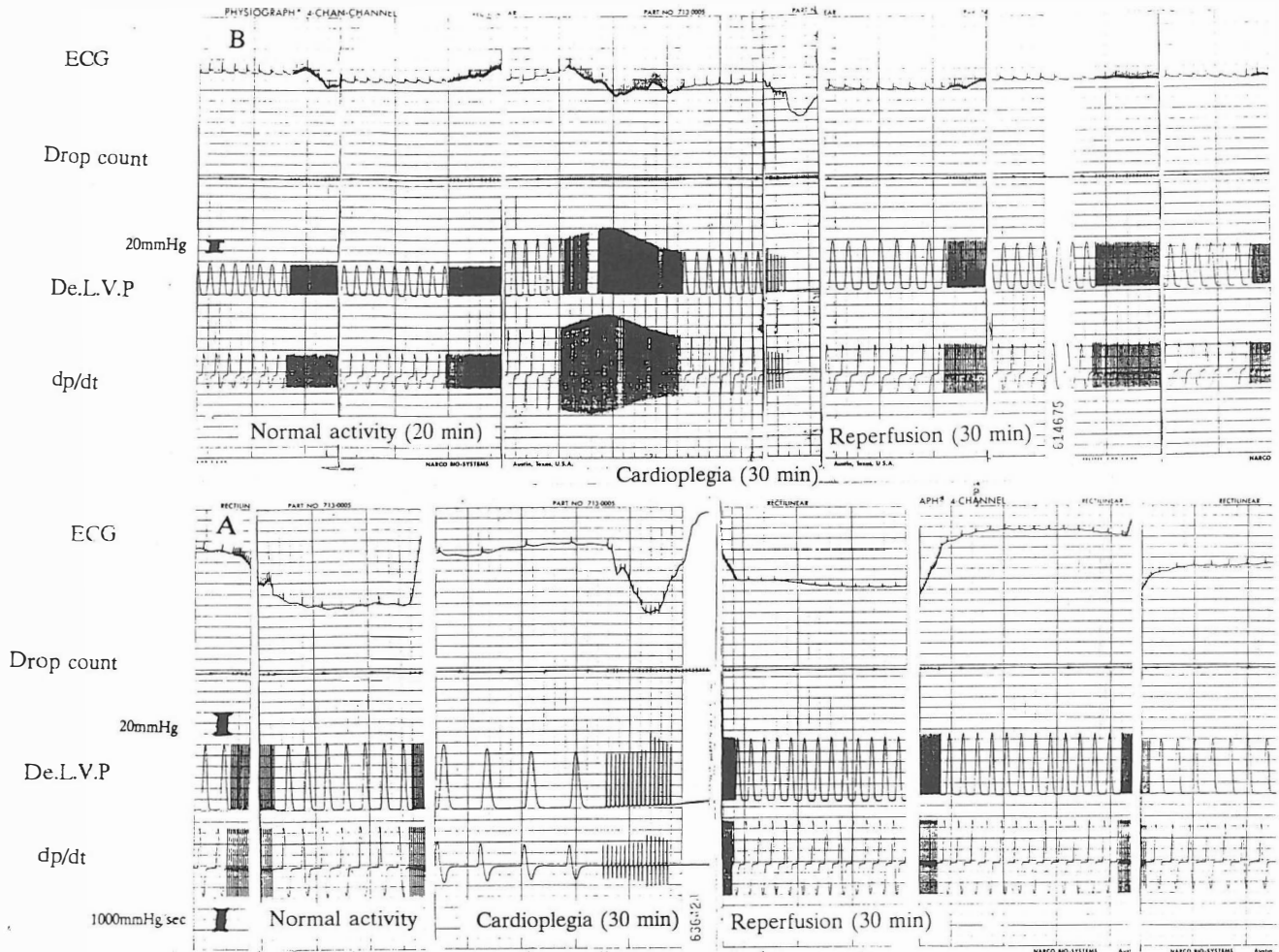


Fig. 1. An example of recorded graphs of the various cardiac parameters in three successive stages in the control (A) and test (B) hearts.

Table I. The mean values of different cardiac parameters before cardioplegia (normal activity stage) and after cardioplegia (reperfusion stage), in control and test groups (Mean±S.E.M.)

Cardiac Parameters	Control		Test	
	Normal activity (Pre-cardioplegia)	Reperfusion	Normal activity (Pre-cardioplegia)	Reperfusion
H.R. (beat/min)	209.44±11.98	197.58±9.21	204.82±9.37	165.699±7.10
De.L.V.P. (mmHg)	59.97±2.07	54.94±2.75	55.82±4.14	86.01±6.68
dp/dt (mmHg/sec.)	2401±260	2369±117.8	1908±191	2498.9±183
C.S.F. (drop/min)	86.4±6.12	79.68±2.74	93.315±7.43	87.87±2.9

these parameters among animals in the control and test groups was calculated and compared with each other. In addition, the amount of changes in cardiac parameters (H.R., De.L.V.P., dp/dt, C.S.F.) in the third stage (reperfusion stage) in comparison with the first stage (normal activity) was measured for the hearts in the test and control groups (in

percentage). Then the mean of these changes among animals was calculated separately and the results were compared with each other. The increase of left ventricular diastolic pressure in the third stage in comparison to the first was measured and it's mean was separately calculated in each group and compared with each other.

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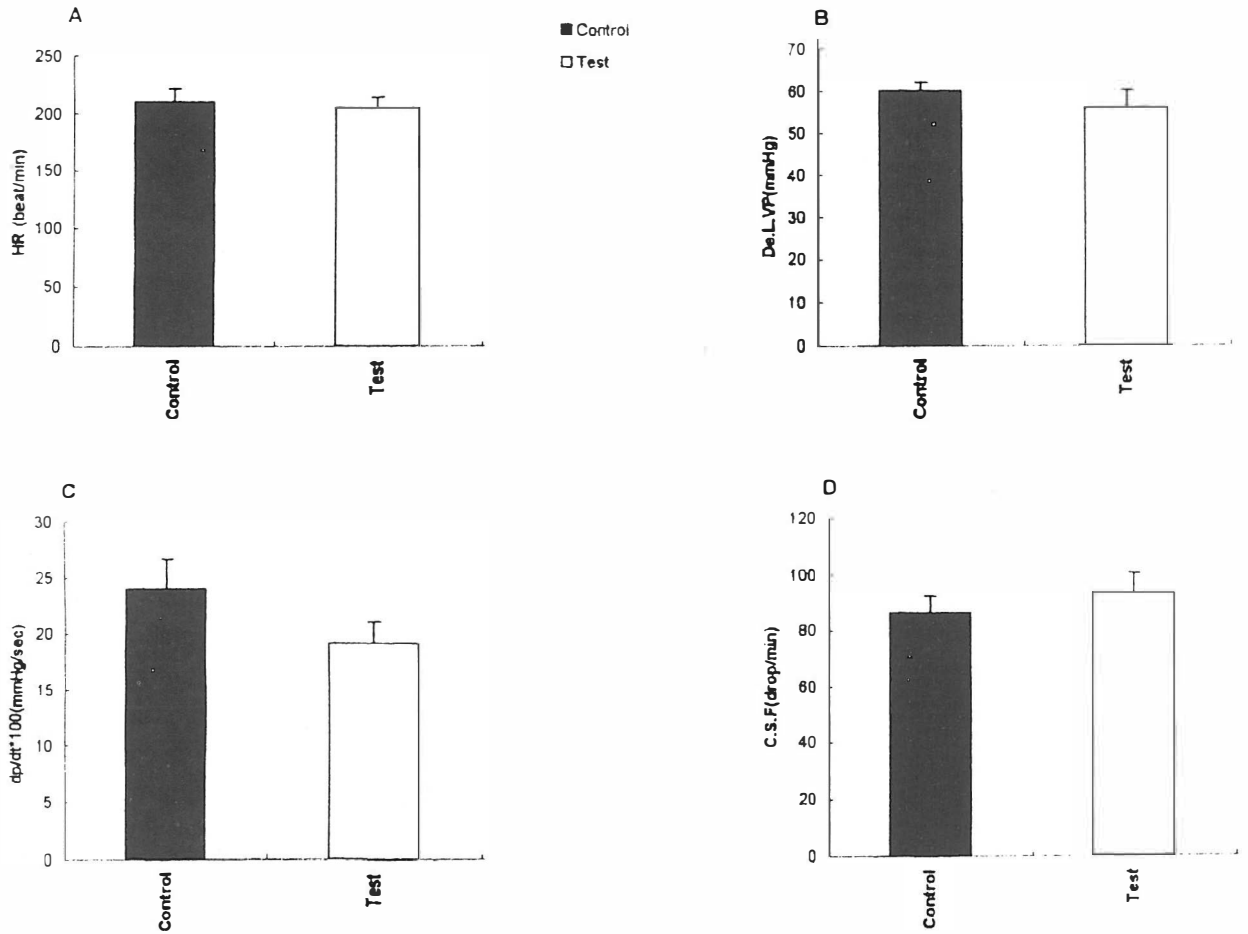


Fig. 2. The mean of different cardiac parameter values in the first stage in control and test groups.

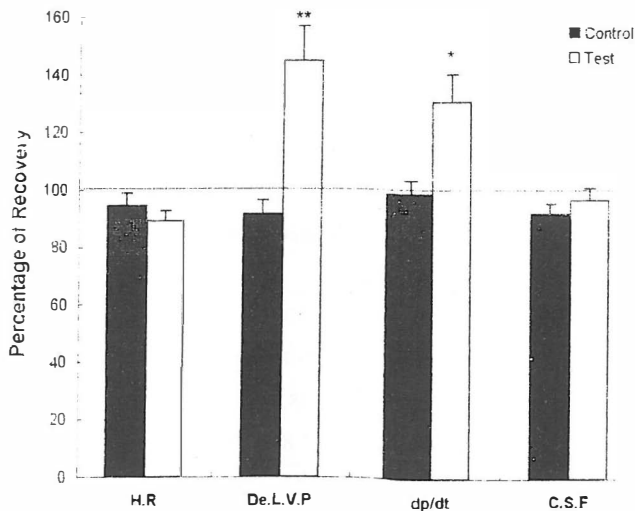


Fig. 3. The mean of recovery percentage of different cardiac parameters in the reperfusion stage in control and test groups. * $p < 0.01$, ** $p < 0.005$.

After the experiment, each heart was studied histologically for probable pathologic lesions in particular necrotic lesions, and the findings were compared with each other.

Statistical analysis

Data are reported as mean \pm S.E.M. To compare the results in both groups F-test was used and on the basis of the results, unpaired t-test with two methods of pooled estimated or separated variance was implemented.

RESULTS

An example of the recorded graphs of the various cardiac parameters related to the hearts of control and test groups are shown in Fig. 1. These parameters have been recorded during successive stages of experimental protocol including pre-cardioplegic, cardioplegic and reperfusion stages.

The mean values of various cardiac parameters, during successive stages, for control and test groups are presented in Table I.

In the first stage of the experiment, the mean of the various cardiac parameters in pre-cardioplegic stage between the two groups (control and test) were compared, indicating no significant difference (Fig. 2).

In the reperfusion stage, there were no significant differences in recovery percentage of HR ($94.34\% \pm 4.4$, $89.1\% \pm 3.37$) and CSF ($92.23\% \pm 3.18$, $96.86\% \pm 4.049$) between control and test groups respectively (Fig. 3) but

De.L.V.P ($91.62\% \pm 4.59$, $145.1\% \pm 11.97$) and dp/dt ($96.1\% \pm 4.91$, $130.97\% \pm 9.619$) changes were significantly different ($p < 0.005$ and $p < 0.01$, respectively) between control and test groups.

Left ventricular diastolic pressure, in the reperfusion stage and in both control and test groups have increased by ratios of 3.227 ± 3.4 mmHg and 6.85 ± 1.39 mmHg respectively (Fig. 4). But these differences were not significant.

Histologically specimens were obtained from the hearts. There were no observed pathological lesions in both control and test groups in this experiment.

DISCUSSION

The results indicate that the frequent application of cyanide with the mentioned dose does not have a significant effect on various cardiac parameters in normal states. As it was observed in the first stage, there were no significant differences between test and control groups. Considering the mechanism of cyanide effect, the use of high doses can lead to severe disturbance in oxygen consumption by the cells. As it is known when the heart muscle is completely deprived of O_2 , glycogen stores are rapidly depleted, and there is insufficient ATP production by glycolysis to maintain normal ATP concentrations. Contraction rapidly diminishes and ceases within about 30 seconds, and irreversible cell damage occurs in about 40 min.¹¹ This point underscores the sensitivity of the cyanide dose. This study shows that the frequent use of cyanide did not show any significant effect on normal cardiac parameters in the test group in comparison with that of the control group, while the responsive pattern of the hearts in recovery stages was different in study and control groups. Thus the amount of cyanide used seems to be the appropriate dose in this experiment.

As it was indicated, in the reperfusion stage the recovery pattern of cardiac parameters was different in test and control groups; particularly cardiac function was better preserved in the test group. It is considered that ischemic pre-

conditioning is the most powerful cardioprotective maneuver known so far,^{4,5,12} which can protect the heart from reperfusion-induced myocardial necrosis and contractile dysfunction. During sustained ischemia, glycolysis, lactate synthesis and glycogenolysis occur at a lower rate in preconditioned myocardium. This is accompanied by a slower degradation of ATP.⁶ In addition, the role of cytochrome oxidase enzyme in adaptation to hypoxia has been known. It is speculated that an ability to suppress aerobic respiration and ATP utilization could be a protective response to prolonged hypoxia. This inhibition appears to be mediated by a regulatory effect of molecular oxygen on the catalytic behaviour of cytochrome-C oxidase.⁸ The utilization of cyanide, on one hand, can induce IPC (by hypoxic stress) and increase the heart tolerance to ischemia, and on the other hand, regarding the specific effect of cyanide on cytochrome-C oxidase, reduces the heart susceptibility to ischemia via changing the cellular metabolic rate under ischemic conditions.

The prominent note is the significant increase in pressure and contractility of the left ventricle during the reperfusion stage in the test group. This positive inotropic effect could be justified by two mechanisms: the first mechanism relates to myocardial norepinephrine release. It is known that myocardial stress promotes norepinephrine release. Selective α_1 -adrenergic stimulation is an external stimulus which exerts the beneficial effects of ischemic preconditioning through the transduction of extracellular signals to an intracellular regulatory site,¹³ and leads to the various routes of cardiac tolerance to ischemia. The second mechanism relates to intracellular calcium increment. Intracellular Ca^{2+} is one of the important intracellular signals involved in PKC activation, and it seems that a preischemic Ca^{2+} load would also provide protection against ischemic reperfusion injury. Indeed, Ca^{2+} may be a clinically acceptable means of activating the Ca^{2+} -dependent protein kinase PKC, which is a central mediator of preconditioning.¹³

Therefore considering the above mechanisms, cardiac function improvement and even positive inotropic effects in the reperfusion stage are possible. But excessive calcium also may produce complications. For instance, it has been known that intracellular Ca^{2+} loading is considered to represent the common denominator of ischemia-reperfusion-induced cell dysfunction and death. Both post-ischemic mechanical dysfunction of reversibly injured myocytes and lethal injury have been related to elevated $[Ca^{2+}]$.¹³ In addition to that, the consequent problems of Ca^{2+} increment related to ischemia include ischemic contracture with a rise in diastolic pressure virtually without systolic activity.¹⁴ In the present study, not only was there no cellular and mechanical dysfunction following ischemia, but as it was shown the mean diastolic pressure increment in the reperfusion stage also did not show any significant difference in the test group relative to the control group; this finding is also against the con-

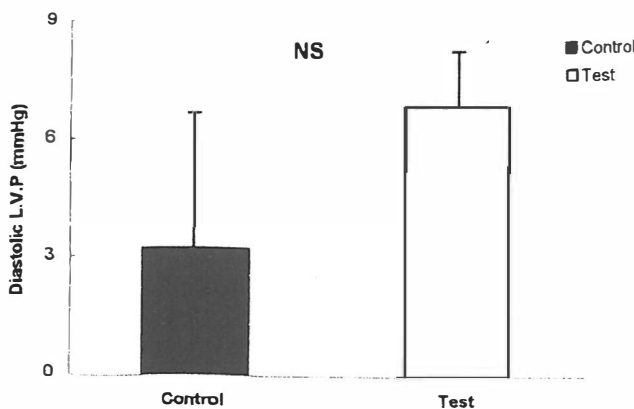


Fig. 4. The mean of increase in the diastolic left ventricular pressure in the reperfusion stage in control and test groups.

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tracture phenomenon. Histological studies did not show any marked tissue lesions in the test group. Following the above results, it seems that cyanide might be a suitable factor for inducing the heart's tolerance to ischemia; particularly the utilization of cyanide in cardioplegic solution involves positive effects.

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