Effect of a Novel Cardioprotective Agent, JTV-519, on Metabolism, Contraction and Relaxation in the Ischemia–Reperfused Rabbit Heart

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The effect of a novel cardioprotective agent, JTV-519 on myocardial metabolism and contraction during ischemia and reperfusion was investigated by means of phosphorus 31-nuclear magnetic resonance (31P-NMR) in Langendorff rabbit hearts. Normothermic, 20-min, global ischemia was followed by 30 min of posts ischemic reperfusion and JTV-519 was administered from 40 min prior to the global ischemia. Adenosine triphosphate (ATP), creatine phosphate (PCr), inorganic phosphate (Pi), intracellular pH (pHi), left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), and coronary flow were measured. Fourteen hearts were divided into 2 experimental groups of 7: Group I were controls and Group II were perfused with JTV-519 (10^-4 mol/L). During ischemia, Group II showed a significant (p<0.01) inhibition of the increase in Pi and LVEDP and the decrease in ATP and pHi, compared with Group I. After posts ischemic reperfusion, Group II also showed a significant (p<0.01) improvement in ATP and pHi as compared with Group I. There were no differences in LVDP or coronary flow during ischemia and reperfusion between the 2 groups. In conclusion, JTV-519 had a significant beneficial effect on myocardial energy metabolism and relaxation during both myocardial ischemia and reperfusion. (Jpn Circ J 2000; 64: 772–776)

Key Words: Cardioprotection; Ischemia; JTV-519; Myocardial metabolism; Myocardial relaxation

The persistence of a contractile dysfunction in reperfused myocardium that has not been irreversibly injured after short periods of ischemia has been called ‘myocardial stunning’. Of the many theories to explain the development of stunning, the most plausible involve cytosolic calcium overload and the formation of oxygen-derived free radicals. Most logically, the delayed resynthesis of ATP, probably as a consequence of the loss of adenine and related compounds during the ischemic period, could be blamed. Nonetheless, even when there is no decrease in ATP and rapid recovery of creatine phosphate (PCr) after short periods of ischemia, there is already stunning. The fact that the stunned myocardium can respond well to inotropic stimulation by dopamine, isoproterenol, calcium infusion, epinephrine or postextrasystolic potentiation suggests that stunning represents a lack of available intracellular calcium or a failure of uptake of calcium by the sarcoplasmic reticulum or a failure of the contractile proteins to respond to the normal calcium concentration. Kiusuka et al showed that the contractile dysfunction in the stunned myocardium could be caused by prior calcium overloading that in turn caused a shift in the calcium sensitivity of the contractile apparatus. The hypothesis is that internal cytosolic calcium overload damages the contractile apparatus, impairing the normal physiologic response to calcium so that there is mechanical stunning.

JTV-519, a new 1,4-benzothiazepine derivative drug, has a cardioprotective effect against myocardial-injury-induced myofibrillar overcontraction, the protective effect being related to its intracellular Ca2+ blocking action. A reduction in myocardial damage and reduced formation of Ca2+ overload in the myocardial cell and mitochondria could all play a role as the effect of JTV-519 on myocardial ischemia–reperfusion injury has not been clearly identified.

The purpose of the present study was to determine whether or not JTV-519 has a cardioprotective effect in the ischemia-reperfused heart and to search for the possible action of this agent, which would suggest future clinical applications. We assessed the effects of this agent to measure myocardial energy metabolism by using phosphorus 31-nuclear magnetic resonance (31P-NMR) spectrometer and cardiac function in isolated rabbit heart. The administration of this agent was found to improve abnormal myocardial energy metabolism and relaxation.

Methods

Isolated Heart Preparation

Male Japanese white rabbits weighing 1.6–1.7 kg were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg). The chest was opened and heparin sodium (1,000 IU/kg) was injected through the right atrial appendage. The heart was quickly removed and after aortic cannulation, retrograde Langendorff perfusion was initiated without delay.

The heart was perfused at a constant pressure of 80 mmHg at 37°C with modified Tyrode’s solution of the following composition (in mmol/L): NaCl 108, KCl 5, MgCl2 1, HEPES 5, CaCl2 2, glucose 10, sodium acetate 20. The perfusate was oxygenated with 100% O2 using a bubble type artificial lung (Japan Medical Supply Co, Ltd.)
Hiroshima, Japan) and pH was adjusted to 7.40. The perfusate was recycled to the artificial lung after passing through the heart. A 40-μm Pall filter (Pall Biomedical Products Co, NY, USA) was placed within this route to remove any contaminants.

To measure left ventricular pressure, a latex balloon was inserted into the left ventricle (LV) via a left atrial incision. The balloon was large enough so that no pressure was generated by it over the range of LV volumes used in the experiment. The balloon was filled with bubble-free saline and attached to a pressure transducer (Viggo-Spectramed Pte. Ltd, Oxnard, USA) connected to a polygraph system RM-6000 (Nihon Kohden Co, Tokyo, Japan). The balloon volume was set to produce a left ventricular end-diastolic pressure (LVEDP) of 10 mmHg. The isovolumic measurement of left ventricular developed pressure (LVDP) was employed as the index of LV contraction.

To drain effluent from the LV Thesbuesi vein, a polyethylene tube was inserted into the LV via the left atrial incision. The heart was paced at 180 beats/min throughout all experiments through the right ventricle with an agar wick soaked in saturated KCl and encased in polyethylene tubing. The pacing was performed by an electronic stimulator SEN-3301 and isolator SS-302J (Nihon Kohden Co). Coronary flow was measured by an electromagnetic flow probe (Nihon Kohden Co) attached around the ascending aorta and data were recorded using a thermal-array recorder WS-681G (Nihon Kohden Co).

Drug Administration

The hearts were divided into the following 2 groups: control group with no administration of JTV-519 (Japan Tobacco, Inc, Japan) throughout all experiments (n=7), and the JTV-519 group perfused with solution containing only JTV-519 (10⁻⁶ mol/L) for the whole experimental period, starting 40 min prior to global ischemia (n=7). JTV-519 was dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO in the perfusate was less than 0.1%.

Measurement of High-Energy Phosphates and Intracellular pH (pHi) by 31P-NMR

Measurement of myocardial energy metabolism was done with a 31P-NMR spectrometer maintained at 37°C. The 31P-NMR spectra were recorded with a JNM-GX 400 FT NMR spectrometer (JEOL, Tokyo, Japan) operating at 161.7 MHz. The spectral parameters were 45° pulse, 1.0s intervals, 300 transients (5 min), 16,384 data points (8,192 sampling points, zero filling), 10,000 Hz sweep width and line broadening of 20 Hz. The heart was placed in a NMR sample tube of 25-mm diameter and quantitative analysis was performed by the relative intensities of the β-ATP, PCR and inorganic phosphate (Pi) peaks. Areas under each peak were integrated 5 times with a planimeter and the mean of 5 such readings was normalized as a percentage of the value given during the initial basal period. The distance of the intracellular Pi peak relative to the intracellular PCR peak (pH-independent) was measured and the pHi was determined using the following equation:

\[ pHi = pk - \log10\left(\frac{\partial\phi}{\partial\phi}\right) \]

where \( pk = 6.79, \partial\phi = 3.25, \delta\phi = 5.75, \) and \( \delta\phi \) is the chemical shift of Pi with respect to PCR.

**Fig 1.** Changes in Pi in control and JTV-519 (10⁻⁶ mol/L) groups. Data are expressed as mean ± SEM. *p<0.05, **p<0.01 vs control.

**Fig 2.** Changes in PCR in the 2 groups. Data are expressed as mean ± SEM. *p<0.05, **p<0.01 vs control.
Experimental Protocol

After a 30-min initial warm-up period, 10 min of basal perfusion, 20 min of continuous normothermic global ischemia caused by clamping the perfusion line and 30 min of posts ischemic reperfusion caused by opening the perfusion line were successively performed. LV pressure and coronary flow were measured at 5-min intervals, and NMR spectra were recorded continuously.

Statistical Analysis

Statistical analysis was performed by one-way ANOVA and unpaired Student's t test. All values were expressed as mean ± SEM, and p < 0.05 was considered significant.

Results

Myocardial Metabolism During Basal Perfusion and Ischemia

Pi (Fig. 1) There was no difference in Pi between the 2 groups during basal perfusion. Pi increased to 640±51% 20 min after the start of global ischemia in the control group, but in the JTV-519 group, Pi was significantly (p < 0.01) lower (441±32%) at the same time period.

PCR (Fig. 2) PCR did not show any difference between the 2 groups during basal perfusion. PCR decreased to 2±2% 20 min after the start of global ischemia in the control group. In the JTV-519 group, PCR was significantly (p < 0.01) higher (50±4%) 5 min after the start of global ischemia than in the control group (26±3%).

ATP (Fig. 3) During basal perfusion, ATP did not show any difference between the 2 groups. ATP decreased to 20±6% 20 min after the start of global ischemia in the control group, but at the same time in the JTV-519 group, it was significantly (p < 0.01) higher (56±9%).

pHi (Fig. 4) pHi also did not show any difference between the 2 groups during basal perfusion. pHi sharply dropped during ischemia in the control group, but remained significantly (p < 0.01) higher in the JTV-519 group (6.35±0.03) compared with the control group (6.17±0.03) 15 min after the start of global ischemia.

Left Ventricular Pressure During Basal Perfusion and Ischemia

LVDP did not show any significant difference between the 2 groups during basal perfusion or global ischemia (Fig. 5). In the control group, LVDP was 94±12 mmHg at the start of basal perfusion and decreased to nearly zero during ischemia.

LVEDP did not show any difference between the 2 groups during basal perfusion, but did during global ischemia (Fig. 6). LVEDP sharply increased during ischemia in the control group, but remained significantly (p < 0.01) lower in the JTV-519 group (15±3 mmHg) compared with the control group (44±10 mmHg) 20 min after the start of global ischemia.

Coronary Flow During Basal Perfusion and Ischemia

There was no difference in coronary flow during basal perfusion or global ischemia between the 2 groups (Table 1). Coronary flow was maintained at approximately 4.5 ml/min·g⁻¹ wet weight during basal perfusion.

Myocardial Metabolism During Reperfusion

Pi There was no difference between the 2 groups in Pi
during reperfusion (Fig 1).

PCR  PCR did not show any significant difference between the 2 groups during reperfusion. In the control group, PCR transiently increased after reperfusion (ie, overshoot phenomenon (Fig 2)).

ATP  ATP recovered to 52±4% 30 min after the start of reperfusion in the control group (Fig 3). In contrast, in the JTV-519 group, ATP was significantly (p<0.01) higher (82±3%) at the same time after reperfusion.

pHi  The pHi in the 5 min after reperfusion, pHi was significantly (p<0.01) higher (7.15±0.08) in the JTV-519 group compared with the control group (6.66±0.09) (Fig 4).

**Left Ventricular Pressure During Reperfusion**

LVDP did not show any significant difference between the 2 groups during reperfusion (Fig 5). In the control group, LVDP gradually elevated during reperfusion and finally recovered to 67±13 mmHg after 30 min of reperfusion.

LVEDP showed a slight, but not statistically significant, reduction (range, p<0.07–0.09) in the JTV-519 group during reperfusion. After 30 min of reperfusion, LVEDP was 17±4 mmHg in the JTV-519 group, and 23±6 mmHg in the control group.

**Coronary Flow During Reperfusion**

As shown in Table 1, coronary flow showed a small rebound increase (ie, hyperemia; 5.0 ml·min⁻¹·g⁻¹ wet weight) in both the groups immediately after reperfusion, other wise there was no difference in coronary flow during reperfusion between the 2 groups.

**Discussion**

We examined the effect of JTV-519 on the metabolic and contractile dysfunction associated with myocardial stunning. In this experimental model, ischemia induced changes in hemodynamic and myocardial metabolism, such as decreasing LVDP, ATP, PCR and pHi, and increasing Pi and LVEDP. Decreasing the amount of ATP available decreases the uptake of calcium by the sarcoplasmic reticulum, causing calcium to accumulate in the cytosol, which in turn increases the resting diastolic tension (ischemic contracture).

The beneficial effect of JTV-519 on myocardial metabolism and relaxation during ischemia is probably mediated through protection of the high-energy phosphate store of the myocardium, such as ATP and PCR, from the damage.

**Table 1 Changes in Coronary Flow in Control and JTV-519 Groups**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>JTV-519</th>
</tr>
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<tbody>
<tr>
<td>Basal 0</td>
<td>4.5±0.1</td>
<td>4.6±0.1</td>
</tr>
<tr>
<td>Ischemia 0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Reperfusion 0</td>
<td>5.0±0.1</td>
<td>5.1±0.2</td>
</tr>
<tr>
<td>30</td>
<td>4.5±0.1</td>
<td>4.5±0.2</td>
</tr>
</tbody>
</table>

Values are expressed in ml·min⁻¹·g⁻¹ wet heart weight as mean±SE.
produced by increased catecholamine activity as well as by preventing the Ca\(^{2+}\) overload. This concept is supported by a study showing a rise in cyclic AMP in the first minutes of developing ischemia, caused by local release of catecholamines in the ischemic zone, which induces activation of the β-receptor and contributes to the increased intracellular calcium that occurs with ischemia. It seems that the suppression of this Ca\(^{2+}\) overload by JTV-519 may play an important role in protecting the mitochondria during ischemia.

In the present study, ischemia induced effects that recovered almost to their pre-ischemic values approximately 30 min after the initiation of reperfusion, with the exception of ATP, LVDP and LVEDP. ATP, contraction and relaxation did not completely recover in either group. During reperfusion, early recovery of ATP and pHi was observed in the JTV-519 group, which shows that JTV-519 improved myocardial metabolism during reperfusion. The JTV-519 group also did not show an increase in diastolic tension during reperfusion. In the ischemia–reperfusion heart, the response of the contractile proteins and ion pump systems, such as the actin–myosin interaction and the calcium pump of the sarcoplasmic reticulum, to the lack of ATP would be inhibition of relaxation, i.e., increased diastolic stiffness throughout reperfusion. In the present study, JTV-519 inhibited ischemic contracture and improved diastolic stiffness during reperfusion. However, myocardial contraction did not show any difference between the 2 groups during reperfusion in spite of the beneficial effect of JTV-519 on myocardial energy metabolism. On this subject, Hoffmeister et al reported that experimental promotion of the recovery of ATP content in the myocardium did not produce any improvement in contractility. It seems that there may be more to the delay in recovery of contraction than ATP content.

In the present experiment, coronary flow showed no significant difference between the 2 groups during the basal state and reperfusion. Generally speaking, the clinical benefit of a calcium influx inhibitor is vasodilation, but we did not observe this effect on coronary flow in the perfused rabbit heart. The possible explanation for the beneficial effect of JTV-519 during reperfusion is that it has a variety of cardioprotective effects against ischemia–reperfusion injury. JTV-519 was found to have a cardioprotective effect against the sudden cardiac cell death caused by myofibrillar overcontraction induced by high Ca\(^{2+}\), and was a more effective cardioprotectant than propranolol, verapamil or diltiazem. JTV-519 binds with the central region of annexin V and inhibits its calcium channel activity. In the isolated rat heart, JTV-519 exerts its protective effect against ischemia–reperfusion injury by specifically inducing activation of δ-protein kinase C and it has been shown to have a frequency- and voltage-dependent blocking effect on the inwardly rectifying Na\(^+\) current in guinea pig ventricular myocytes. During reperfusion, there was the preservation in high-energy phosphate store and relaxation, produced by JTV-519. It seems that the suppression of Ca\(^{2+}\) overload during reperfusion by JTV-519 may play a more important role against the mitochondrial damage.

In the present study, JTV-519 had a pronounced cardioprotective effect against ischemia–reperfusion injury by more completely inhibiting the Ca\(^{2+}\) overload in cardiac cells.

Study Limitations

Our studies were performed in Langendorff perfused rabbit hearts using modified Tyrode's solution; results may differ in human hearts under normal blood circulation. The present study did not directly reveal the mechanism by which suppression of the cytosolic calcium ion efflux or control of cytosolic calcium ion influx through the cell membrane occurs.

**Conclusion**

The present study indicates that JTV-519 preserved the myocardial energy store above the level of the control group and inhibited the increase in diastolic tension during ischemia–reperfusion. Therefore JTV-519 has the potential to protect the myocardium from abnormalities of energy metabolism and relaxation caused by ischemia–reperfusion injury.

**Acknowledgments**

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**References**