

Effect of Mechanical Assist Devices for Ischemic Myocardial Damage —Cardiomyocyte Apoptosis and TNF α —

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Objective: It has been demonstrated that tumor necrosis factor-alpha (TNF α) induces cardiomyocyte apoptosis. Apoptosis has been elucidated as playing an important role as one of the mechanisms of myocardial disorders. However, it is not known whether mechanical support with a left ventricular assist device (LVAD) and percutaneous cardiopulmonary support (PCPS) influence cardiomyocyte apoptosis. The aim of this study was to examine cardiomyocyte apoptosis and TNF α in an experimental acute myocardial infarction model after mechanical support in pigs.

Method: The animals were divided into three groups: the CONT group, LVAD group, and PCPS group. The CONT group was left unassisted after ligation, while the LVAD group was assisted by LVAD and the PCPS group by venoarterial bypass. Acute myocardial infarction was induced by ligation of the left anterior descending coronary artery. As hemodynamic data, aortic pressure (AoP), end-systolic pressure volume relationship (ESPVR), and pressure volume area (PVA) were calculated. The serum TNF α level was measured and terminal deoxynucleotidyl transferase-mediated dUTP in situ nick end labeling (TUNEL) was performed in sections.

Results: ESPVR in the LVAD group and PCPS group was improved ($p < 0.05$ versus the CONT group). Cardiomyocyte apoptosis was restrained by mechanical support ($p < 0.05$ versus the CONT group). TNF α in the LVAD group showed a low value ($p < 0.05$ versus the CONT group). These results were statistically significant.

Conclusions: This study suggests that VADs participate in proinflammatory cytokines and depression of apoptosis, and are effective in recovery from myocardial injury. (*Ann Thorac Cardiovasc Surg* 2003; 9: 233–40)

Key words: cardiomyocyte apoptosis, tumor necrosis factor-alpha (TNF α), left ventricular assist device (LVAD), percutaneous cardiopulmonary support (PCPS), mechanical support

Introduction

Left ventricular assist devices (LVAD) are available for postcardiotomy cardiac support, mechanical circulatory assist for end-stage heart failure, and as a bridge to transplantation.¹⁻³⁾ In addition, some patients with LVAD may

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experience recovery of myocardial contractility, and thus a new application for LVAD is as a therapeutic ventricular assist device and as a bridge to recovery.^{4,5)}

On the other hand, percutaneous cardiopulmonary support (PCPS) is inferior in left ventricle unloading in comparison with LVAD, but the use of PCPS has become clinically widespread, such as in emergency cardiopulmonary resuscitation, because of its simplicity.^{6,7)} However, the timing as to when to wean the patient from mechanical support and for migration from PCPS to stronger mechanical support, such as LVAD, is not clear.^{8,9)}

Recently it has been elucidated that apoptosis plays an

important role as one of the mechanisms of myocardial disorder.¹⁰⁻¹³ A change in the concept of conventional cardiomyocyte death is being urged. Proinflammatory cytokines are expressed after acute hemodynamic overloading and myocardial ischemia/infarction¹⁴ and induce apoptosis in cardiomyocytes. Tumor necrosis factor- α (TNF α) is a proinflammatory cytokine. Cardiomyocytes have been shown to be an important source of TNF α production in disease conditions such as myocardial infarction and heart failure.¹⁵⁻¹⁷ Above all, it is assumed that there is little influence by extracorporeal circulation in the acute stage as in the case of TNF α .¹⁸

It is not known whether mechanical support with LVAD/PCPS influence cardiomyocyte apoptosis. The aim of this study was to examine cardiomyocyte apoptosis and TNF α in an experimental acute myocardial infarction model after mechanical support in pigs.

Materials and Methods

Experimental design

Twenty-one male pigs (45-55 kg in weight) were used. All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research, as well as with the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (NIH publication 86-23, revised, 1996). Intramuscular injection of pentobarbital sodium (20-25 mg/kg) was administered as premedication and the anesthesia was maintained with continuous intravenous infusion of ketamine chloride (1 mg/kg/h). After intubation via tracheostomy, controlled mechanical ventilation was established at a tidal volume of 10-15 ml/kg by means of a volume-cycle ventilator (Servo 900-E, Siemems-Elcoma Inc., Stockholm, Sweden). The venous line was inserted into the right carotid vein. The left external jugular vein was exposed and a catheter (CX-654U, Katex, Tokyo, Japan) was inserted into the coronary sinus for blood sampling. The common carotid artery was exposed, a conductance catheter (2012-6-27-P, Alpha Medical Instruments Inc., CA, USA) was inserted through the aortic valve into the left ventricle with the catheter tip placed at the apex. A catheter tip manometer (811-195S/ANP534, Sentron Inc., Roden, the Netherlands) was also inserted into the left ventricle to monitor the continuous left ventricular pressure volume loop (P-V loop). The left femoral artery was cannulated for arterial pressure monitor-

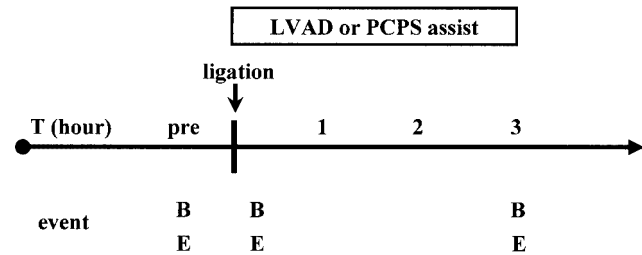


Fig. 1. Overall design of the experiments and the timing of data sampling.

B, blood sampling; E, hemodynamic analysis.

ing. Median sternotomy was performed. Acute myocardial infarction was induced by ligation of the left anterior descending coronary artery and systolic blood pressure was decreased to less than 90 mmHg as an acute myocardial infarction model. The infarcted area was about 40% in the free wall of the left ventricle (Fig. 1).

The animals were divided into three groups: the CONT group (n=7), LVAD group (n=7) and PCPS group (n=7). In the CONT group, animal was left unassisted after ligation for three hours. After coronary ligation, in the LVAD group, the animals were assisted by LVAD with a centrifugal pump (GyroC3E5, Kyocera Corporation, Kyoto, Japan), drained from the left atrium (LA) and an outflow cannula was inserted into the ascending aorta. The assist rate was maintained at 50%. In the PCPS group, the animals were assisted by venoarterial (VA) bypass with the same pump, drained from the right atrium and an outflow cannula was inserted into the right femoral artery. The assist rate was maintained at 50%. Ultrasonic flow transducers (HT207, Transonic Systems Inc., Ithaca, NY, USA) were placed into the ascending aorta and the outflow cannula to measure native heart flow and pump flow. The assist rate (pump flow/total flow; total flow = native flow + pump flow) was maintained at 50%.

Hemodynamic assessment

The left femoral artery was cannulated to monitor continuous aortic pressure. The P-V loop was recorded by Sigma-5 (CardioDynamics Inc., Zoetermeer, the Netherlands). Hemodynamic data were calculated by analyzing the P-V loop with Conduct PC (CardioDynamics Inc.). As hemodynamic data, aortic pressure (AoP) and the end-systolic pressure volume relationship (ESPVR) were calculated. The pressure volume area (PVA) was calculated as myocardial oxygen consumption. Hemodynamic data were registered before coronary ligation (baseline), just

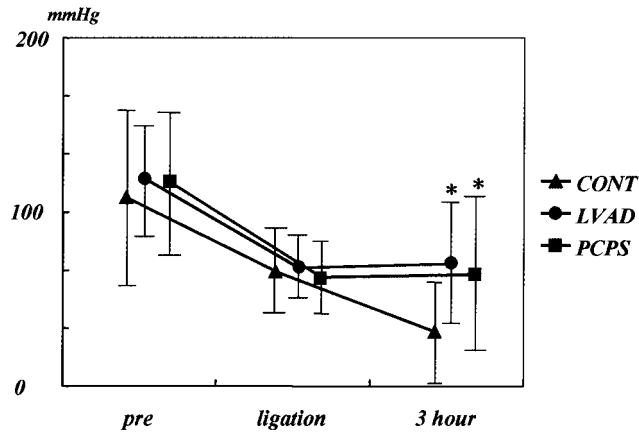


Fig. 2. Aortic pressure (systolic phase). Hemodynamic results.
* $p < 0.05$ versus the CONT group

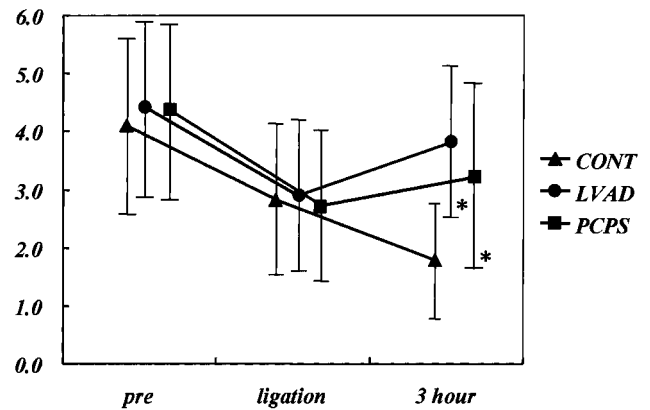


Fig. 3. End-systolic pressure volume relationship (ESPVR). Hemodynamic results.
* $p < 0.05$ versus the CONT group

after ligation and three hours after ligation.

TNF α

Blood samples for laboratory analysis were drawn from the coronary sinus before coronary ligation (baseline), just after ligation and three hours after ligation. The serum TNF α level was measured with enzyme-linked immunometric assay from Endrogen.

Histopathology

After excision of the heart, the heart was sliced perpendicular to the long axis. Sections were divided into the ischemic area, the border area and the normal area. Terminal deoxynucleotidyl transferase-mediated dUTP in situ nick end labeling (TUNEL) was performed in sections with a Colorimetric Apoptosis Detection System, (Promega, Tokyo, Japan). Slides were then counterstained using haematoxylin and were monitored by light microscopy. The assay was standardized using normal cardiomyocyte treated with DNaseI as a positive control of apoptosis. The results were counted as the mean of 30 fields, every 40 high power field (HPF). Sections were examined in an electron microscope (JEOL JEM-1200EX, Nihondenshi, Tokyo, Japan).

Statistical analysis

All data are expressed as mean \pm SD. The statistical significance of differences was tested by the analysis of variance (ANOVA) for repeated measures, followed by the post-hoc test to detect any overall time related changes during the study period. The differences between the groups were tested with Fisher's test. A p value of less than 0.05 was considered statistically significant.

Results

AoP

In all groups, AoP decreased after coronary ligation in comparison with baseline but the difference was not statistically significant. AoP in the LVAD group (77 ± 34 mmHg versus the CONT group, $p < 0.05$) and the PCPS group (69 ± 44 mmHg versus the CONT group, $p < 0.05$) improved in significance in comparison with the CONT group (34 ± 30 mmHg) after assisting. The difference was not statistically significant between the LVAD group and the PCPS group (77 ± 34 versus 69 ± 44 mmHg) (Fig. 2).

ESPVR

ESPVR in the CONT group decreased after ligation. It fell after ligation in the LVAD group, but improvement of ESPVR was noted after assisting. The PCPS group showed a similar result. ESPVR, which correlated with myocardial contractility in the LVAD group (3.83 ± 1.31 versus the CONT group, $p < 0.05$) and the PCPS group (3.34 ± 1.59 versus the CONT group, $p < 0.05$), was improved in comparison with the CONT group (1.82 ± 1.0) after mechanical support. No significant difference was seen between the LVAD and the PCPS groups, but the LVAD group tended to improve in comparison with the PCPS group (Fig. 3).

PVA

PVA in the CONT group decreased after ligation in comparison with baseline. PVA in the mechanical support groups decreased in the same way. The difference was

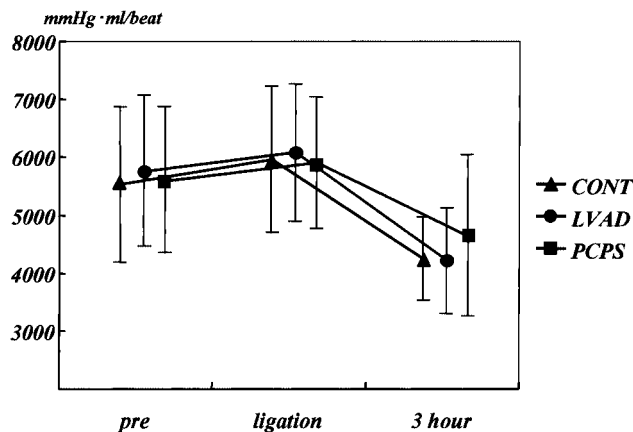


Fig. 4. Pressure volume area (PVA). Hemodynamic results.

not statistically significant among any of the groups (Fig. 4).

TNF α

Plasma concentrations of TNF α remained at the baseline just after coronary ligation but were elevated after three hours in all groups. TNF α in the LVAD group (562 ± 432 ng/L) showed a statistically lower value ($p < 0.05$) in comparison with the CONT group (880 ± 419 ng/L). No difference was seen between the LVAD and the PCPS groups (562 ± 432 versus 749 ± 398 ng/L), but TNF α in the LVAD group tended to be lower in comparison with the PCPS group (Fig. 5).

TUNEL at the light microscopy

TUNEL-positive cardiomyocyte nuclei were mainly observed in the ischemic area, but there were no TUNEL-positive cardiomyocytes in either the border area or the normal area in any of the groups (Fig. 6). TUNEL-positive cardiomyocytes with chromatin condensation were observed around the necrotic area in all groups.

TUNEL-positive cardiomyocytes

The number of TUNEL-positive cardiomyocytes in the microscopic field at a magnification of $\times 40$ was counted in the ischemic area. Thirty fields were examined in each slice and the counts were averaged. The number of TUNEL-positive cells in the mechanical support groups was significantly reduced in comparison with the CONT group: 10.3 ± 4.2 (/40 HPF) in the LVAD group ($p < 0.05$ versus the CONT group), 12.3 ± 2.9 (/40 HPF) in the PCPS group ($p < 0.05$ versus the CONT group), 30.6 ± 5.3 (/40 HPF) in the CONT group. The amount of apoptotic

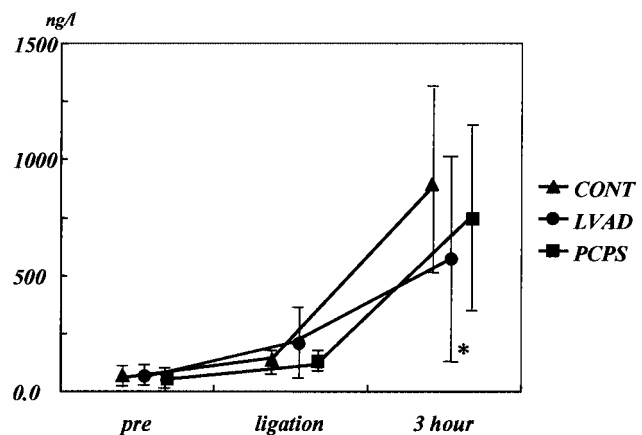


Fig. 5. Plasma concentrations of tumor necrosis factor-alpha (TNF α).

TNF α in the LVAD group showed a statistically lower value ($p < 0.05$) in comparison with the CONT group.

* $p < 0.05$ versus the CONT group

cardiomyocytes tended to be lower in the LVAD group when compared with the PCPS group, and so there was no significant difference between the LVAD and the PCPS (Fig. 7).

Electron microscopy

No typical apoptotic ultrastructure was observed at electron microscopy. A contraction band with high density, which suggested necrosis, was observed in the CONT group. Lipid infiltration in mitochondria and swollen mitochondria were noted in the LVAD group. Deposition of a woolly density in mitochondria was observed in the PCPS group. There were oncotic cardiomyocytes, which suggested damaged cells in the LVAD and the PCPS groups (Fig. 8).

Discussion

Various mechanical supports such as intraaortic balloon pumping (IABP), PCPS and VAD have been useful for end-stage heart failure and have improved the survival rate of heart failure. PCPS has become clinically widespread, such as in cases of emergency cardiopulmonary resuscitation, acute myocardial infarction, myocarditis, cardiomyopathy, postoperative LOS, and so on, because of its simplicity and low cost.^{6,7)} However, it is difficult to support for a longer duration with PCPS. The durability of the device and antithrombogenicity limit the chronic use of PCPS.⁹⁾ On the other hand, VAD is able to provide stronger support than PCPS and can assist long term.

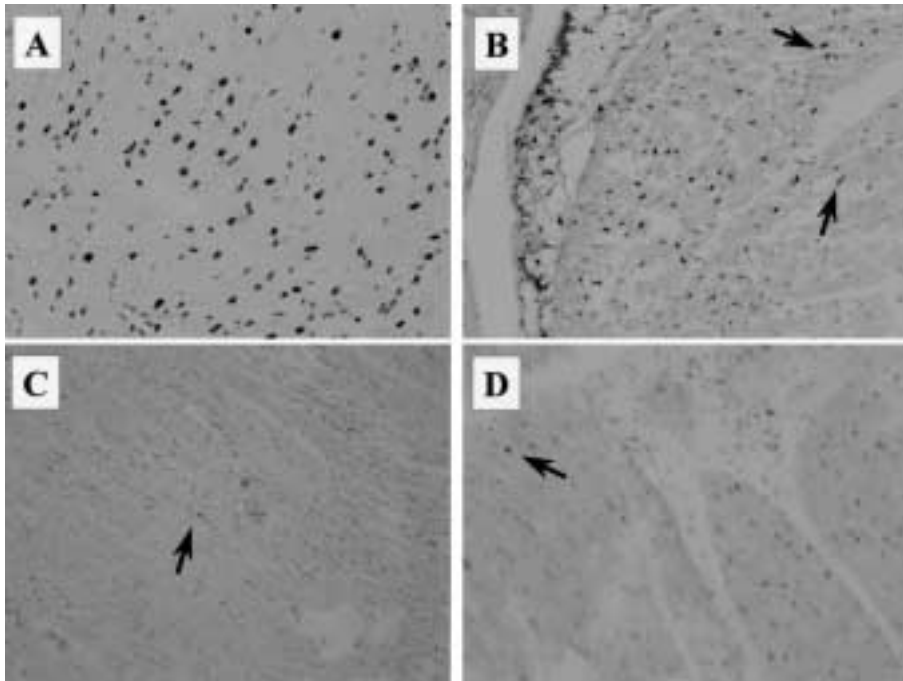


Fig. 6. TUNEL at microscopy. Terminal deoxynucleotidyl transferase-mediated dUTP in situ nick end labeling (TUNEL) was performed in sections. A: The assay was standardized using normal cardiomyocyte treated with DNaseI as a positive control of apoptosis. B-D: TUNEL-positive cardiomyocytes nuclei were mainly observed in the ischemic area. TUNEL-positive cardiomyocytes with chromatin condensation were seen around the necrotic area in all groups (arrows).

LVAD is available for not only support of a patient with acute heart failure due to various cardiogenic diseases, but also using circulatory assist devices as a temporary support until cardiac transplantation.¹⁻³⁾ In addition, a new application for LVAD is as a therapeutic ventricular assist device and as a bridge to recovery.^{4,5)} However, both the timing for initiating LVAD support and optimal LVAD weaning protocols have yet to be determined.

Features of apoptosis include nuclear changes accompanying a half-moon-like or horseshoe-like condensation of chromatin, cytoplasmic shrinkage, and apoptosis body. It has been tacitly believed that apoptosis does not occur in terminally differentiated adult heart muscle cells. In recent years, it has been reported that apoptosis participates widely in various heart diseases.¹⁰⁻¹³⁾ In this study, DNA fragmentation was observed in heart failure such as dilated and ischemic cardiomyopathy, and BCL-2, which protects cells against apoptosis, was twice as high in the hearts of patients with cardiac failure as in normal hearts.¹¹⁾ On the other hand, most reports are mainly based on DNA fragmentation, a biochemical hallmark of apoptosis, detected from the DNA ladder pattern on gel electrophoresis and/or TUNEL. The incidence of apoptotic myocytes detected by TUNEL is too high. The apoptotic process progresses rapidly, and continuous and massive myocyte loss without corresponding replication of myocytes instantly causes clinical crisis in patients. It has also been reported that so-called apoptotic myocytes

based on positive TUNEL in infarcted areas are ultra-structurally oncotic myocytes with DNA fragmentation, while TUNEL-positive myocytes in dilated cardiomyopathy are neither apoptotic nor necrotic, but are living cells with DNA repair.¹⁹⁾ Apoptosis is regarded as controllable cell death. If cardiomyocyte apoptosis is reduced, it is possible to treat for heart failure.

There have been few fundamental experiments that show that mechanical support with LVAD/PCPS influences cardiomyocyte apoptosis. The aim of this study was to examine cardiac function, cardiomyocyte apoptosis and TNF α in an experimental acute myocardial infarction model during mechanical support. In each group, TUNEL-positive cells with chromatin condensation were observed in the ischemic area, while there were no TUNEL-positive cardiomyocytes in either the border area or the normal area. TUNEL-positive cells were mainly observed around necrotic cells with a rupture of the plasma membrane. The number of TUNEL-positive cells in the mechanical support groups was significantly decreased in comparison with the CONT group. Based on these results, it is suggested that cardiomyocyte apoptosis is reduced by mechanical support. However, there was no apoptotic change such as condensation at electron microscopy. Lipid infiltration in the mitochondria, swollen mitochondria and deposition of a wooly density in the mitochondria were observed. These findings were oncotic features. The same TUNEL-positive cells were not seen

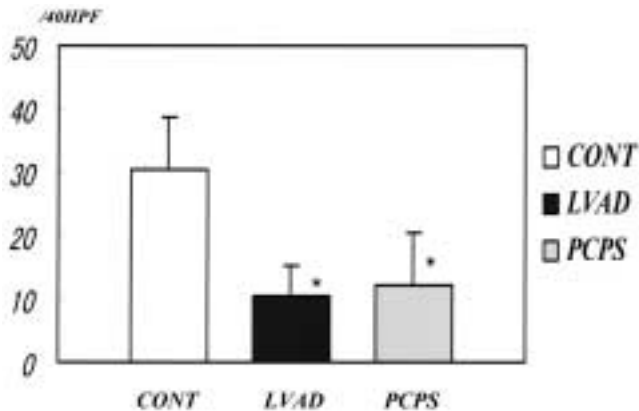


Fig. 7. TUNEL-positive cardiomyocytes.

The number of TUNEL-positive cells in the mechanical support groups was significantly reduced in comparison with the CONT group.

* $p < 0.05$ versus the CONT group

at electron microscopy in this study. Therefore, oncotic changes at electron microscopy may not be TUNEL-positive cardiomyocytes. As a result, TUNEL-positive cells may be apoptotic biochemically and oncotic in morphology. Biochemical hallmarks of apoptosis, such as DNA ladder pattern on gel electrophoresis and/or TUNEL, are generally used, but studies to detect ultrastructural features of apoptosis at electron microscopy and the examination of genes are necessary.

ESPVR, which correlates with myocardial contractility in the LVAD group and the PCPS group, was improved after mechanical support. Furthermore, PVA, which has a strong correlation with cardiac oxygen consumption, decreased after three hours in each group.^{20,21} ESPVR in the CONT group decreased due to cardiogenic shock, and then end-diastolic volume (EDV) increased while PVA decreased. After mechanical support ESPVR improved, and then EDV decreased with left ventricular (LV) unloading. As a result, PVA decreased. In the PCPS group, LV function improved due to volume unloading, but its effect was less than in the LVAD group because of an increasing afterload due to retrograde perfusion. LV cannulation has the advantage of a lower rate of thromboembolism and higher pump flow than LA cannulation.^{22,23} Therefore, LV cannulation is better for long-term circulatory support than LA cannulation and it is able to decompress LV volume. However, we decided on LA cannulation in order to evade physical cardiomyocyte injury due to LV drainage.

Several proinflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), TNF α , are ex-

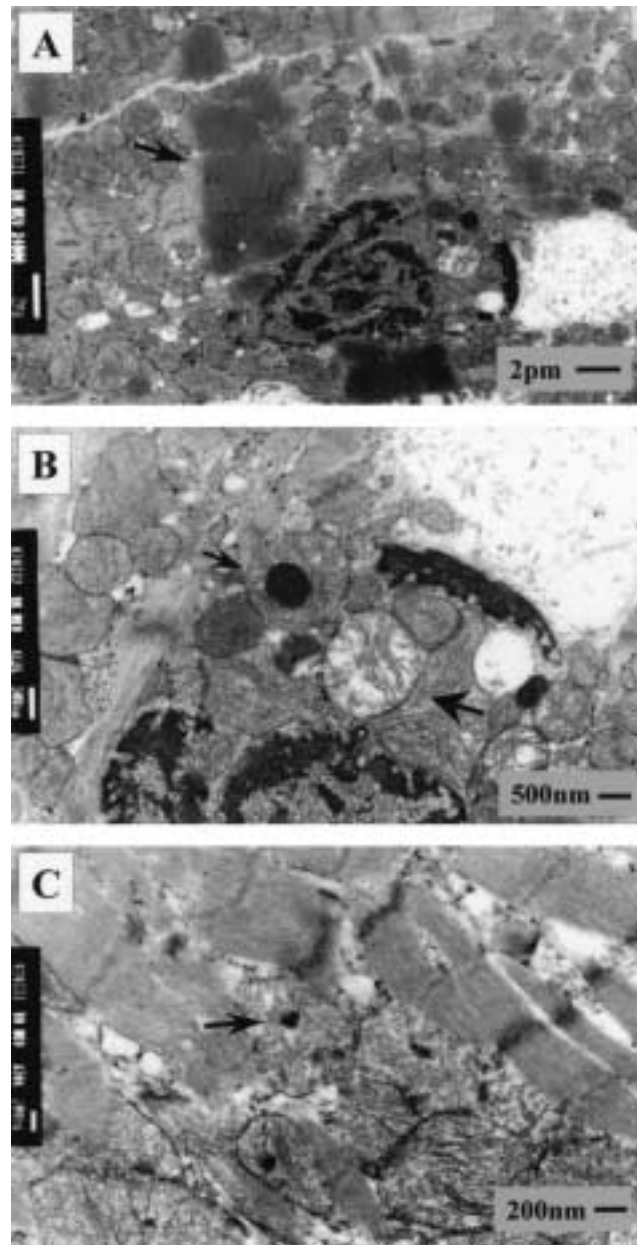


Fig. 8. Electron microscopy.

No typical apoptotic ultrastructure was observed. A: A contraction band with high density was seen in the CONT group (arrow). B: Lipid infiltration in mitochondria (small arrow) and swollen mitochondria (large arrow) were noted in the LVAD group. C: Deposition of woolly density in mitochondria (arrow) was observed in the PCPS group.

pressed within the myocardium in response to either mechanical overload or ischemic injury.¹³⁻¹⁶ Cytokines that have been reported to change during extracorporeal circulation are IL-1, interleukin-2 (IL-2), interleukin-4 (IL-4), IL-6, interleukin-8 (IL-8), interleukin-10 (IL-10), TNF α .²⁴⁻²⁷ With regard to IL-1, IL-6, a change is noted

after extracorporeal circulation at a comparatively early stage. IL-1 increased only once several hours after extracorporeal circulation, while IL-6 increased rapidly during extracorporeal circulation. TNF α did not change during extracorporeal circulation. TNF α increased gradually after 24 hours and reached the maximum level after one week.¹⁸⁾ Physical agents to assist circulation did not reduce TNF α . In addition, Torre et al. reported that chronic mechanical circulatory assistance decreases TNF α content in failing myocardium, and they also suggest that the magnitude of the change may predict which patients will recover cardiac function.²⁸⁾ TNF α is a protein with a molecular weight of 17.000 and is induced by activated macrophage and monocytes. TNF α is a proinflammatory cytokine that has been implicated in the pathogenesis of cardiovascular disease. In fact, cardiomyocytes have recently been shown to be an important source of TNF α production in conditions such as myocardial infarction and heart failure. TNF α inhibits myocardial contractility and induces apoptosis of cardiomyocytes. Proinflammatory cytokines are expressed in response to various forms of myocardial injury, such as acute viral myocarditis, myocardial infarction, myocardial reperfusion injury, heart failure, and hemodynamic overloading, and so on.²⁹⁻³⁴⁾

Cardiomyocyte apoptosis was reduced after mechanical support as the number of TUNEL-positive cells in the mechanical support groups were significantly decreased in comparison with the CONT group. If cardiomyocyte apoptosis was restrained by LVAD and PCPS, the trigger, which caused apoptosis, should have been restrained. In this study, we measured serum TNF α , which has little influence during assist circulation. TNF α in the LVAD group showed a significantly lower value in comparison with the CONT group. It is suggested that LV unloading due to mechanical support reduces TNF α .

Macrophage and monocytes are activated by the first attack of ischemia, and proinflammatory cytokines such as TNF α and IL-1 β are induced. Proinflammatory cytokines produce other inflammatory cytokines and cause activation of neutrophils, an organization disorder. Furthermore, it is suggested that overloading due to LV dysfunction induced TNF α in this study. TNF α in the LVAD group showed a statistically lower value in comparison with the CONT group, and fewer apoptotic cardiomyocytes were observed than in the CONT group. TNF α and apoptotic cells in the LVAD group tended to be lower in comparison with the PCPS group. It is suggested that apoptotic cells are decreased, so that LV un-

loading reduces TNF α in the LVAD.

The flow pattern of a blood pump in LVAD is divided into pulsatile flow and non-pulsatile flow. In this study, we used an LVAD of the non-pulsatile flow type with a centrifugal pump. At the position of cannulation, we decided on LA drainage to protect cardiomyocyte injury. This experiment was an acute study; we need to study LV cannulation, the pulsatile pump and the chronic stage in the future. Cytokines have various physiological activities and the network of cytokines is complicated, so a careful interpretation of TNF α is needed. Moreover, the examination of genes is required as the next step.

Conclusions

ESPVR, which correlates with myocardial contractility in the LVAD and PCPS groups, was improved after mechanical support. No significant difference was seen between the LVAD and the PCPS groups. Cardiomyocyte apoptosis was restrained by mechanical support. TNF α in the LVAD group showed a statistically lower value in comparison with the CONT group. This study suggests that mechanical ventricular assist devices (VADs) participate in proinflammatory cytokines, depression of apoptosis and, as a result, VADs are effective in recovery from myocardial injury in this study.

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