Cardioprotective Effects of Melatonin on Recovery of Rat Donor Hearts after 12-Hour Preservation

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Summary: The cardioprotective effects of melatonin on recovery of rat donor hearts after 12 h of preservation were investigated. Wistar rats weighing 200 to 250 g ($n=24$) were randomly divided into 3 groups. In the non-storage group ($n=8$), donor hearts were not stored. In the melatonin group ($n=8$), donor hearts were stored in 4 ℃ St. Thomas solution with melatonin (0. 1 mmol/L). In the control group ($n=8$), donor hearts were stored in 4 ℃ St. Thomas solution only. The coronary flow (CF), cardiac function, coronary vasodilatory response, creatine kinase (CK) and high energy phosphate levels were measured after the hearts had been preserved for 12 h. Transmission electron microscopy was used to examine the microstructural changes after 12 h of preservation. The recovery of cardiac function and coronary vasodilatory response were significantly improved in the melatonin group ($P<0.01$). CK release decreased greatly in the melatonin group ($P<0.01$). High energy phosphate levels were significantly better preserved in the melatonin group ($P<0.01$). Histological findings were much better in the melatonin group than in the control group. These results suggest that melatonin has cardioprotective effects on the recovery of rat donor hearts after 12 h of preservation.

Key words: melatonin; donor heart preservation; transplantation; ischemia-reperfusion

Heart transplantation is an accepted therapeutic modality for select patients with end-stage heart disease. However, the "safe" ischemic time for cardiac allografts in clinical practice is still limited to 4 to 6 h. This limitation greatly exacerbates the critical shortage of donor organs and precludes long-distance organ procurement and sharing. Furthermore, inadequate myocardial preservation continues to be a significant cause of early graft failure and recipient mortality[1].

The key to extending the safe ischemic time of cardiac allografts is the elucidation of the mechanisms underlying the etiology of myocardial ischemia-reperfusion injury. Recent studies have shown that the hydroxyl radicals has notable characteristics in taking part in ischemia-reperfusion injury[2].

Melatonin, the pineal secretory production, has recently been confirmed to be a potent scavenger of hydroxyl radicals[3]. In this study, we supplement melatonin in 4 ℃ St. Thomas solution to investigate the effects of melatonin on recovery of rat donor hearts after 12 h of preservation.

1 MATERIALS AND METHODS

1.1 Donor Heart Preservation and Grouping

Twenty-four male Wistar rats weighing 200 to 250 g were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and mechanically ventilated by means of a tracheostomy. In each rat, the heart was exposed by median sternotomy. The rat received systemic heparin (1000 U/Kg), and then the heart was arrested with an intravenous injection of 1. 5 to 3 ml of 4 ℃ St. Thomas solution. The heart was excised, immedi-

ately immersed in 4 ℃ Krebs-Henseleit buffer (KHB) solution, and quickly perfused from the aorta with 60 ml/kg of 4 ℃ St. Thomas solution, using less than 38 mmHg pressure continuously. 24 Wistar rats were randomly divided into three groups. In the non-storage group ($n=8$), donor hearts were not stored. The hearts in the melatonin group ($n=8$) and the control group($n=8$) were submersed in 40 ml of 4 ℃ St. Thomas solution with melatonin (0. 1 mmol/L) and 4 ℃ St. Thomas solution for 12 h, respectively.

1.2 Perfusion Technique

Each heart was mounted on a Langendorff apparatus by the aorta and perfused with KHB solution at a constant pressure of 60 mmHg for 15 min in the Langendorff model (L mode). KHB solution was filtered (40-μm-filter), equilibrated with 95 % O_2 and 5 % CO_2 and maintained at 37 ℃. Following cannulation of the left atrium through the auricle, the Langendorff apparatus was switched to the working model (W mode), with a left atrium perfusion pressure of 10 mmHg and an afterload of 60 mmHg for measurements of hemodynamic indices. These include aortic flow (AF), coronary flow (CF), systolic pressure (SP), left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure(LVEDP), and rate of left ventricular pressure rise or fall ($\pm$dp/dt). Aortic flow was recorded with an electromagnetic flowmeter. Left ventricular minute work (LVW), calculated as cardiac output $\times$ (systolic pressure— left atrial preload), served as an index of mechanical function. The LVDP, LVEDP and $\pm$dp/dt (in mmHg/s) were measured by puncturing the left ventricular apex with a pressure transducer (EQ-601G Type, Nihon Koden Inc. , Japan). The beats were paced in an atrial mode at 200 beats/min.

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1.3 Coronary Flow and Creatine Kinase

Coronary effluent was collected 5, 10, 20, and 40 min after reperfusion. Meanwhile, CF (in ml/min) was also measured. CK level (IU/L) in the coronary effluent was measured 15 and 30 min after reperfusion by DRI-CHEM 5500 (Fujifile Inc. , Japan).

1.4 Coronary Vasodilatory Response to Acetylcholine Chloride

The vascular dilatory function of endothelial cells was assessed by the increased percentage of CF in response to acetylcholine chloride (Ach) at 10^{-8} mol/L. The basal CF was measured for 1 min, after the hearts had been perfused with the KHB solution on L mode for 15 min. Then the hearts were perfused with KHB solution containing 10^{-8} mol/L of Ach for 3 min. The content of CF was measured 1, 2, and 3 min after the infusion of Ach. An increased percentage of CF after administration of Ach was calculated and expressed per minute using the following formula: increase of CF (%) = [CF (with Ach) − basal CF]/basal CF × 100

1.5 Myocardial High Energy Phosphate

Phosphocreatine (PCr), inorganic phosphate (Pi) and β-adenosine triphosphate (β-ATP) were measured using ^{31}P-nuclear magnetic resonance (NMR) spectroscopy (GS × 270W spectrometer. JEOL Inc. , Japan) immediately after excision of the heart, and 12 h after cold storage. β-ATP, PCr and Pi were calculated by integrating the peak are-as on the spectrum. These values were expressed as a percentage of control values that were obtained at the onset of cold storage. Intracellular high energy phosphate values were expressed as a ratio to Pi.

1.6 Transmission Electron Microscopy

After 12 h of preservation, the hearts were taken from the preservation solution for histological examination. Specimens were fixed in 4 % buffered formalin and were embedded in paraffin. Sections of 4 μm were stained with eosin and hematoxylin. Transmission electron microscopy was used to examine the microstructural changes.

1.7 Statistical Analysis

All data were analyzed statistically by SAS statistical software. $\bar{x}\pm s$ of each group was calculated. $P<0.05$ was regarded as the threshold of the significance of differences.

2 RESULTS

2.1 Recovery of Cardiac Function

Indices of cardiac function measured in W mode are summarized in table 1 and 2. Recovery of aortic flow (AF), CF, cardiac output (CO), systolic pressure (SP), and LVW in the melatonin group were significantly better than corresponding values in the control group ($P<0.01$). Indices of LVDP, LVEDP and ± dp/dt$_{max}$ in the melatonin group were significantly enhanced compared with the control group ($P<0.01$).

Table 1   Recovery of cardiac function ($n=8$ in each group)

Groups	AF (ml/min)	CF (ml/min)	CO (ml/min)	SP (mmHg)	LVW (L/min/mmHg)
Nonstorage	39.27±3.2*	11.02±0.9*	51.23±3.4*	91.15±8.4*	4.21±0.35*
Control	7.06±2.1	2.13±0.7	10.15±2.6	41.34±3.3	0.33±0.2
Melatonin	26.95±3.0*	9.04±0.8*	43.25±3.0*	80.15±7.6*	3.02±0.28*

$^{\triangle}P<0.05$, * $P<0.01$ as compared with control group

Table 2   Recovery of cardiac function ($n=8$ in each group)

Groups	LVDP (mmHg)	LVEDP (mmHg)	+dp/dt$_{max}$ (mmHg/s)	-dp/dt$_{max}$ (mmHg/s)
Nonstorage	105.67±5.3*	5.96±0.4*	2261.65±271.5*	1713.76±134.3*
Control	40.39±13.1	10.15±1.0	612.81±291.3	418.22±123.6
Melatonin	80.49±9.6*	5.92±0.9*	2034.23±161.5*	1129.30±126.8*

$^{\triangle}P<0.05$, * $P<0.01$ as compared with control group

2.2 Changes of Coronary Flow in L Mode

The CF in L mode was significantly higher in the melatonin group than in the control group ($P<0.01$, table 3).

Table 3   Coronary flow (ml/min) in L mode

Groups	After reperfusion			
	5 min	10 min	20 min	40 min
Control	10.2±1.3	6.3±1.4	4.4±1.1	2.3±0.8
Melatonin	16.7±1.9*	13.2±1.6*	11.5±1.4*	8.4±1.3*

$^{\triangle}P<0.05$, * $P<0.01$ as compared with control group

2.3 Coronary Vasodilatory Response to Acetylcholine Chloride

Administration of a vasodilatory agent resulted in a reproducible increase in CF without causing any significant changes in the systolic pressure. The vasodilatory response to ACh is shown in table 4. The increased percentage of CF in the melatonin group was significantly higher than in the control group ($P<0.01$).

2.4 Changes of Creatine Kinase Level

The CK levels in the coronary effluent in the melatonin group 15 min after reperfusion were significantly lower than those in the control group ($P<0.01$, table 5).

2.5 Myocardial High Energy Phosphate

Myocardial high energy phosphate levels, which were expressed as PCr/Pi and β-ATP/Pi, were significantly higher 12 h after storage in the melatonin group than those in the control group ($P<0.01$, table 6).

2.6 Histological Findings

In transmission electron microscopic findings,

degeneration and swelling of the mitochondria were obvious in the control group. Loss of normal dense granules and acute clearing matrix of the mitochondria were observed in the control group. However, in the melatonin group, only slight clearing matrix of the mitochondria was observed and glycogen granules were well-preserved (fig. 1—2).

Table 4   Increased percentage of CF (%)

Groups	After reperfusion of Ach		
	1 min	2 min	3 min
Control	2.5±0.4	2.1±0.4	1.5±0.4
Melatonin	10.1±1.3*	12.4±1.5*	9.6±1.1*

△ $P<0.05$, * $P<0.01$ as compared with control group

Table 5   Changes of CK (IU/L)

Groups	After reperfusion	
	15 min	30 min
Control	700.4±21.5	300.3±23.1
Melatonin	300.9±17.3*	100.7±11.5*

△$P<0.05$, * $P<0.01$ as compared with control group

Table 6   Changes of PCr/Pi and β-ATP/Pi (%) #

Groups	PCr/Pi		β-ATP/Pi	
	0 min storage	12 h storage	0 min storage	12 h storage
Control	100%	1.7±0.4%	100%	20±3.5%
Melatonin	100%	20±3.6%*	100%	60±0.4%*

PCr/Pi and β-ATP/Pi values are expressed as the percentage of baseline values which were obtained immediately after harvesting of hearts
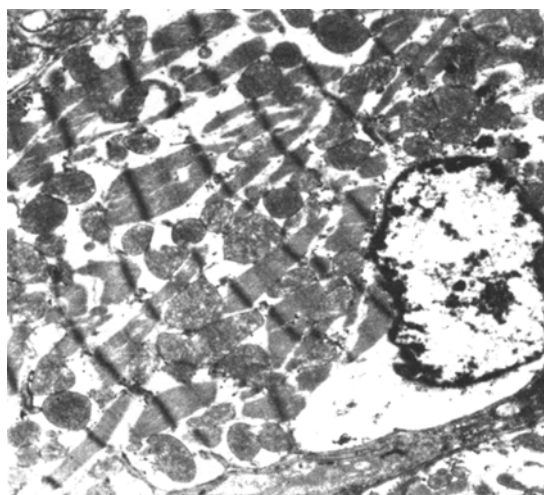△$P<0.05$, * $P<0.01$ as compared with control group

Fig. 1   Degeneration and swelling of the mitochondria were obvious. Granules lose normal density and the mitochondria's matrix clear acutely.
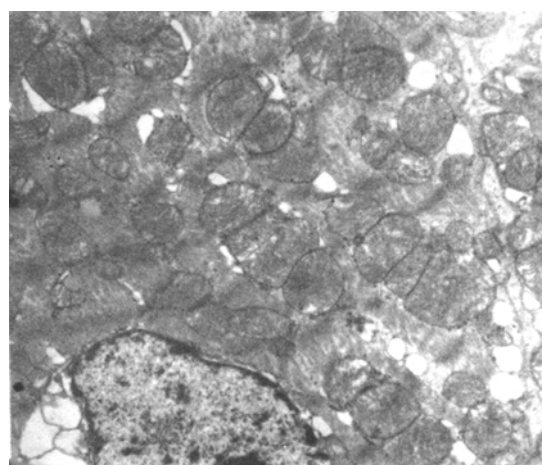

Fig. 2   Glycogen granules were preserved well. Slight clearing matrix of the mitochondria could be found.

3   DISCUSSION

This study demonstrated that when melatonin was added to heart preservation solution, the recovery of cardiac function, coronary vasodilatory response, myocardial high energy phosphate and histological findings were much better than those in the control group after 12 h of hypothermic ischemia. These results suggest that the administration of melatonin as an additive to the heart preservation solution may be a useful therapeutic maneuver.

Recent studies have proposed the essential role of reactive oxygen species (ROS) in the pathogene-

sis of myocardial ischemia-reperfusion injury[4]. ROS may result in celluar defects including a depression in the sarcolemmal (SL) Ca^{2+}-pump ATPase and Na^+-K^+ ATPase activites which lead to decreased Ca^{2+}-efflux and increased Ca^{2+}-influx, respectively. The depression in Ca^{2+}-regulatory mechanism by ROS ultimately results in intracellular Ca^{2+} overload and cell death.

The hydroxyl radical, one of ROS, is potently cytotoxic and has been shown to interact with proteins, phospholipids, nucleic acids, and sugars to produce irreversible damage[5]. It displays much greater chemical reactivity than superoxide radical or hydrogen peroxide and plays a significant role in post-ischemic reperfusion injury[4].

Melatonin, an indoleamide that the pineal gland secretes, has recently been confirmed to be a potent scavenger of hydroxyl and peroxyl radicals. Melatonin works via electron donation to directly detoxify highly toxic hydroxyl radicals with no enzymatical reaction[3].

The results of this study suggested that melatonin supplement in heart preservation solution can significantly improve the cardiac function. The possible mechanisms responsible for the effect may include: (a) melatonin is a potent hydroxyl radical scavenger; (b) melatonin can have easy access to the inside of myocardial cells thereby protecting DNA, protein and lipid from oxidative damage[5]; (c) melatonin can increase glutathione peroxidase activity[6]; (d) melatonin reduces iutracellular Ca^{2+} by inhibiting both extracellular Ca^{2+} entry and Ca^{2+} release from intracellular stores[7].

In conclusion, melatonin supplement in heart preservation solution showed significant protective effects against post-ischemic myocardial dysfunction and reperfusion injury.

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