# Heat shock protein 70 induced by heat stress protects heterotopically transplanted hearts in rats

LI-WEI DIAO[1], LIN-LIN ZHAO[2], FENG QI[1], ZHEN-DONG SUN[1], QING-HUA ZHANG[1] and NAI-SHI WU[1]

[1]Department of Surgery, Division of Cardiac Surgery, The 2nd Affiliated Hospital of Harbin Medical University, Harbin 150086; [2]Division of Cardiac Surgery, The People's Hospital of Liaoning Province, Shenyang 110016, P.R. China

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**Abstract.** Heat shock protein 70 (HSP70) protects cardiac function against ischemia-reperfusion injury through gene transfection, although it is not a clinically practical and economical method. This study investigated whether heat stress-induced HSP70 protects heterotopically transplanted donor hearts. A total of 60 donor rats were randomly divided into 6 groups. Five of those received heat stress and one was a control group. Donor hearts were heterotopically transplanted into recipient rats at five time points, following the heat stress (0, 24, 48, 96 and 192 h). The levels of HSP70 expression in donor hearts and the variation of myocardial enzymes in receptor blood or donor hearts were measured 24 h after transplantation. The donated hearts were also examined under a microscope for pathological changes. HSP70 expression was the highest in the 24-h group ($p \leq 0.01$) and decreased gradually in the 48- and 96-h groups. No statistically significant difference was found in the HSP70 expression in the control, the 0- and 192-h groups ($p \geq 0.05$). Of all the groups, the 24-h group had the lowest lactate dehydrogenase and creatine kinase muscle band concentrations in receptor blood. Moreover, this group showed the lowest malondialdehyde concentration and the highest atriphosphate concentration ($p \leq 0.01$), demonstrated by the mildest inflammatory injury in the transplanted hearts. We found a time-dose-effect relationship among heat stress, HSP70 and the protection of donor hearts. Heat stress is a practical method that can be clinically applied to protect donor hearts against ischemia-reperfusion injury by inducing endogenous HSP70, which indicates the future direction of clinical practice.

*Correspondence to:* Dr Nai-Shi Wu, Department of Surgery, Division of Cardiac Surgery, The 2nd Affiliated Hospital of Harbin Medical University, 148 Baojian Rd, Harbin 150086, P.R. China
E-mail: doctor_dlw@126.com

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## Introduction

Successful heart preservation is essential for clinical heart transplantation. Previous studies have shown that heat shock protein 70 (HSP70) induced by gene transfection can protect the mechanical and endothelial function of the donor heart against ischemia-reperfusion injury (1,2). However, gene transfection is a relatively complex method for clinical practice, especially in developing countries. Therefore, finding a practical and cost-effective method to using HSP70 to protect donor hearts is crucial.

Studies have shown that HSP70 can be induced by heat stress (3). Heat stress is much easier to achieve clinically than gene transfection. Therefore, we investigated whether heat stress-induced HSP70 protects donor hearts by measuring the enzyme levels in recipients' blood and examining the transplanted hearts for pathological changes.

## Materials and methods

*Animals*. Sixty male Wistar rats (weight, 200-250 g) were purchased from the Experimental Animal Center of the 2nd Affiliated Hospital of Harbin Medical University and cared for in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

*Heat stress procedure and animal grouping*. The donor rats were subjected to hyperthermia in an incubator for 15 min, while the rectal temperature (at 42±0.5˚C) was monitored. Subsequently, the animals were transferred to a room temperature (24˚C) environment and were randomly divided into the following groups (n=10 per group): control group (donor hearts transplanted without heat stress) and experimental groups (0-, 24-, 48-, 96- and 192-h groups), according to the time points of examination after heat stress.

*Heterotopic heart transplantation:*. Heterotopic abdominal heart transplantation was performed using improved microsurgical techniques (4). In brief, rats were anesthetized by intraperitoneal administration of ketamine (75 mg/kg), and dexmedetomidine (0.25 mg/kg). The donor hearts were transplanted into the recipients by end-to-side anastomoses

of the aorta and the pulmonary artery to the abdominal aorta and inferior vena cava, respectively, using 8-0 monofilament sutures. During surgery the heart was wrapped in gauze and kept cold by the use of topical ice-cold saline solution. After the operation, the rats recovered with oxygen in a warm environment. Viability of the grafts was verified by palpation of the beating transplanted heart.

*Specimen collection*. The donor hearts and receptor blood were collected 24 h after transplantation.

*Detection of HSP70*. HSP70 levels of donor hearts were measured by using the enzyme-linked immunosorbent assay (ELISA) Kit of R&D Systems (DYC1663E, Minneapolis, MN, USA). The optical density was measured at λ=490 nm (reference at λ=620 nm). The detection range of the assay was 0.05-2000 ng/ml, the intra/inter-assay variability <10/<16%, respectively.

*Detection of lactate dehydrogenase (LDH) and creatine kinase muscle band (CK-MB)*. Receptor blood samples with heparin were centrifuged to obtain a serum to detect the leakage of LDH and CK-MB using enzyme kits (Human UV test, Hamburg, Germany).

*Detection of adenosine atriphosphate (ATP) and malondialdehyde (MDA)*. Two separate samples from each donor heart were removed for myocardial ATP and MDA determinations. The tissue ATP content was determined using an enzymatic spectrophotometric kit (Sigma, St. Louis, MO, USA) after the tissue was homogenized in perchloric acid (60% w/v) and $K_2CO_3$ solution was added for neutralization. In this way, we determined the decrease in absorbency at 340 nm resulting from the oxidization of NADH to NAD. Tissue ATP levels were expressed as mmol/g wet tissue. Tissue homogenates were prepared for MDA analyses in a ratio of 1 g wet tissue to 9 mmol KCl using Heidolph RZR 2021 equipment. The MDA content in the homogenate was determined by using a calorimetric reaction with thiobarbituric acid as described by Buege and Aust (5). The tissue MDA levels were expressed as nmol/g wet tissue.

*Statistical analysis*. Values were presented as the mean ± SEM. Statistical comparison was carried out by variance analysis and an unpaired Student's t-test, using the statistical packages SPSS 18.0 and R 2.10.1. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Detection of HSP70 expression level in donor hearts by ELISA*. HSP70 expression was the highest in the 24-h group ($p \leq 0.01$) and decreased gradually in the 48- and 96-h groups. There was no statistically significant difference in HSP70 expression in the control, 0- and 192-h groups ($p \geq 0.05$; Table I).

Table I. HSP70 expression level in donor heart.

| Groups (n=10) | HSP70 expression (ng/ml) |
|---|---|
| Control | 36.18±12.15 |
| 0 h | 38.31±13.11 |
| 24 h | 116.86±16.67[b] |
| 48 h | 90.11±12.56[b] |
| 96 h | 52.02±11.60[a] |
| 192 h | 41.26±10.11 |

[a]Compared with the control group, $p \leq 0.05$; [b]compared with the control group, $p \leq 0.01$

*Cardiac enzyme concentration in receptor blood and donor heart tissue*. The 24-h group, which had the highest HSP70 expression, had the lowest LDH and CK-MB concentrations in receptor blood of all the groups. This group also showed the lowest MDA concentrations and the highest ATP concentrations in the transplanted hearts ($p \leq 0.01$). There was no statistically significant difference in the concentrations of cardiac enzymes in the control, 0- and 192-h groups ($p \geq 0.05$; Table II).

*Pathological changes in donor hearts*. The cardiac tissue of donor hearts in the control group showed sarcomere contraction, muscle fiber edema, myocardial necrosis and inflammatory cell infiltration. The 24- and 48-h groups, which had a higher HSP70 expression, had significantly better pathological outcomes (Fig. 1).

## Discussion

Heart transplantation has become an established treatment for patients with end-stage heart failure. However, current methods of donor heart preservation are limited by the detrimental effects of ischemia-reperfusion injury. Previous studies have shown that HSP70 gene transfection protects the mitochondrial and ventricular function from ischemia-reperfusion injury (2). Similarly, recent reports demonstrate that the myocardial expression of HSP70i protects early postoperative right ventricular function in cyanotic tetralogy of Fallot (6). Moreover, the leukocyte expression of HSP70 enhances the inflammatory response in coronary artery bypass-grafting patients (7).

Heat stress induces the production of HSP70 (8), which may improve heart recovery from injury and protect the heart from ischemia (9). In the present study, we found a significant increase in the HSP70 expression in donor hearts subsequent to heat stress. Recipients of hearts from animals that had undergone heat stress had lower blood levels of cardiac enzymes than the recipients of hearts from controls. Similarly, hearts transplanted from the treated animals showed milder pathological changes compared to hearts from the controls. A previous study has also demonstrated that HSP70 contributes to the late-phase protection of ischemic pre-conditioning in ischemic hearts (10). However, in that study, hearts were studied at a fixed time point subsequent to HSP70 induction, whereas our results were observed at successive time points after heat stress, which demonstrated, for what appears to be the first time, the HSP70 time-dose change *in vivo*.

Table II. Myocardial enzyme concentrations in receptor blood (LDH and CK-MB) and donor heart (ATP and MDA) after transplantation in each group.

| Groups | LDH (U/l) | CK-MB (U/l) | ATP (mmol/g) | MDA (nmol/g) |
|---|---|---|---|---|
| Control | 89.68±6.07 | 158.62±12.35 | 4.68±0.85 | 12.86±0.41 |
| 0 h | 86.32±5.72 | 148.96±11.78 | 4.82±0.93 | 12.79±0.37 |
| 24 h | 39.17±2.49 | 60.27±4.96 | 12.78±1.87 | 7.16±0.19 |
| 48 h | 51.97±2.56 | 98.52±4.21 | 9.09±2.14 | 9.14±0.17 |
| 96 h | 76.08±4.83 | 127.83±9.76 | 5.79±1.24 | 11.37±0.27 |
| 192 h | 87.54±5.87 | 152.78±10.65 | 4.57±0.75 | 12.76±0.34 |

The 24-h group had significantly lower enzyme levels than the other groups ($p \leq 0.01$).

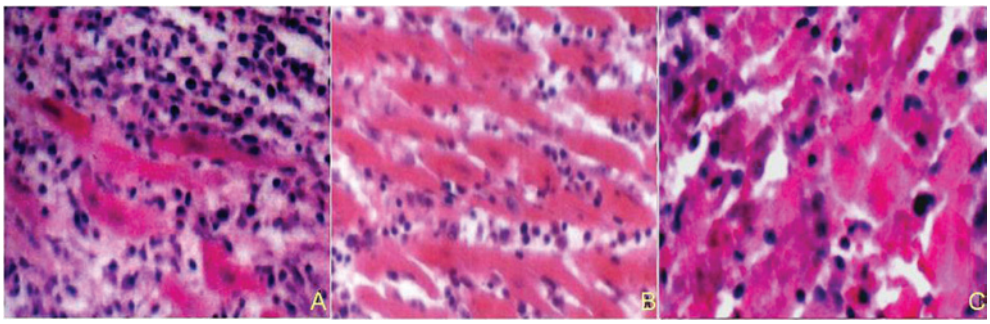


Figure 1. Myocardial tissue in the (A) control, (B) 24-h and (C) 48-h groups.

The important role of HSP70 in protecting hearts from ischemia-reperfusion injury has been clearly proven by experiments with gene transfection (1,2). In these experiments, *ex vivo* donor rat hearts with HSP70 gene transfection demonstrated a better mechanical and endothelial function after a period of ischemia and reperfusion. However, the *in vivo* performance of donor hearts with HSP70 overexpression against ischemia-reperfusion injury has yet to be adequately assessed. Gene transfection protocols are relatively complex regarding clinical practice, whereas our protocol used heat stress as an easier and more economical induction method.

To study the relationship of heat stress, HSP70 and their protective effects on donor hearts *in vivo*, we constructed the transplantation model at different time points after donor rats received heat stress. Using this method, we observed a time-dose relationship between heat shock and HSP70, and compared the dose-effect relationship with HSP70 and markers of cardiac injury, such as the cardiac enzymes in receptor blood and donor heart and the pathological changes of cardiac tissue.

Our results indicate that heat stress may have beneficial effects on the donor heart despite inducing endogenous HSP70, and these effects are time-dependent. The peak of the HSP70 concentration in the donor heart occurred at 24 h after heat stress, and hearts transplanted during this time period showed the lowest cardiac enzyme levels both in the recipient blood and the donated heart, as well as the slightest pathological changes in donor heart tissue. This finding is consistent with that of previous studies indicating that heat stress leads to HSP70 induction in the heart (11) and that HSP70 protects the heart during cardiac surgery (12). Furthermore, our finding that HSP70 expression peaks at 24 h after heat stress may be clinically useful.

Advantages of the present study include the use of heat stress as a practical and cost-effective method to induce endogenous protective ability, and the use of the time-dose-effect relationship among heat stress, HSP70 and donor heart protection. Limitations of the present study include the use of rats as study subjects and the use of an incubator to produce heat stress. The extent to which the findings are applicable in humans is unclear at present. Furthermore, the use of an incubator is a relatively inefficient and uncomfortable way to produce heat stress. Alternatives that might be worth investigating include lasers, ultrasound and microwaves.

Rectal temperature at 42˚C during a 30-min heat stress was required in this study. Endogenous HSP70 expression kinetics is the basis of this design. This time period allows for an optimal level of HSP70 expression between 24 and 48 h after heat stress (the stress-induced rise in HSP70 levels returns to the pre-stress level by 192 h) (13). Collection of the donor hearts one week after transplantation eliminated the tissue injury effect from surgery, which may increase the cardiac enzyme level. Furthermore, one week is sufficient for cardiac pathological changes to develop. Thus, the present study provided an *in vivo* model for investigating heat stress in cardiac protection. Advances in heat stress may allow a more practical and cost-effective induction of HSP70 expression, particularly when the method of heat stress may be achieved by

higher thermal input rates. Notably, patients with high initial myocardial levels of endogenous HSP70 had lower ischemia-reperfusion injury during cardiac surgery, thus HSP70 may have clinical application for myocardial protection (8,12,14).

In conclusion, we found that the induction of HSP70 in donor hearts through heat stress resulted in decreased enzyme level alterations and improved preservation of myocardial cells subsequent to heart transplantation. Our findings suggest that heat stress is a promising approach towards myocardial protection in clinical transplantation.

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