

Expression of Heat Shock Proteins 25, 60, 70 and 90 in Rat Myocardium Following Transmyocardial Laser Revascularization

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Abstract: Background: Transmyocardial laser revascularization has been shown to relieve symptomatic ischemia but laser tissue effects have potential complications. In order to define the mechanism of laser action, heat shock protein (hsp) expression was evaluated in rat hearts after Transmyocardial laser revascularization. **Methods:** Under general anesthesia, hearts were removed from 10 rats and immediately placed in oxygenated physiologic buffered solution (PBS) at 0°C. After the various treatments, hearts were homogenized and hsp25, 60, 70 and 90 were measured with Western Blotting. Group 1 (n=3) hearts were immediately homogenized; Group 2 (n=3) hearts were perfused with the PBS in a Langendorff setup for 6 h; Group 3 (n=3) hearts were lased (50 channels) using a Ho:Yag laser via a 600 m core fiber at 3 Hz and 280 mJ/pulse and perfused up to 6 h. Group 4 (n=1) rat was heated to 42°C for 15 min then recovered at 23°C for 6h prior to hsp measurement. **Results:** There was a significantly lower hsp70 expression in Group 3 and higher in Group 4 than that the control Groups 1, 2. Hsp25 and 60 were expressed in the 4 groups and there was no significant difference among the groups. There was no expression of hsp90 in any of the 4 groups. **Conclusion and Discussions:** In isolated rat hearts under stress, lasing lowered the expression of hsp70. This could be related to laser inhibition of hsp70 expression or enhancement of its degradation. Transmyocardial laser revascularization may protect myocardial cells from stress related expression of hsp. [Researcher. 2009;1(1):86-89]. (ISSN: 1553-9865).

Keywords: heat shock protein (hsp); ischemia; laser; transmyocardial; revascularization;

Abbreviations: hsp, heat shock protein; PBS, physiologic buffered solution; TMLR, transmyocardial laser revascularization

Introduction

Heat shock proteins (hsps), also called stress proteins, are a group of proteins that are present in all cells of all life forms. They are induced when a cell undergoes various types of environmental stresses like heat, cold, chemical, electricity, and hurt, etc (Katschinski, 2004). Hsps exist in cells under normal conditions, so called molecular chaperones, to help the cell's proteins folding/keeping in the right shape and place at the right time, which is essential for the proteins' function (Mogk, et al, 2004). They shuttle proteins from one compartment to another inside the cell (Welch, 1993). Hsps are also believed to play a role in the presentation of pieces of proteins (or peptides) on the cell surface to help the immune system recognize diseased cells (Papp, et al. 2003; Falkowska-Podstawka, et al. 2003). The literatures provided convincing proofs that cardiomyocytes that are subjected to hyperthermia or many other stress factors are reacting increased synthesis of hsp what guaranteed protection against further, stronger episodes of different stresses (Latchman, 2001).

Transmyocardial laser revascularization (TMLR) has been shown to relieve symptomatic ischemia but laser tissue effects have potential complications. Laser irradiation caused a significant influence in the content of inducible hsps (Yaakobi, et al, 2001). In order to define the mechanism of laser action, the expression of hsps25, 60, 70, 90 was evaluated in rat hearts after TMLR.

Material and Methods

Under general anesthesia, hearts were removed from 10 rats and immediately placed in oxygenated physiologic buffered solution (PBS) at 0°C. After the various treatments, hearts were homogenized and hsps25, 60, 70 and 90 were measured with Western Blotting. Group 1 (n=3) hearts were immediately homogenized for hsp measurements as the control-control; Group 2 (n=3) hearts were perfused with the

PBS in a Langendorff setup (Figure 1) at 34°C for 6 hours then homogenized for hsp measurements as the control (Neely, et al, 1975); Group 3 (n=3) hearts were lased 50 channels each blast using a Ho:Yag laser of 2100 nm via a 600 μ m core fiber at 3 Hz and 280 mJ/pulse through the full thickness of the myocardium after 1 hour perfusion then performed as Group 2 as the laser group; Group 4 (n=1) rat was heated to 42°C for 15 min then recovered at 23°C for 6 hours then the heart was removed and hsp were measured as above.

Western Blotting method: Heart tissue was collected after Langendorff perfusion and homogenized in 3 volume of extract buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 0.1 mg/ml phenylmethylsulfonyl fluoride, 0.001 mg/ml aprotinin, 1% Nonidet P-40) under iced condition. The homogenized sample was centrifuged at 10,000 rpm for 10 min at 4°C and the supernatant was collected for hsp measurement. Hsps were detected by Western Blotting method with monoclonal antibodies (hsps25, 60 and 90 antibodies were obtained from Sigma, St. Louis, MO, USA; hsp70 antibody was from StressGen Biotechnologies Corp, Victoria, BC, Canada). Secondary antibody was measured by alkaline phosphatase method.

Results

Hsps25 and 60 were expressed in all 4 groups and there was no significant difference among the groups. There was a significant higher expression of hsp70 in group 4 than that of other groups and lower in group 3 than that of groups 1 and 2. There was no expression of hsp90 in any of the 4 groups. Lasing 50 channels/heart decreased hsp70 by 78% (0.87±0.10 vs. 0.19±0.03; p<0.01) (Table 1, Table 2, Figure 2).

Table 1. Heat Shock Protein 70 in Rat Hearts

	Group 1 *	Group 2 *	Group 3 **	Group 4
Relative OD _{600nm}	1	0.87±0.10	0.19±0.03	3.47

* to *: p=ns; * to **: p<0.003

Table 2. Heat Shock Proteins 25, 60, 90 in Rat Hearts

	Group 1	Group 2	Group 3	Group 4
Hsp25	++	++	++	++
Hsp60	++	++	++	++
Hsp90	-	-	-	-

p=ns among groups.

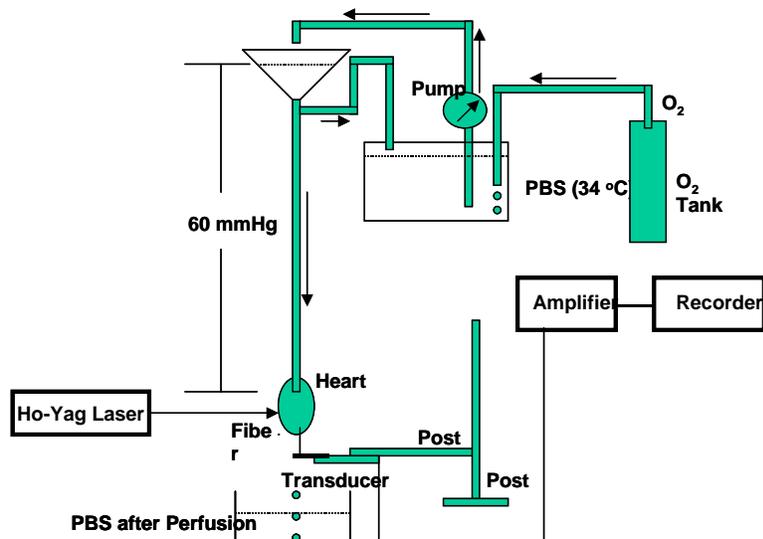


Figure 1. Langendorff Setup. Rat hearts were perfused with PBS at 34°C and lased 50 channels using a Ho:Yag laser of 2100 nm via a 600 nm core fiber at 3 Hz and 280 mJ/pulse through the full thickness of the myocardium after 1 hr perfusion.

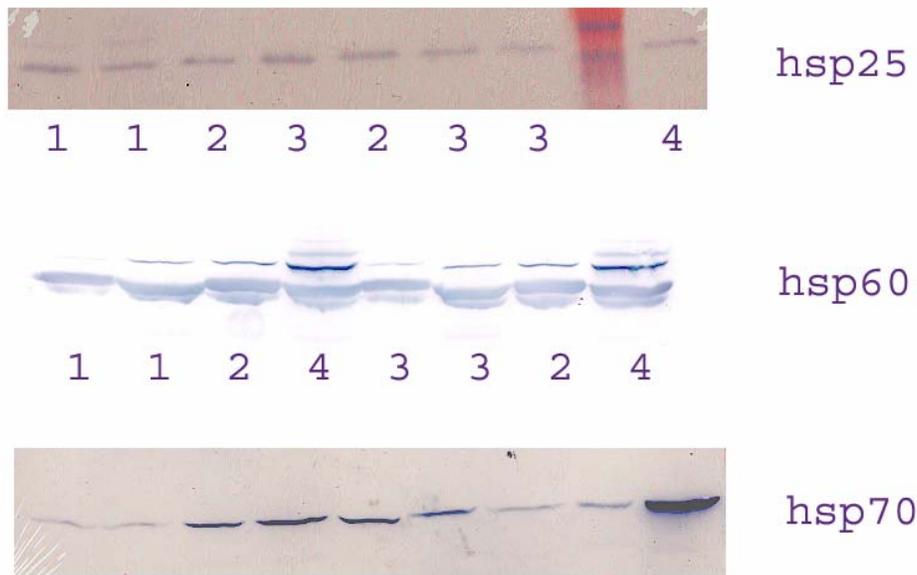


Figure 2. Heat shock proteins expressed in rat hearts measured by Western Blotting. 1: Group 1 (Control control); 2: Group 2 (Control); 3: Group 3 (Laser); 4: Group 4 (Heat 42°C).

Discussions

Hsp synthesis arises transiently as a tool to protect cellular homeostasis after exposure to heat and a wide spectrum of stressful and potentially deleterious stimuli (Delogu, et al, 2002). Isolated heart is under the severe adverse circumstances and the hsp expression will increase under the adverse condition. In this study we showed that lasing lowered the expression of hsp70. This is possibly a result of laser protect cells from injury by isolated condition. This result could be related to laser inhibition of hsp70 expression or enhancement of its degradation. The isolated rat hearts were under stress and TMLR may protect myocardial cells from stress related expression of hsp. Furthermore, lasing made holes in the rat hearts that could increase the contact of heart cells with solution, that improved the heart physical condition.

According to Garrido's describes, hsp70 is expressed in response to a wide variety of physiological and environmental insults including anticancer chemotherapy, thus allowing the cell to survive to lethal conditions. Several mechanisms account for the cytoprotective effect of hsp70. Hsp70 is a powerful chaperones and it inhibits key effectors of the apoptotic machinery at the pre and post-mitochondrial level, and it participates in the proteasome-mediated degradation of proteins under stress conditions, thereby contributing to the so called "protein triage". In cancer cells, the expression of hsp70 is abnormally high and it may participate in oncogenesis and in resistance to chemotherapy. In rodent models, Hsp70 over-expression increases tumor growth and metastatic potential. The depletion or inhibition of hsp70 frequently reduces the size of the tumors and even can cause its complete involution. Therefore, the inhibition of hsp70 has become a novel strategy of cancer therapy (Garrido, 2006). Our results showed that there was a significant higher expression of hsp70 in group 4 than that of other groups and lower in group 3 than that of groups 1 and 2. In isolated rat hearts under stress, lasing lowered the expression of hsp70. This could be related to laser inhibition of hsp70 expression or enhancement of its degradation. Transmyocardial laser revascularization may protect myocardial cells from stress related expression of hsp.

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