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Original Research Article

Effect of Resuscitation Fluids on the Expression of hsp90α in Cardiac Muscles of Hemorrhagic Shocked Rats

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Abstract

Purpose: *To investigate the protective effects of various resuscitating fluids on severe hemorrhagic shocked (HS) rats by comparing the expression changes of hsp90α in cardiac muscles and survival of rats.*
Methods: *Western-blot and immunohistochemistry methods were performed to determine hsp90α expressions in cardiac muscles of HS rats following treatment with different resuscitation fluids. In addition, the survival rates of of all the test groups were investigated.*
Results: *The expression of hsp90α decreased following treatment with resuscitation fluids based on both Western blot and immunohistochemistry data. Ringer lactate solution (RLS) was the most effective therapeutic fluid in this regard, and this was buttressed by survival rate data (90 %).*
Conclusion: *The expression of hsp90α in rat cardiac muscle is decreased to various degrees by treatment with different resuscitating fluids, with the effect most pronounced for ringer lactate solution. Furthermore, hsp90α plays an important role in hemorrhagic rat cardiac muscle as a regulatory factor.*

Keywords: *Resuscitating fluids, Hemorrhagic shock, Heat shock protein 90a, hsp90a*

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INTRODUCTION

Fluid resuscitation is one of the most important methods for early treatment of shock, and effective fluid resuscitation may increase the long-term survival of patients after hemorrhagic shock (HS) [1-4]. Currently, the fluids used for resuscitation mainly include crystalloids and colloids [1]; the common form of crystalloids is the ringer lactate solution, and blood plasma and the blood substitute is the main form of colloids; in addition, hypertonic sodium chloride dextran was reported to have good treatment effects for recovery of the blood volume after HS [5,6].

Secondary multiple organ failure (MOF) may be induced after severe HS. Heart is one of the target organs susceptible to damage by HS, and the change in heart function can affect the haemodynamics and functions of other vital organs, leading to irreversible damage. Therefore, how to protect cardiac function in HS or alleviat dysfunction of cardiac function caused by HS is a serious problem.

The molecular chaperone, heat shock protein (hsp90), is a universally expressed and abundant

molecular chaperone, and mammalian hsp90α and hsp90β are two major cellular proteins of the hsp90 subfamily and are highly homologous (86%) to each other [7-9]. In our previous study [10], we found that hsp90α can indirectly participate in the protection of myocardial tissue together with other molecules (including IF-1α and HIF-1α). Therefore, the present investigation is designed to evaluate the protective effects of some resuscitation fluids on myocardial tissue of HS rats by comparing the expression changes of hsp90α in cardiac muscles and survival of the rats.

Ringer lactate solution (RLS) and hypertonic sodium chloride dextran (HSD) were purchased from Sichuan Kelun Pharmaceutical Co. LTD. (Sichuan, China); Polygeline (PG) was obtained from Wuhan Hualong Bio-chemical Pharmaceutical Co. LTD. (Wuhan, China); Goat anti-rat hsp90α polyclonal IgG was purchased from Merck Drug & Biotechnology (Germany); Rabbit anti-goat IgG/HRP was purchased from Boster Bio-engineering LTD. (Wuhan, China); nitrocellulose filters were purchased from Beijing Dingguo Biotechnology Co.(Beijing, China); Coomassie Protein Assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China); immunohistochemistry kit was purchased from Beijing Zhongshan Co. (Beijing, China). All other chemicals used in this study were of analytical reagent grade.

Grouping of animals and protocols

A total of 240 SD rats were equally divided into the following 4 groups (n=60): Control group, RLS group, PG group, and HSD group. All the rats were fasted and had free access to water before the experiment. Cannulations were performed in the right femoral artery, right femoral vein and right carotid artery. In addition, a three limb tube, connecting the arterial cannulation, was used to observe blood pressure, exsanguinate, and administer heparin sodium (500 U/kg) and test resuscitation fluids. The shock model was established by exsanguinating 45 % of the blood of the rats. For the control group, the same volume of physiological saline as blood loss was administered; for the RLS group, the volume of fluid replacement and blood loss were the same; for the PG group, the volume of fluid replacement was 20 ml/kg; for the HSD group, the volume was 6 ml/kg [12]. Six anesthetized rats were sacrificed to obtain cardiac muscles at 1, 3, 6, 12 and 24 h after treatment with the various resuscitation fluids. For survival analysis, the remaining 30 animals of each group were freed from the cannula, the opening sutured, and observed for 24h.

Western blotting

Total proteins of cardiac muscles were extracted, and then equal amounts of protein (40 μg) were separated by sodium dodecyl sulfate/ polyacrylamide gel electrophoresis (SDS/PAGE), blotted on nitrocellulose filters (NCF), and probed with Goat anti-rat hsp90α polyclonal IgG and anti-c-Jun rabbit polyclonal IgG, and subsequently with Rabbit anti-goat IgG/HRP, and detected by chemiluminescence. To measure protein loading, antibody directed against β-actin was used.

Immunohistochemistry test

The immunohistochemistry assay was performed following the method described on the commercial kits to examine the expressions of hsp90α in the cardiac muscles.

Statistical analysis

All the experiments were conducted at least in triplicate and the data are presented as mean ± SEM. Chi-square of exact test was used to analyze the significance of mouse mortality differences among groups. All other data were evaluated with one-way ANOVA followed by Dunnett multiple comparison test between different groups. Statistical significance of differences was analyzed using SPSS software (SPSS for Windows 15.0, SPSS Inc, USA) at a significance level of $p < 0.05$.

RESULTS

Expressions of hsp90α

The expressions of hsp90α proteins were determined by western blotting. The expressions of hsp90α proteins (obtained by Western blot) increased after hemorrhagic shock; however, the expressions of hsp90α proteins decreased progressively with the treatment of fluid resuscitation; in addition, it increased rapidly at the 24h after HS (Figs 1 and 2). Furthermore, the expressions of hsp90α protein in cardiac muscles of RLS group were the lowest among all the test groups, and this was followed by the PG group, HSD group, and control group (Fig 2) ($p < 0.05$). The relative protein level was normalized to the Western blot intensity of β-actin.

Expression of hsp90α proteins in cardiac muscles

The expression of hsp90α increased after HS. As can be seen in Fig 3, the expression of hsp90α protein in the cardiac muscles of RLS group was the lowest among all the test groups, followed by PG group, HSD group.

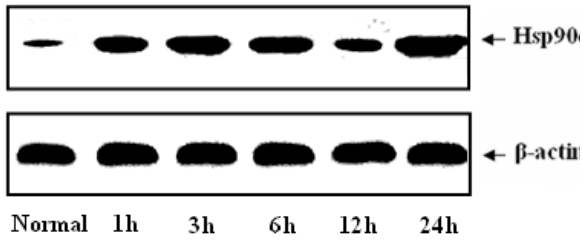

Figure 1: Changes in expression of hsp90α proteins of cardiac muscles after HS

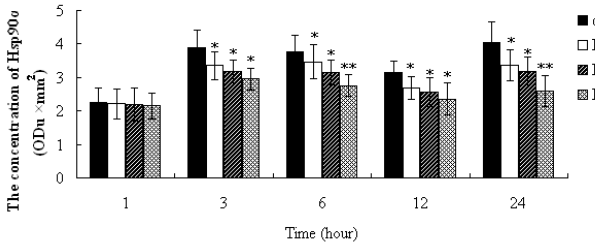

Figure 2: The expressions of hsp90α proteins in cardiac muscles of 4 test groups at different time intervals. (ODu ×mm^2, mean ± SEM, n = 6). Differences amongst the test groups were significant at 3, 6, 12 and 24 h after HS (p < 0. 05), compared with the control group.

Survival rate

As can be seen from Table 1, survival increased significantly following treatment with fluid resuscitation, and this was most pronounced for those administered with RLS (90 %, p < 0.01), compared with the control group.

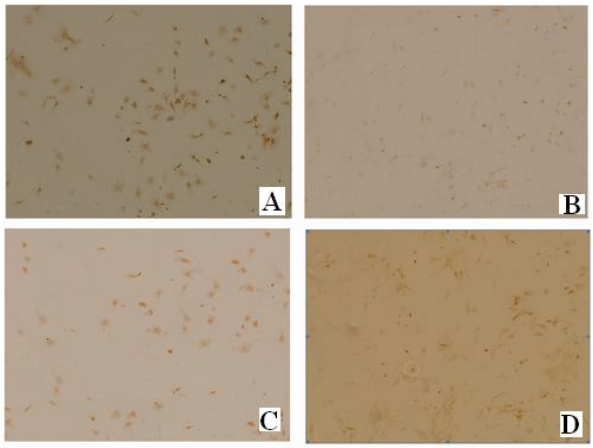

Figure 3: Results of immunohistochemistry test ***Note:*** A = Control group; B = RLS group; C = PG group; D = HSD group

DISCUSSION

HS can induce a high mortality and morbidity because of hypoperfusion and tissue hypoxia, which tend to induce multiple-organ failure (especially the cardiovascular organs) [13-15]. Heat shock protein Hsp90α is a ubiquitously expressed molecular chaperone, which is necessary for the maintenance of eukaryotic homeostasis. Hsp90α can also be secreted extracellularly and is associated with several physiological and pathological processes including shock, infectious diseases, wound healing, cancer and diabetes [9]. In addition, hsp90α proteins play very important roles in protecting the body from damage resulting from shock, hyperpyrexia, anoxia and infection [16]. In our previous study, we found that hsp90α protein plays an important protective effect on cardiac muscles in the early phase of shock. In addition, the protective effect of hsp90α protein on cardiac muscles is mainly based on its effect as a molecular chaperone by combining with the bHLH-PAS domain of hypoxia inducible factor 1α (HIF-1α) [11].

Table 1: Survival rate of the animals

Group	Outcome of intervention		
	Death	Survival (N)	Survival (%)
Control	22	8	26.67
HSD	12	18	60.00
PG	7	23	76.67
RLS	3	27	90.00

Significant differences were observed between the groups using X^2 analysis (p < 0.01).

In this study, we investigated the effects of different resuscitation fluids on the expression of hsp90α in HS rats' cardiac muscles, so as to find a safe and effective therapeutic method to treat HS. From the results of our present study, we can come to the conclusion that the hsp90α expressions can be affected significantly by treating with different resuscitation fluids after HS. In addition, the expressions and lasting time of hsp90α in RLS groups is the lowest among all the tested groups, and this is followed by PG group, HSD group. These results indicate that RLS is the most effective resuscitation fluid for the treatment of HS, and also demonstrate that RLS has a very potent protective effect against mortality induced by HS in rats.

CONCLUSION

In conclusion, the results obtained in this work are noteworthy, and our results demonstrate that resuscitation fluids have favorable protective effects on cardiac muscles of rats with HS, especially RLS, and we suggest that the RLS can be utilized as an effective and safe disease preventive or therapeutic agent for the treatment of HS. However, more investigations are necessary to fully elucidate the mechanism of action of the different resuscitation fluids in the future.

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