行政院國家科學委員會補助專題研究計畫成果報告

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※ 熱休克蛋白質在敗血症實驗動物中細胞能量轉換的保護機制 ※
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計畫類別：☑ 個別型計畫 □整合型計畫

計畫編號：NSC 89-2320-B-041-009-

執行期間：89 年 08 月 01 日至 90 年 07 月 31 日

計畫主持人：陳香文

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：嘉南藥理科技大學

中 華 民 國 90 年 07 月 31 日

熱休克蛋白質在敗血症實驗動物中細胞能量轉換的保護機制

Protective mechanism of heat shock protein for energy regulation during sepsis

計畫編號：NSC 89-2320-B-041-009-;
執行期限：89 年 08 月 01 日至 90 年 07 月 31 日
主持人：陳香文　嘉南藥理科技大學
計畫參與人員：王淑容、呂宗禧、呂美津 高雄醫學大學

中文摘要

在敗血症的病程發展下，往往由於多器官衰竭的產生，導致敗血症死亡率的高居不下。至今仍是各科臨床住院病人中最常見的致死性診斷。隨著醫學及科學的發展，對於敗血症複雜的生理病理變化已有進一步的了解，無論如何目前對於造成多器官衰竭及最終導致死亡的細胞機制仍不清楚。本計劃的目的將從細胞能量代謝的方面來進行，探討敗血症期間心臟粒腺體的功能和細胞內 ATP 含量的改變，及其熱休克處置可能造成的效應。本實驗利用盲腸結紮與穿孔手術來誘發實驗動物敗血症的產生。以全身加熱的方式誘發熱休克反應。實驗結果顯示，敗血症晚期心臟之粒腺體的活性有顯的下降，且組織中 ATP 的含量亦有明顯的減低($P<0.05$)，然而在實行盲腸結紮誘發敗血症之手術前，先進行熱休克處置誘發熱休克蛋白質 72 的大量表現，可避免粒腺體中 NSSR、SCCR、COO 活性及組織中 ATP 的含量的下降。除此之外，熱休克處置亦可誘發 Grp75 表現增加。再者，從西方轉漬及免疫染色結果顯示，在敗血症期間粒腺體中 complex I、complex II、complex III、complex IV 的表現並無明顯的改變。由此實驗結果吾等認為，能量代謝的障礙，確實參與了敗血症的致病機轉。而熱休克前處置，確實可避免敗血症期間造成的粒腺體酵素活性及 ATP 含量的明顯下降，熱休克蛋白質的大量表現於此效應上，應扮演著重要的角色。

關鍵詞：敗血症，粒腺體，熱休克蛋白質，盲腸結紮與穿孔

Abstract

The present study was designed to investigate the role of mitochondria activity in heart in influencing the outcome of sepsis modulated by previous heat shock treatment. Sepsis was induced in rats by CLP method. Rats of heated group were heated by whole-bodily hyperthermia 24 before CLP operation. Mitochondria from freshly heart muscle were collected 9 hr and 18 hr after CLP as early and late sepsis, respectively. The expressions of Hsp72, Grp75 and mitochondrial complex I、complex II、complex III、complex IV were evaluated by Western blot and immunochemical analysis. Mitochondrial enzyme activity of NCCR, SCCR and COO were measured following the reduction or oxidation of cytochome c at 550nm by spectrophotometer. The results showed that mitochondrial enzyme activity of NCCR, SCCR and COO were apparently suppressed during late stage of sepsis. Previously treated by heat shock, late-sepsis rats emerged high preservation of mitochondrial enzyme activity, while Hsp72 was over-expressed. Moreover, ATP content decrease during sepsis is also prevented by heat shock pretreatment. In conclusion, decrease of mitochondrial enzyme activity and ATP content showed correlation with the deterioration of sepsis, while heat shock response could contribute to maintain or regulation the energy metabolism during late sepsis.

Keywords: sepsis, heat shock protein, cecal ligation and puncture, mitochondria

Introduction

Despite significant advances in the management of sepsis, its sequelae (such as septic shock and multiple organ failure) are still main causes for morbidity and mortality in the intensive care unit. Although much effort has been focused on the mediators released in large quantities following sepsis, blockade of mediators such as proinflammatory cytokines has not yet resulted in a successful therapy. However, as more studies are forthcoming, the mechanisms responsible for cell and organ dysfunctions following sepsis are becoming better understood, and promising new therapeutic approaches are currently being evaluated. In order to understand the precise mechanisms responsible for cellular dysfunction and consequently irreversible organ damage and multiple organ failure, it is important to correlate various pathophysiological changes with mediators and signal transduction pathways at the cellular and subcellular level.

Recently, knowledge about a family of protective proteins seems to offer a novel therapeutic or prophylactic strategy to sepsis. Since first mentioned by Rittosa in 1962, it is well accepted that living cells, from plant to human, react to heat and other physiological or pathological stress by synthesizing a group of highly conserved proteins known as heat shock proteins (Hsps). Recently, we and others found that Hsps can not be induced significantly from the onset till late phase of sepsis in CLP-induced animal models although a number of adverse metabolic alterations similar to that in septic patients were present. The basis for this phenomenon remains a mystery, however, it is reasonable to consider that the absence in Hsps induction may correlate with the poor outcome in the disease entity. In fact, the clinical outcome in rats, using protective potential of Hsps, seems promising.

The aim of this study is to evaluate the possible mechanisms of heat shock protein induced by hyperthermia to regulate the change of mitochondrial structure and function during sepsis by detection the expressions of Hsp (Grp75, Hsp72,), the changes of activity of mitochondrial electron transport complex, the intracellar ATP content in experimental sepsis animal. We hope that this study, through the understanding of the relationship between Hsps and mitochondrial function, may offer a successful management in achieving the goal to treat a severe infection.

Result

Fig 1: Activities of mitochondrial enzyme in hearts of septic rats.
Mitocohindrial was collected from freshly heart muscle. Mitochondrial enzyme activity of NCCR, SCCR and COO were measured following the reduction or oxidation of cytochome c at 550nm by spectrophotometer. Enzyme activities are given as units/gram protein. Data as means±SD. S: sham operation, E: early stage of sespsis, L: late stage sepsis, HE: early stage of sepsis with previous heat shock treatment and HL: late stage of sepsis with previous heat shock treatment Significant difference to sham control (*) or late stage of sepsis (+), $P < 0.05$

Heart Enz	S (n=10)	E (n=11)	L (n=10)	HE (n=11)	HL (n=11)
NCCR	1.45±0.33	1.61 ±0.43^{+}	1.13 ±0.30*	1.68 ±0.28^{+}	1.63 ±0.21^{+}
SCCR	0.88 ±0.09	0.92 ±0.12^{+}	0.74 ±0.10 *	0.95 ±0.29^{+}	0.87 ±0.11^{+}
COO	9.82 ±0.64	10.79 ±0.79^{+}	6.70 ±2.89 *	10.69 ±1.02^{+}	9.83 ±0.56^{+}

Fig 2: heat shock pretreamt prevent the ATP content depletion during sepsis in heart.
Data as means±SD. S: sham operation, E: early stage of sespsis, L: late stage sepsis, HE: early stage of sepsis with previous heat shock treatment and HL: late stage of sepsis with previous heat shock treatment Significant difference to sham control (*) or late stage of sepsis (+), $P < 0.05$

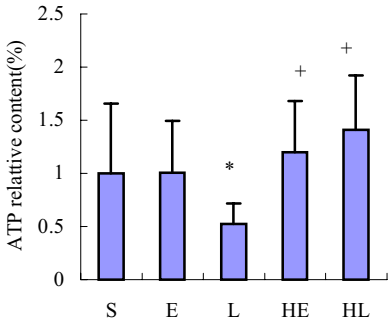


Fig 3: Expression of mitochondrial enzyme in heart during sepsis.
Detection of mitochondrial enzyme complex in the heart by Western blotting and immunochemical study. Equal amount of cellular extract was loaded to each lane. S: sham operation, E: early stage of sespsis, L: late stage sepsis, HE: early stage of sepsis with previous heat shock treatment and HL: late stage of sepsis with previous heat shock treatment

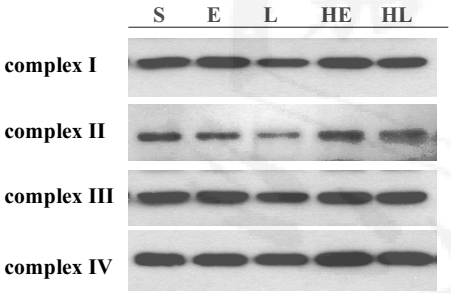


Fig 4: heat shock induces the Hsp72 over-expression in heart.
Detection of Hsp72 in the heart by Western blotting and immunochemical study. Equal amount of cellular extract was loaded to each lane. S: sham operation, E: early stage of sespsis, L: late stage sepsis, HE: early stage of sepsis with previous heat shock treatment and HL: late stage of sepsis with previous heat shock treatment Beta-tubulin was co-reacted as the internal standard.

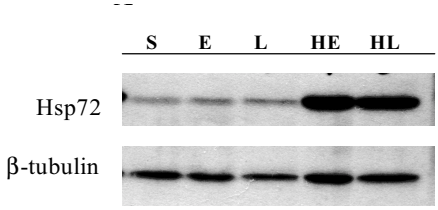
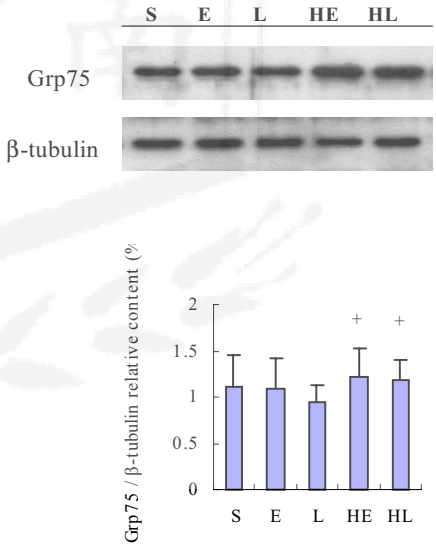


Fig 5: heat shock pretreatment increase the Grp75 expression in heart.
Detection of Grp75 in the heart by Western blotting and immunochemical study. Equal amount of cellular extract was loaded to each lane. Bands ofGrp75 and β-tubulin, acting as the internal standard, were quantified using a densitometer. Samples from preheated rats were statistically compared with those of non-heated rats. Upper panel: immunochemical study. Lower panel : statistical analysis of relative content of Grp75 (ratio of ODHsp72/tubulin). S: sham operation, E: early stage of sespsis, L: late stage sepsis, HE: early stage of sepsis with previous heat shock treatment and HL: late stage of sepsis with previous heat shock treatment. Significant difference to sham control (*) or late stage of sepsis (+), $P < 0.05$



Conclusion

Decrease of mitochondrial enzyme activity and ATP content showed correlation with the deterioration of sepsis, while heat shock response could contribute to maintain or regulation the energy metabolism during late sepsis.