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Expression change of TNF-α in myocardium and hepatic tissue of rats with compound stress of hyperthermia and lipopolysaccharide

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ABSTRACT

Objective: To investigate the expression characteristics of TNF-α in myocardium and hepatic tissue of rats with compound stress of hyperthermia and lipopolysaccharide (LPS). **Methods:** Male SPF Wistar rats were randomly divided into room temperature+physiological saline group (Group C), hyperthermia+physiological saline group (Group H), room temperature+LPS group (Group L) and hyperthermia+LPS group (Group HL). The rats were put in simulated climate cabin. Group HL and Group H were exposed in the environment at a dry bulb temperature (TDB) of (35.0±0.5) ℃, while Group L and Group C were exposed in the environment at a TDB of (26.0±0.5) ℃. The rats in Group HL and Group L were given tail intravenous injection of LPS 10 mg/kg, while the rats in Group H and Group C were given tail intravenous injection of 9 g/L NaCl 10 mL/kg. After the stress, immunohistochemical SABC staining method was used to detect the expression characteristics of TNF-α in myocardium and hepatic tissue of rats, and those rats were given routine pathological examinations. **Results:** The expression of TNF-α in myocardium and hepatic tissue in Group HL was enhanced remarkably, and the tissue damages of Group HL were severest. **Conclusions:** The eardiotoxieity and hepatotoxicity caused by compound stress of hyperthermia and LPS is closely related to the expression of TNF-α.

1. Introduction

Strong and lasting heat stress can result in hyperthermia, and the interaction of cytotoxicity and systemic inflammatory response of host usually lead to thermal damage, heat stroke and even multiple organ dysfunction syndrome (MODS), and more than 50% of MODS patients could suffer concurrent infection which increases the difficulty of clinical treatment[1]. Seventy percent of MODS are caused by infection which is mainly caused by Gram-negative bacteria. Lipopolysaccharide (LPS) is the main component of cell wall of Gram-negative bacteria, and the key factor which leads to systemic inflammatory response and endotoxemia. Along with global warming and the yearly increase of hot wave attack frequency, time length and intensity, it is significant to prepare for the outburst of thermal damage events[2]. Studies have showed that thermal damage is highly relevant with endogenous endotoxemia[1,3,4].

As an important proinflammatory cytokines, TNF-α has strong toxicity on myocardium and hepatic tissue, and it can cause tissue damage by multiple mechanism. We monitored the characteristics of the immunohistochemical staining change of TNF-α in rat myocardium and hepatic tissue with compound stress of environmental hyperthermia and LPS, and provide basis for the study of thermal damage combined with septic shock and MODS, especially systemic inflammatory response syndrome starting mechanism.

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2. Materials and methods[4]

2.1. Materials

Twenty SPF male Wistar rats were provided by the Experimental Animal Center of Shandong University with body weight of 190-210 g per rat. LPS (*Escherichia coli*, 0111:B4) was purchased from German Sigma-Aldrich company. All other reagents were products of Wuhan Boster Bio- Engineering Co., Ltd.

2.2. Methods

Rats were randomly divided into 4 group: room temperature+physiological saline group (Group C) (n=5), hyperthermia+physiological saline group (Group H) (n=5), room temperature+LPS group (Group L) (n=5) and hyperthermia+LPS group (Group HL) (n=5). The rats were put in simulated climate cabin. Group HL and Group H were exposed in the environment at a dry bulb temperature (TDB) of (35.0±0.5) ℃, while Group L and Group C were exposed in the environment at a TDB of (26.0±0.5) ℃. The rats in Group HL and Group L were given intravenous injection of LPS 10 mg/kg via tails, while the rats in Group H and Group C were given tail intravenous injection of 9 g/L NaCl 10 mL/kg. Powlab/8 sp polygraph was connected, and left femoral artery was cannulated to monitor and trace mean arterial pressure (MAP) of experimental animal.

The myocardium and livers of rats were taken out 120 min after the beginning of stress. And immunohistochemical method was used to observe the staining characteristics of TNF-α in myocardium and hepatic tissues of rats in the 4 stressed groups. Immunohistochemical SABC staining method was adopted, and the procedures were taken according to instruction manual. Equation: rabbit anti-TNF-α antibody+BSA confining liquid+biotinylated goat anti-rabbit IgG+strept avidin-biotin complex (SABC)+DAB. Brief procedures: Fixation by adding neutral formaldehyde buffered solution into the tissue, routine paraffin imbedding → poly-L-lysine slide anti-creep processing, tissue slicing of 4 μm, routine de-waxing → Inactivation of endogenous enzyme by 30 mL/L H_2O_2 → Antigen repairing by use of citrate buffer solution and heating → BSA confining liquid → rabbit anti-TNF-α antibody → second antibody (biotinylated goat anti-rabbit IgG) → SABC → DAB coloration→ hematoxylin staining, differentiation with ethanol-hydrochloric acid, washed and bluing, mounting. PBS replacing first antibody was used as negative control to rule out nonspecific staining. Results determination: positive staining of TNF-α was yellowish brown or brown. The percentage of stained cells in 1 000 cells which were randomly counted under light microscope was calculated. If the percentage of stained cells ⩾20 %, it is positive; and if the percentage of stained cells < 20%, it is negative. Staining intensity was classified as - (negative, unstained), + (weakly positive, light yellow), ++ (positive, yellow), +++ (strongly positive, brownish yellow).

2.3. Statistical analysis

Measuremental date was expressed as mean±SD. SPSS10.0 software package was used to conduct One-way analysis of variance (ANOVA), and multiple comparisons among means were carried out by least significant difference (LSD)-t test. Kruskal Wallis test was employed to do the comparisons of multi-level data, and P<0.05 indicated that there is statistical difference.

3. Results

3.1. Dynamic change of MAP

MAP of animals in Group H and Group HL showed a trend of rising first and then decreasing. It rose prominently 80 min after the beginning of stress (P<0.01 while compared with Group C and Group L), peaked 100-110 min after the beginning of stress, and then had circulatory collapse immediately (Table 1).

3.2. SABC staining observation of TNF-α

Myocardium: TNF-α staining of myocardium in Group C were all negative (Figure 1A); Group L had 3 negative cases and 2 weakly positive cases; Group H had 1 negative case, 2 weakly positive cases and 2 positive cases; there were 3 positive cases and 2 strongly positive cases in Group HL (Figure 1B). Kruskal Wallis test showed that there was statistic difference in TNF-α staining intensity of myocardium among these experimental groups (χ^2 = 14.265, P = 0.003).

Hepatic tissue: TNF-α staining of hepatic tissue in Group

Table 1
Impact of compound stress of hyperthermia and endotoxin on MAP of rats (mmHg, n=5).

Group	0 min	40 min	80 min	90 min	100 min	110 min	120 min
C	97.0±4.4	97.4±3.0	96.4±3.4	97.2±4.3	96.8±4.3	96.8±4.3	96.8±4.3
L	96.8±3.4	93.8±1.3	96.4±2.0	97.0±2.7	97.2±2.3	97.2±2.3	97.2±2.3
H	96.0±5.2	97.2±4.8	118.0±1.6^{ac}	125.6±3.0^{ac}	132.6±3.4^{ac}	136.8±1.5^{ac}	67.2±4.8^{ac}
HL	97.2±4.4	94.0±2.6	117.0±2.4^{ac}	122.2±3.1^{ac}	128.4±2.7^{ac}	117.8±10.5^{ace}	49.0±3.5^{ace}

a: Compared with Group C, P<0.01; c: Compared with Group L, P<0.01; e: Compared with Group H, P<0.01.

C were all negative (Figure 2A); Group L had 3 weakly positive cases and 2 positive cases; Group H had 3 negative cases, 2 weakly positive cases; Group HL had 2 positive cases and 3 strongly positive cases (Figure 2B). Kruskal Wallis test showed that there was statistic difference in TNF-α staining intensity of hepatic tissue among these experimental groups (χ^2 =16.188, P = 0.001) (Table 2).

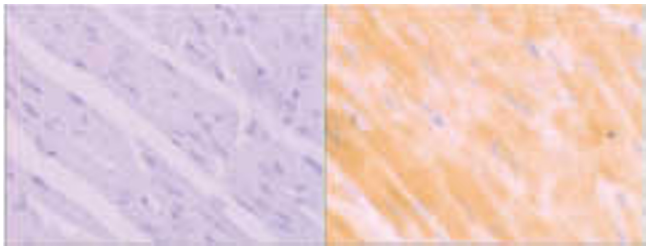

Figure 1. Expression of TNF-α in myocardium of rats with compound stress of hyperthermia and LPS (SABC×400).
1A: C-Group; 1B: HL-Group.

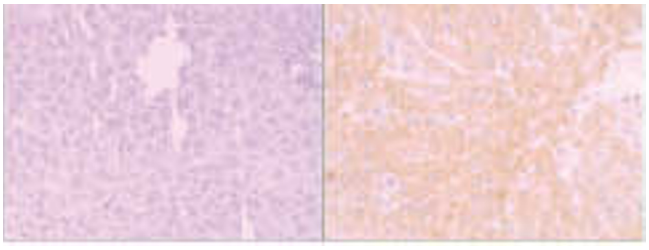

Figure 2. Expression of TNF-α in hepatic tissue of rats with compound stress of hyperthermia and LPS (SABC×400).
2A: C-Group; 2B: HL-Group.

3.3. HE staining observation

It was observed under microscope that, 120 min after injury, Myocardium: as showed in myocardium HE staining pathological examination, the cellular morphology and cytoarchitecture of myocardium in Group C were intact, and there was no congestion, edema and inflammatory cell infiltration among muscles (Figure 3A); Myocardium cells in Group HL had obvious edema, degeneration, intramuscular vascular dilatation and congestion, interstitial edema as well as inflammatory cell infiltration (Figure 3B); Hepatic tissue: the shape and structure of hepatic lobules in Group C were intact, and there was no degeneration and necrosis of hepatic cells,or congestion in central vein and hepatic sinusoid (Figure 4A); Group HL had obvious congestion in central vein and hepatic sinusoid as well as edema in hepatic cells (Figure 4B).

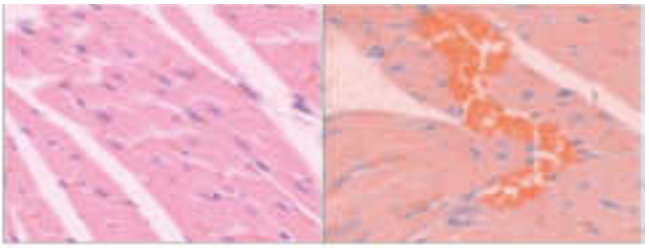

Figure 3. Histomorphological changes of myocardium of rats with compound stress of hyperthermia and LPS (HE×400).
3A: C-Group; 3B: HL-Group.

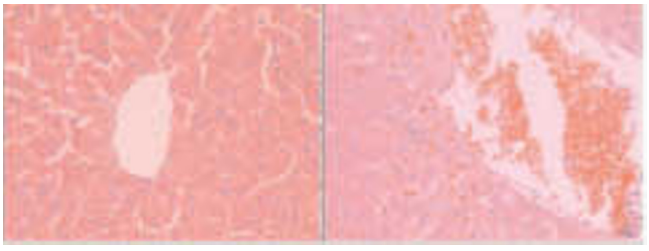

Figure 4. Histomorphological changes of hepatic tissue of rats with compound stress of hyperthermia and LPS (HE×400).
4A: C-Group; 4B: HL-Group.

4. Discussion

As stressors, both hyperthermia and LPS can stimulate organism to produce stress response and inflammatory reaction[5-9]. Appropriate stress response and inflammatory reaction would have synergistic effect[10] which can prevent expansion of tissue injury and promote tissue repair, and they are a part of the whole defense system of organism, and necessary for organism repair and survival[11,12]. However, the intensive and lasting effect of injury factors could cause malignant stress and out of control of the inflammatory reaction, which leads to systemic inflammatory response featured by self-destruction of cells, resulting in tissue damage[13]. In this experiment, compared with Group C, myocardial and hepatic cells of the rats in Group HL had significant inflammatory response; The myocardium of rats in Group H with a higher expression of TNF-α in hepatic tissue were more easily get injured than Group C and Group H. Myocardium was more susceptible to be injured

Table 2
SABC staining results of TNF-α expression in myocardium and hepatic tissue of the experimental groups.

Results/grouping	Group C		Group H		Group L		Group HL	
	Myocardium	Hepatic tissue	Myocardium	Hepatic tissue	Myocardium	Hepatic tissue	Myocardium	Hepatic tissue
Negative	5	5	1	3	3	0	0	0
Weakly positive	0	0	2	2	2	3	0	0
Positive	0	0	2	0	0	2	3	2
Strongly positive	0	0	0	0	0	0	2	3

by hyperthermia rather than LPS; while hepatic tissue was more easily damaged by LPS than hyperthermia. It might be that hyperthermia stimulates the expression of TNF-α in myocardial cells, although normally TNF-α is only produced in myocardial interstitial macrophages. The studies of Giroir *et al*[14] show that mature myocardial cells also have the ability to produce TNF-α mRNA and its protein in some stressed circumstances, and the TNF-α produced at this moment can affect cardiac function directly. Liver is one of the target organs of TNF-α, and also is the main organ which intakes and metabolizes LPS. LPS receptors on the surface of many kinds of cells in hepatic tissue is the initiating step to make LPS to activate cells to release pro inflammatory cytokines, which could be the reason why the expression level of TNF-α in the livers of rats in Group L in this experiment. Douzinas *et al*[15] believe that on the one hand, TNF-α combine with receptors on organ cell membrane, on the other hand, cytokines such as TNF-α combine with soluble receptors in circulation, which leads to the decrease of cytokines and yet the increase of expression in tissues, and its excessive production in some part causes damage to organs.

Mass of studies show that hyperthermia is highly associated with elevation of blood pressure, and hyperthermia is one of independent risk factors resulting in hypertension[16,17], which might be the reason why the MAP levels of experimental rats in Group H and Group HL were obviously higher than those of Group C and Group L at the early stage of heat stress in this study. And the reason for the later immediate falling of MAP might be the reduction of total blood volume in circulatory system which is ascribable to plasma hyperosmolality caused by mass water loss in organism. In addition, hyperthermia can bring injury to tissue directly and the lasting time decides the order of severity. Heat stress can lead to resistance tissue damage involving endothelial cells, eucocytes and epithelial cells, and acute phase reaction promoting repair[18]. Present study found that the increase of the levels of plasma inflammatory cytokines (TNF-α, IL-1β, IFN-γ) and anti-inflammatory factors (IL-6, soluble TNF receptor 55, p75 and IL-10) of heat stroke patients elevates, and lowering the body temperature to the normal level can notinhibit the inflammatory cytokines[19,20]. The interaction of cytokines leads to continuous increase of cytokines, and forms a giant web system of cytokines which makes inflammatory reaction expand constantly. When this exceeds the compensatory capability of organism, excessive inflammatory reaction occurs in organism, which causes extensive tissue cell damage and SIRS[21], and then leads to necrosis, disseminated intravascular coagulation, multiple organ failure and other common symptoms of heat stroke. The experiment proves that making animals achieve endotoxin tolerance trough peripheral infection caused by LPS and antibiotic therapy can improve the short-term (<24 h) survival rate of heat stroke patients[22]. Bouchama *et al*[23] generated secondary stress via hyperthermia and endogenous endotoxemia, and leaded to SIRS, and then further developed into MODS. This experiment shows, with compound stress of hyperthermia and LPS, the contents of TNF-α in myocardium and hepatic tissue increases remarkably, upon which and the other experimental data[4,24-26], it can be speculated that infection and hyperthermia stress simultaneously or sequentially can cause damage to myocardium and liver via TNF-α pathway.

Conflict of interest statement

We declare that we have no conflict of interest.

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