Pre-existing Inflammatory State Compromises Heat Tolerance In Rats Exposed To Heat Stress

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INTRODUCTION

- Heat stroke is a condition that is associated with systemic inflammation, multiorgan failure, and CNS dysfunction.[1]
- For more than three centuries, heat stroke has been thought to occur due to heat-induced tissue damage, where the direct thermal effect of heat causes proteins in tissue cells to denature.
- While this model explains the clinical presentation of heat stroke victims which is similar to that of sepsis, it cannot explain the lack of a consistent core temperature (Tc) threshold that triggers heat stroke.
- A dual-pathway mechanism has thus been proposed, suggesting that heat stroke could be triggered by heat-induced endotoxemia or heat-induced tissue damage separately.
- Endotoxemia refers to the presence of bacteria endotoxin, known as LPS, in the circulation, which has been proposed to also trigger heat stroke, independent of heat-induced tissue damage. Exposure to heat stress compromises the gut mucosal integrity, resulting in gut translocation of LPS into circulation. The resultant endotoxemia drives the systemic inflammation seen in heat stroke.
- A better understanding of the triggers of heat stroke can help aid in the prevention of heat stroke in individuals
- Hypotheses
    1. Heat stroke can be triggered by heat-induced endotoxemia or heat-induced tissue damage.
    2. Pre-existing local inflammation reduces heat tolerance.
    3. Preventing endotoxemia increases heat tolerance.

AIM

To address the knowledge gap of the triggers of heat stroke by investigating a dual-pathway mechanism[2] involving both heat-induced endotoxemia and heat-induced tissue damage.

METHODOLOGY

4 groups of rats were sedated

Group 1 had no drug given, group 2 received intramuscular turpentine (T), group 3 received intravenous dexamethasone (D) and group 4 received a combination of both intramuscular turpentine and intravenous dexamethasone (TD)

Rats rested for 2h before undergoing heat exposure (HE) until their Tc were 42°C for 15 min. Concurrently, control rats also received T, D, and TD but without HE

Blood samples were collected before treatment (baseline I), after 2 h of passive rest (baseline II), at Tc 40°C, and 15 min after achieving Tc 42°C

Liver enzymes (AST, ALT), cytokines (IL-1β, IL-6) and LPS were analysed. Survival rates of rats were recorded.

Statistical analysis

RESULTS

Table 1. *Effects of heat stress on plasma concentrations of ALT and AST*

	ALT				AST			
Groups	*Baseline I*	*Baseline II*	T40	T42	*Baseline I*	*Baseline II*	T40	T42
HE	42.7 (9.0)	48.2 (15.9)	49.1 (15.3)	73.3 (22.6)[a]	88.4 (25.6)	104.7 (47.7)	131.7 (45.9)	162.9 (48.1)[a]
T	39.0 (10.6)	44.1 (12.0)	46.7 (9.2)	49.7 (13.6)	83.9 (30.6)	106.2 (32.2)	111.1 (27.7)	113.6 (24.5)
T+HE	42.1 (14.8)	48.0 (13.8)	53.0 (16.0)	79.8 (24.3)[a]	77.5 (23.2)	131.6 (43.8)	155.0 (53.1)	200.5 (63.0)[a,b,c,d]
D	39.9 (7.1)	40.2 (11.6)	40.5 (9.9)	40.7 (11.5)	77.1 (7.9)	82.71 (12.1)	88.73 (18.1)	89.7 (14.7)
D+HE	41.0 (7.9)	43.7 (9.0)	44.3 (9.1)	50.1 (20.8)	86.5 (17.9)	89.9 (37.0)	98.4 (27.6)	102.4 (35.6)
TD	42.9 (8.1)	49.3 (16.9)	50.0 (17.0)	63.2 (30.0)	77.2 (17.6)	121.3 (44.0)	120.8 (43.5)	130.1 (42.3)
TD+HE	42.3 (7.8)	61.4 (18.4)	63.8 (21.3)	100 (42.2)[a,d]	87.3 (32.5)	132.0 (43.8)	168.1 (69.1)	252.8 (105.5)[a,b,c]

Values are expressed as means ± SD (in parentheses) concentrations of plasma alanine aminotransferase (ALT) and aspartate transaminase (AST) (U/l) in rats treated with heat exposure only (HE), turpentine only (T), T combined with heat stress (T+HE), dexamethasone only (D), D combined with heat stress (D+HE), T and D combined without heat stress (TD), and TD with heat stress (TD+HE). T40, core temperature 40°C; T45, core temperature 45°C. [a]Significant changes in ALT and AST concentrations in each group ($P < 0.001$). [b]$P < 0.05$ and [c]$P < 0.01$, significant difference at the specific time point between the heat-stressed and control rats within each treatment group. For comparisons between the heat-stressed groups, [d]$P < 0.05$ and [e]$P < 0.001$ represent a significant difference from the D+HE rats at the specific time point.

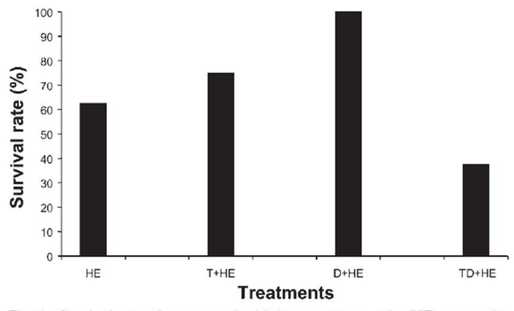

Fig. 1. Survival rate of rats treated with heat exposure only (HE), turpentine combined with heat stress (T+HE), dexamethasone combined with heat stress (D+HE) and a combination of turpentine, dexamethasone, and heat stress (TD+HE). The data were analyzed in categorical scale (died vs. survived) using χ^2 statistics. The survival rates among the heat-stressed rats are significantly different ($P < 0.05$).

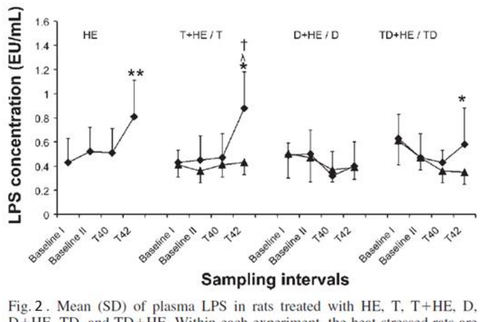

Fig. 2. Mean (SD) of plasma LPS in rats treated with HE, T, T+HE, D, D+HE, TD, and TD+HE. Within each experiment, the heat-stressed rats are represented by solid diamonds (◆), and the nonheat-stressed rats are represented by solid triangles (▲). *$P < 0.05$ and **$P < 0.01$, significant change in LPS concentrations within each group. †Significant difference at the specific time point between the heat-stressed and control rats within each treatment group, $P < 0.05$. For comparisons between the heat-stressed groups, significant difference from the D+HE rats at the specific time point, λ$P < 0.05$.

RESULTS (CONTINUED)

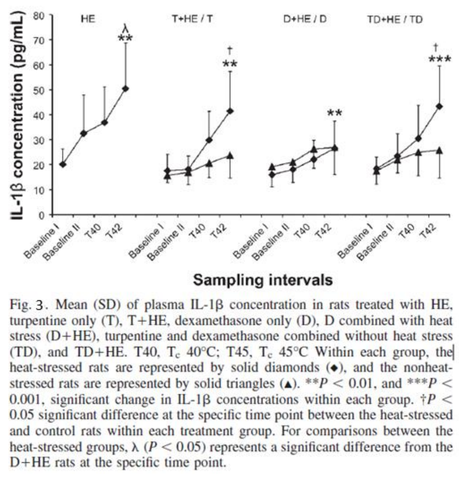

Fig. 3. Mean (SD) of plasma IL-1β concentration in rats treated with HE, turpentine only (T), T+HE, dexamethasone only (D), D combined with heat stress (D+HE), turpentine and dexamethasone combined without heat stress (TD), and TD+HE. T40, T_c 40°C; T45, T_c 45°C Within each group, the heat-stressed rats are represented by solid diamonds (◆), and the nonheat-stressed rats are represented by solid triangles (▲). **$P < 0.01$, and ***$P < 0.001$, significant change in IL-1β concentrations within each group. †$P < 0.05$ significant difference at the specific time point between the heat-stressed and control rats within each treatment group. For comparisons between the heat-stressed groups, λ ($P < 0.05$) represents a significant difference from the D+HE rats at the specific time point.

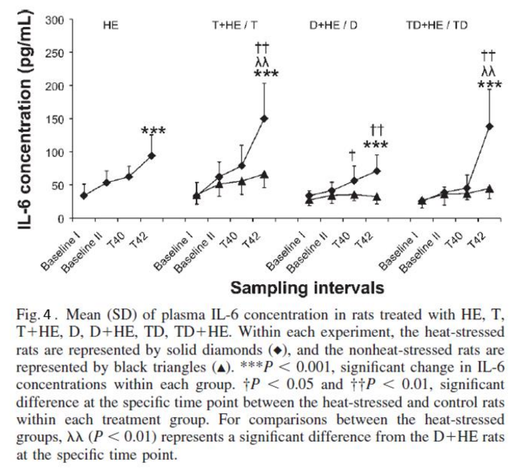

Fig. 4. Mean (SD) of plasma IL-6 concentration in rats treated with HE, T, T+HE, D, D+HE, TD, TD+HE. Within each experiment, the heat-stressed rats are represented by solid diamonds (◆), and the nonheat-stressed rats are represented by black triangles (▲). ***$P < 0.001$, significant change in IL-6 concentrations within each group. †$P < 0.05$ and ††$P < 0.01$, significant difference at the specific time point between the heat-stressed and control rats within each treatment group. For comparisons between the heat-stressed groups, λλ ($P < 0.01$) represents a significant difference from the D+HE rats at the specific time point.

- Survival rate of rats after heat exposure was the highest with dexamethasone (D+HE) treatment alone but lowest when dexamethasone treatment was combined with turpentine (TD+HE) (Fig 1).
- TD+HE rats had much higher concentrations of plasma IL-1β (Fig 3) and IL-6 (Fig 4) than the D+HE rats. In addition, ALT and AST concentrations (Table 1) were more than twice as high in the TD+HE rats than in the D+HE rats.
- The concentrations of plasma IL-6 (Fig 4) in turpentine-treated rats (T+HE and TD+HE) were significantly higher than in the D+HE rats
- In the HE and T+HE rats, the high concentrations and the magnitude of increase in plasma LPS (Fig 2) coupled with the relatively lower magnitude of increase in the concentrations of plasma ALT and AST (Table 1)
- The near-resting plasma LPS concentrations (Fig 2) and the higher increase in plasma ALT and AST concentrations in the TD+HE rats (Table 1).
- Plasma LPS concentrations (Fig 2) increased significantly in the HE and T+HE rats but decreased below resting concentrations in the D+HE rats during the experiment, suggesting that dexamethasone treatment protects the rats from the lethality of heat stroke
- AST and ALT concentrations (Table 1) increased significantly in all the heat-stressed rats, except in the D+HE rats.
- Plasma IL-1β concentrations (Fig 3) increased significantly in all the heat-stressed groups, including D+HE, but comparisons with the respective control groups showed that the increases in IL-1β concentrations were due to the heat stress in the HE, T+HE, and TD+HE rats, but not in the D+HE rats.

CONCLUSION

- Survival rate was the lowest when dexamethasone treatment was combined with turpentine, possibly due to their contribution to an overdriven acute phase response.
- Heat stroke is triggered by heat-induced endotoxemia or heat-induced tissue damage.
- Blocking endotoxemia protected the rats from mortality in lethal heat stress
- Turpentine treatment reduces heat tolerance by enhancing the inflammatory response and tissue damage at a given level of heat stress.
- Dexamethasone treatment increased heat tolerance, possibly by preventing endotoxemia.

Practical Implication

- The dual-pathway mechanism of heat stroke highlights the importance of a holistic health approach in managing heat stroke such as protecting the immune system and managing stress rather than a narrow approach of just reducing the body temperature or limiting physical training.

Possible Research Question

- In athletes undergoing intense training, is dexamethasone administration effective in preventing heat stroke?

REFERENCES

1. Bouchama A, Knochel JP. Heat stroke. N Engl J Med. 2002 Jun 20;346(25):1978-88. doi: 10.1056/NEJMra011089
2. Lim CL, Mackinnon LT. The roles of exercise-induced immune system disturbances in the pathology of heat stroke : the dual pathway model of heat stroke. Sports Med. 2006;36(1):39-64. doi: 10.2165/00007256-200636010-00004
3. Lim CL, Wilson G, Brown L, Coombes JS, Mackinnon LT. Pre-existing inflammatory state compromises heat tolerance in rats exposed to heat stress. Am J Physiol Regul Integr Comp Physiol. 2007 Jan;292(1):R186-94. doi: 10.1152/ajpregu.00921.2005

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