Pre-existing Inflammatory State Compromises Heat Tolerance in Rats Exposed to Heat Stress

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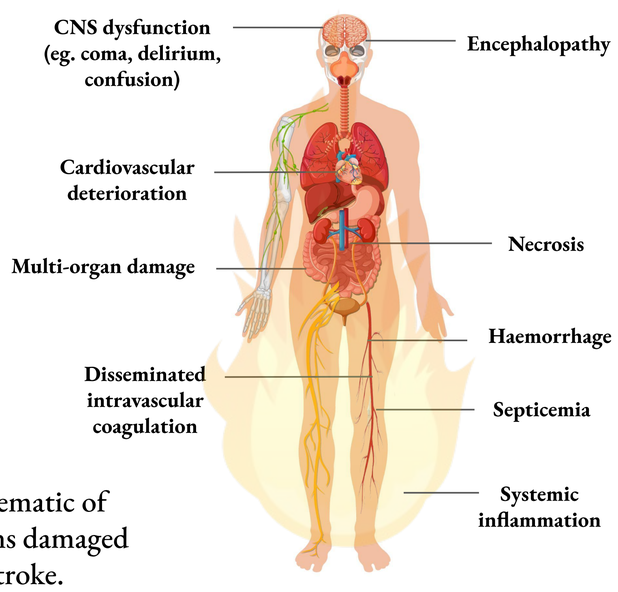
Introduction

Heat Stroke is a hyperthermic condition with core temperature (Tc) >40°C and presents with multiorgan dysfunction (*Fig. 1*).[1]

The dual pathway model of heat stroke suggests that heat stroke is due to 2 independent but sequential pathways: a) heat sepsis resulting from gut related endotoxemia, leading to sepsis and b) thermolysis due to extreme hyperthermia.[2]

The effects of a pre-existing inflammation under lethal heat stress on the pathology of heat stroke, with and without endotoxemia, have not been investigated before.



► **Fig. 1** Schematic of various organs damaged due to heat stroke.

Objectives

Investigate the dual pathway of heatstroke (heat induced endotoxemia vs heat induced tissue damage)

Investigate the effects of local inflammation (turpentine induced), in the presence or absence of endotoxemia, under lethal heat stress

Methodology

Experimental Set-ups

56 rats were assigned into four groups to investigate the effects of pre-existing inflammation under lethal heat stress on plasma LPS inflammatory cytokines and heat tolerance (*Fig. 2*). Each rat was cannulated at the jugular vein for administration of relevant drugs in their respective set-ups and blood collection.

Blood Sampling

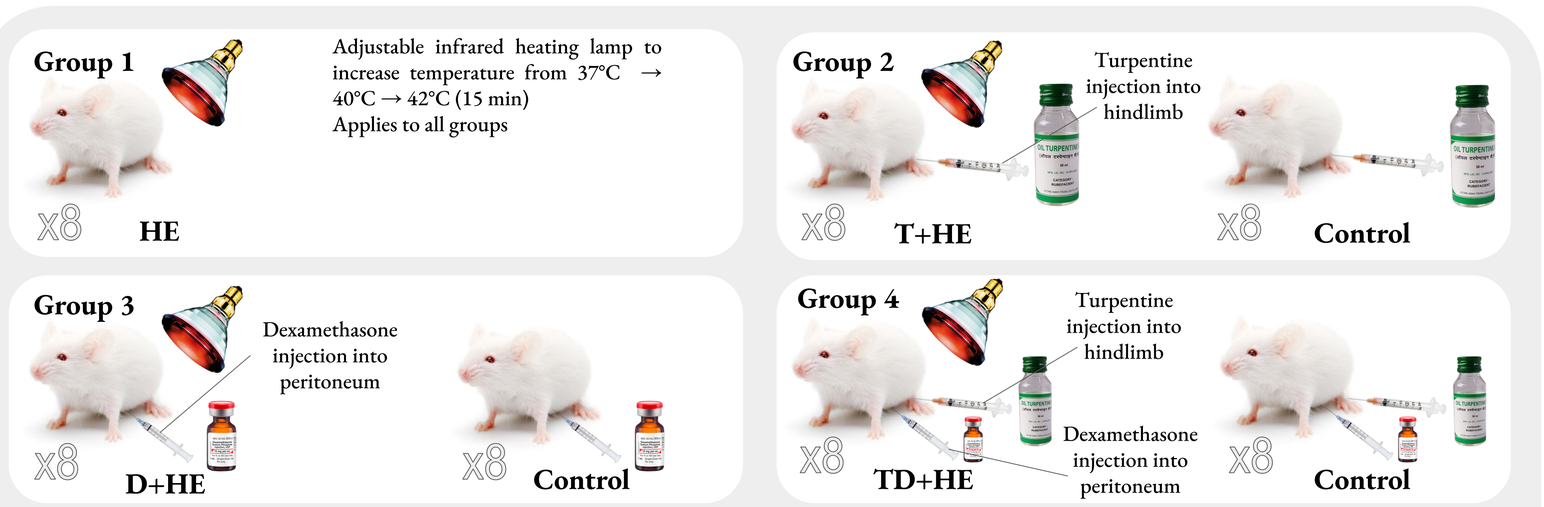
1 ml extracted from rats at four time points:

1. After cannulation (baseline 1)
2. 2h after drug administration (baseline 2), just before heat exposure
3. Tc = 40°C
4. 15 mins after Tc = 42°C or instantly upon the rat's death, whichever is earlier

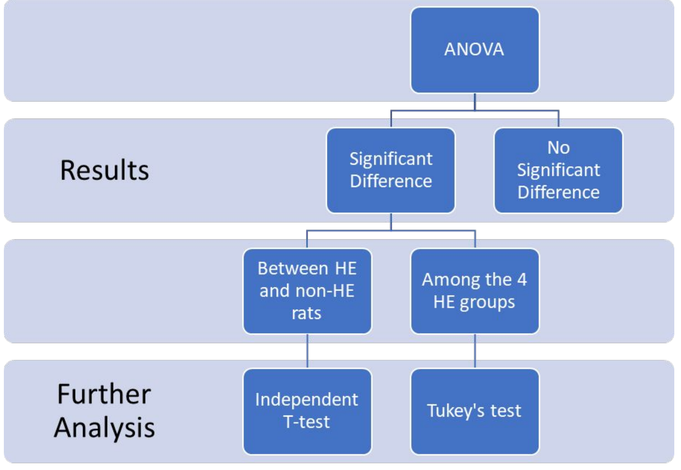
Chemical Analysis

Blood collected was centrifuged and plasma extracted from it was used for analysing:

- Inflammatory cytokines (IL-1β, TNF-α, IL-6)
- Alanine transaminase (ALT) & aspartate transaminase (AST)
- Lipopolysaccharides (LPS)



▲ **Fig. 2** Overview of the four groups testing for the effect of different variables (group 1: heat exposure (HE); group 2: turpentine and heat exposure (T+HE); group 3: dexamethasone and heat exposure (D+HE); group 4: turpentine and dexamethasone with heat exposure (TD+HE) on plasma cytokine and LPS levels).

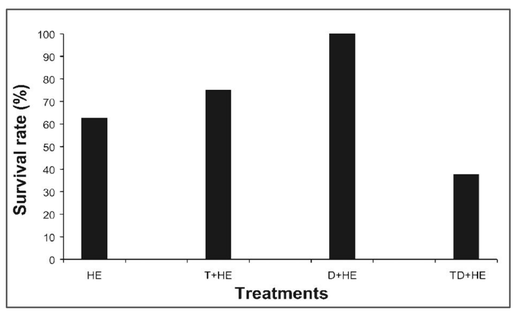


◄ **Fig. 3** Flowchart of the statistical tools used for different groups of comparison.

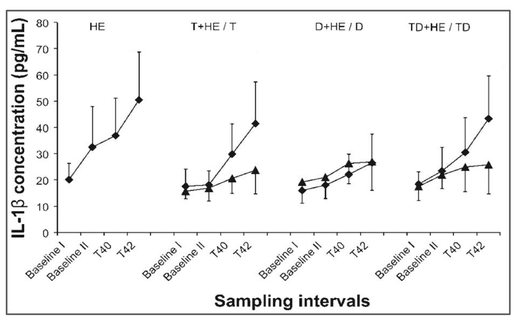
Other Statistical Analyses

- **χ^2-test**: categorical survival rate (survived vs died)
- **Mann-whitney U test**: duration of survival during Tc = 42°C

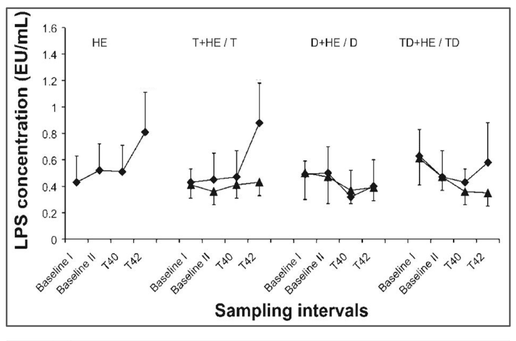
Results & Discussion



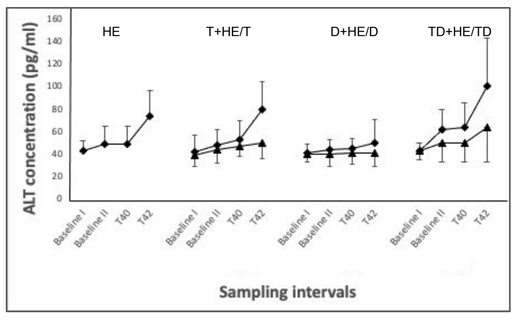
◄ **Fig. 4** Survival rates for the different groups of rats: 100% for D+HE, 75% for T+HE, 62.5% for HE, 37.5% for TD+HE ($P<0.05$)



◄ **Fig. 5** Sharp significant increase in IL-1β concentration when rats were heat stressed in all groups except D+HE. Similar trends were observed for IL-6 concentration



◄ **Fig. 6a** Anomalous decrease in LPS concentration for TD+HE group before increasing back to baseline at T42



◄ **Fig. 6b** Significant increase in ALT concentration for TD+HE group. Similar trend observed for AST concentrations

Dual pathway - a) Heat stroke is driven by endotoxemia, as seen in the HE and D+HE rats. A macro-perspective shows higher survival rates (*Fig. 4*) in D+HE rats (100%) than HE rats (62.5%). Further cytokine analysis presents an overall decrease in LPS levels (*Fig. 6a*), with suppressed increases in IL-1β, IL-6, ALT and AST (*Figs. 5 and 6b*) as Tc increased in D+HE rats as compared to HE rats. Dexamethasone treatment protected the rats from the lethality of heat stroke. By maintaining the integrity of the gut mucosa tissue and inhibiting gut-related LPS translocation[3, 4, 5], dexamethasone helps to prevent endotoxemia, tissue damage, and the inflammatory response.

b) With dexamethasone established as an inhibitor of endotoxemia, heat toxicity as the other proposed pathway of heat stroke can be observed in the TD+HE rats, where there is near baseline LPS concentration but a higher spike in plasma ALT and AST levels, which are markers of heat-induced tissue damage, especially as Tc rose from 40°C to 42°C (*Figs. 6a and 6b*).

Discrepancy in the Effects of Dexamethasone Between Groups 3 (D+HE) and 4 (TD+HE)
The administration of dexamethasone with turpentine exacerbated the acute phase response (APR) triggered by the former in order to suppress the systemic inflammatory response, inhibiting turpentine-induced endotoxemia. [5] This paradoxically results in systemic inflammation on top of tissue damage, as evidenced by the increase in pro-inflammatory cytokines (IL-1β and IL-6), ALT and AST (*Figs. 5 and 6b*). Ironically, heat-induced tissue damage caused LPS leakage as Tc increased from 40°C to 42°C (*Fig. 6a*), hence also contributing to the decreased survival duration and rates in the TD+HE group.

Inflammation - Turpentine treatment compromised heat tolerance by enhancing the inflammatory response and tissue damage. The concentrations of plasma IL-6 (an inflammatory responsive cytokine stimulated by IL-1β and TNF-α) in turpentine-treated rats (T+HE and TD+HE) were significantly higher than in the D+HE rats.

Conclusion - The results of this study supports the proposed dual pathway of heat stroke. Local inflammation compromises heat tolerance due to exaggerated proinflammatory cytokine response in the presence of endotoxemia. Meanwhile, suppression of gut mucosal LPS translocation prevents endotoxemia-related heat stroke. While dexamethasone has achieved positive outcomes in this study, it is unfeasible for long term usage in high functioning athletes (higher risk of exercise-induced immune suppression[6] and musculoskeletal injuries[7]) due to its vast side effects. Future works could focus on intervention at the level of LPS binding protein even after translocation.

References

1. Bouchama A, Knochel JP. Heat stroke. N Engl J Med 346: 1978–1988, 2002.
2. Lim CL, Mackinnon LT. The role of exercise-induced immune system disturbances in the pathology of heat stroke: the dual pathway model of heat stroke. Sports Med 36: 39–64, 2006.
3. Gathiram P, Gaffin SL, Brock Utne JG, Wells MT. Prophylactic corticosteroid suppresses endotoxemia in heat-stressed primates. Aviat Space Environ Med 59: 142–145, 1988.
4. Gathiram P, Wells MT, Brock Utne JG, Gaffin SL. Prophylactic corticosteroid increases survival in experimental heat stroke in primates. Aviat Space Environ Med 59: 352–355, 1988.
5. Bauer J, Birmelin M, Northoff GH, Northemann W, Tran-Thi TA, Ueberberg H, Decker K, Heinrich PC. Induction of rat 2-macroglobulin in vivo and in hepatocyte primary cultures: synergistic action of glucocorticoids and a Kupffer cell-derived factor. FEBS Lett 177: 89–94, 1984.
6. Nieman DC. Exercise immunology: nutritional countermeasures. Can J Appl Physiol 26: S45–S55, 2001.
7. Smith LL. Overtraining, excessive exercise, and altered immunity. Sports Med 33: 347–364, 2003.