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The Protective Effect of Etanercept on Heatstroke-Induced Hypotension and Mortality

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Background: Mortality and multiorgan dysfunction in heatstroke have been attributed to the host's inflammatory responses to heat stress, suggesting that anti-inflammatory therapy may improve the outcomes. We tested the beneficial effect of a tumor necrosis factor-alpha (TNF-α inhibitor etanercept on heatstroke-induced hypotension and hyperthermia. **Methods:** Rats were assigned randomly to four groups: normothermic control rats, heatstroke (HS) rats, HS rats with etanercept pretreatment or cotreatment. Rats under anesthesia were exposed to heat stress (ambient temperature,43 °C) until mean arterial pressure (MAP) began to drop from its peak level, which was arbitrarily defined as the onset of heatstroke. The MAP, colonic temperature (Tco) and plasma TNF-α and interleukin-6 (IL-6) levels were measured in the rats. **Results:** Plasma TNF-α levels were significantly lower in HS rats treated and pretreated with etanercept than in those without treatment. HS-induced elevation in plasma IL-6 levels was further augmented in those with either etanercept pretreatment or treatment. The MAP at heatstroke onset was also significantly increased in HS rats with etanercept pretreatment or treatment. Etanercept pretreatment significantly increased the tolerance of hyperthermia as indicated by prolongation of the latent period and increase in the magnitude of the MAP that triggered the onset of heatstroke. **Conclusion:** Etanercept treatment could attenuate the HS-induced circulating TNF-α and arterial hypotension and improve the survival rate, suggesting that the etanercept could be a potential target drug for the treatment of heatstroke.

Key words: heatstroke, etanercept, tumor necrosis factor-alpha, rats

INTRODUCTION

Heatstroke, defined as core temperature (Tco) > 40 °C and multiorgan dysfunction[1] including hypotension, renal and hepatic failure, a hypercoagulable state, central nervous systemic abnormalities and systemic inflammation, is a life-threatening illness in humans[2]. Evidence has accumulated to indicate that the pathophysiological responses to heatstroke may be the result of a systemic inflammatory response following thermal injury[3]. For example, an increased circulating concentration of cytokines was noted in patients and experimental animals in end-stage heatstroke[4-6] and induction of heat shock symptoms (e.g., hypotensive shock and tissue injury) following interleukin-1 (IL-1) or tumor necrosis factor-alpha (TNF-α) injection have been reported in previous studies[7,8]. Cytokines are important regulators of acute inflammation and have thus been implicated as mediators of a systemic inflammatory response syndrome in heatstroke.

Etanercept, a soluble fusion protein that binds specifically to human TNF, has been approved for use via subcutaneous injection in the treatment of patients with moderate to severe active rheumatoid arthritis[9], juvenile rheumatoid arthritis[10], psoriatic arthritis[11], ankylosing arthritis[12] and plaque psoriasis[13] in the US, Italy, the rest of the EU and in other countries. In the present study, we tested the role of TNF-α in the development of HS and the potential therapeutic effect of etanercept pretreatment and cotreatment before and after the onset of HS.

METHODS

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Animals

Male Sprague Dawley rats weighting 300-350 g were obtained from the Animal Resource Center of the National Science Council in Taipei, Taiwan. The animals were housed in an AAALAC-certified animal center in the National Defense Medical Center, Taipei, Taiwan. Pelleted rat chow and tap water were available ad libitum. All animals were handled and housed according to the guidelines and manual set by the committee of the Care and Use of Laboratory Animals of this institute.

Experimental Groups

Rats were randomly assigned to four groups. The first group was exposed to ambient temperature of 25 °C to reach thermal equilibrium and was used as the normothermic control (CONT). The second group was exposed to an ambient temperature of 43 °C (with relative humidity of 60% in a temperature-controlled chamber) for heatstroke (HS). The third group was pretreated with etanercept (0.9 mg/kg body weight, i.p.) one day before the start of heat stress (HS + Etanercept pretreatment).The fourth group was treated with etanercept (0.9 mg/kg body weight, i.v.) immediately after the onset of heatstroke (HS + Etanercept cotreatment). The time at which the mean arterial pressure (MAP) dropped more than 25 mmHg from the peak level was defined as the onset of heatstroke (time 0 shown in Fig. 1). After the onset of heatstroke, the animals were allowed to recover at room temperature (25 °C). The latent period for the onset of heatstroke was defined as the interval between the start of heat exposure and the onset of heatstroke, indicating the degree of tolerance of hyperthermia. The selected dose of etanercept was modified from a previous study[14] and also tested for its efficacy in our pilot study.

Surgery and the Monitoring of Physiological Parameters

In rats under anesthesia (pentobarbital, 50 mg/kg i.p.), the right femoral artery and vein were cannulated with a polyethylene tubing (PE50) under anesthesia for blood pressure monitoring and drug administration. Physiologic monitoring such as colonic temperature (Tco) was monitored continuously by a thermocouple, and both mean arterial pressure (MAP) and heat rate (HR) continuously monitored through a pressure transducer.

Measurement of Plasma TNF-α and IL-6

Blood samples were taken at baseline, pre-heatstroke (Pre-HS), heatstroke onset (HS onset) and 10, 20, 30, 45, 60 min post-heatstroke (Post-HS 10, 20, 30, 45, 60 min) for determination of TNF-α and IL-6 levels. The concentrations of cytokines were determined using commercial ELISA kits (R&D system, Minneapolis, MN, USA) and were expressed as picograms per milliliter.

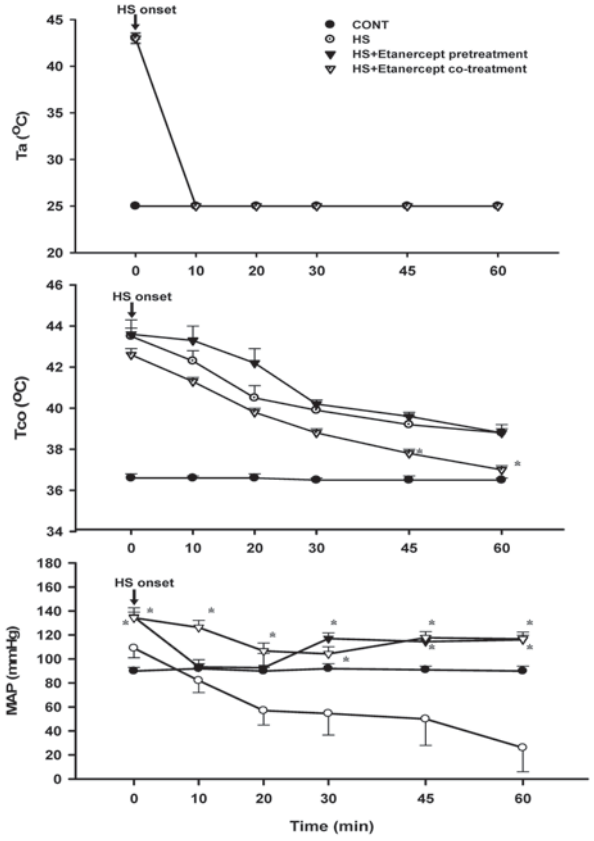

Fig 1. Time-course changes in ambient temperature (Ta), colonic temperature (Tco), and mean arterial pressure (MAP) evaluated in normothermic control rats (●), in rats subjected to heat stress (43°C Ta) (○), in rats subjected to heat stress (43°C Ta) with etanercept (0.9 mg/kg, i.p.) pretreatment 1 day before the start of heat stress (▼), in rats subjected to heat stress (43°C Ta) with etanercept (0.9 mg/kg, i.v.) co-treatment immediately after the onset of heatstroke (▽). The onset of heat stroke is indicated by the arrow. Data are the means±SEM; CONT (n=6), HS (n=12), HS+Etanercept pretreatment (n=8), and HS+Etanercept co-treatment (n=7). ***P < 0.05 versus HS group**. The onset of heatstroke is indicated by the arrowhead.

Statistical Analysis

Statistical analysis was performed with a repeated measure one-way analysis of variance (ANOVA) followed by the Bonferroni test (SPSS Inc., Chicago, IL, USA). A probability of $P < 0.05$ was taken to indicate a significant

Table 1 Plasma level of TNF-α in different heatstroke groups

TNF-α (pg/ml)	Baseline	Pre-HS	HS onset	Post-HS(min) 10	20	30	45	60
CONT	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
HS	0 ± 0	84 ± 10*	229 ± 90*	349 ± 65*	375 ± 77*	401 ± 89*	427 ± 101*	453 ± 114*
HS+Etanercept pretreatment	0 ± 0	89 ± 30*	120 ± 20*	181 ± 23*+	250 ± 46*	302 ± 92*+	328 ± 130*	302 ± 98*+
HS+Etanercept co-treatment	2 ± 1	74 ± 33*	81 ± 15*	89 ± 11*+	161 ± 59*	104 ± 37*+	164 ± 57*+	75 ± 10*+

TNF-α Tumor necrosis factor-α. HS, Heatstroke. Heat exposure in HS rats was withdrawn after 40 min exposure and then were allowed to recover at room temperature (25 °C) for 2 hrs. CONT (n=6), rats with normothermic control; HS (n=7), rats subjected to heat stress (43 °C); HS+Etanercept pretreatment (n=6), rats subjected to heat stress with etanercept pretreatment 1 day before the start of heat stress; HS+Etancecept co-treatment (n=5), rats subjected to heat stress with etanercept immediately after the onset of heatstroke. Data are means±SEM ; ***P < 0.05 vs. CONT**; **+ P < 0.05 vs. HS**

Table 2 Plasma level of IL-6 in different heatstroke groups

IL-6 (pg/ml	Baseline	Pre-HS	HS onset	Post-HS (min) 10	20	30	45	60
CONT	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
HS	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	277 ± 78*	344 ± 67*	391 ± 193*
HS+Etanercept pretreatment	0 ± 0	30 ± 13*+	246 ± 118*+	407 ± 221*+	438 ± 173*+	702 ± 130*+	748 ± 38*+	1000 ± 69*+
HS+Etanercept co-treatment	0 ± 0	0 ± 0	29 ± 18*+	55 ± 21*+	222 ± 74*+	419 ± 138*	614 ± 220*	609 ± 174*

IL-6, Interleukin-6. HS, Heatstroke. Heat exposure in HS rats was withdrawn after 40 min exposure and then were allowed to recover at room temperature (25 °C) for 2 hrs. CONT (n=6), rats with normothermic control; HS (n=7), rats subjected to heat stress (43 °C); HS+Etanercept pretreatment (n=6), rats subjected to heat stress with etanercept pretreatment 1 day before the start of heat stress; HS+Etancecept co-treatment (n=5), rats subjected to heat stress with etanercept immediately after the onset of heatstroke. Data are means ± SEM ; ***P < 0.05 vs. CONT**; **+ P < 0.05 vs. HS**

difference between means. Values are expressed as means ±SEM.

RESULTS

Physiological Parameters

Figure 1 shows the effects of a high ambient temperature (43 °C) on Tco and MAP in experimental groups. Compared with HS rats, the values of Tco were significantly lower at times 45 to 60 min after heatstroke onset (time 0) in rats cotreated with etanercept. Etanercept treatment did not significantly change the Tco value. On the other hand, the MAP at heatstroke onset was significantly higher in rats pretreated and cotreated than in those without treatment. Both etanercept cotreatment and pretreatment could significantly attenuate heatstroke-induced hypotension during the observation period.

Plasma TNF-α

Table 1 shows the effect of heat exposure on plasma TNF-α in experimental rats. Plasma levels of TNF-α were significantly increased in HS rats as compared with normothermic controls from the start of heat stress to the end of the study. Etanercept cotreatment significantly attenuated the heat stress-induced increased levels of TNF-α at 10, 30, 45, and 60 min after heatstroke onset (Post-HS) compared with untreated rats. The etanercept pretreatment also significantly suppressed the plasma TNF-α levels at 10, 30, and 60 min after heatstroke onset (Post-HS) compared with untreated rats.

Plasma IL-6

Table 2 shows the effect of heat exposure on plasma IL-6 in experimental rats. Plasma IL-6 levels were significantly increased compared with control rats from the start of heat stress to the end of the study. Etanercept pretreatment significantly increased the plasma IL-6 levels before heatstroke onset (Pre-HS; Tco = 41.2 °C), heatstroke onset (HS onset) and at 10 to 60 min after the heatstroke onset (Post-HS), whereas etanercept cotreatment significantly increased the plasma IL-6 levels at HS onset and at 10 to 20 min after heatstroke onset (Post-HS).

Survival Rate, MAP and the Latency and Core Temperature at Heatstroke Onset

Table 3 summarizes the latent period, survival rate, MAP and Tco at heatstroke onset in rats. Etanercept pretreatment, but not cotreatment, significantly increased

Table 3 The values of the survival rate, MAP, the latency and Tco at heatstroke onset

Treatment groups	Latency at heatstroke onset (min)	Survival rats /total rats	MAP at heatstroke onset (mmHg)	Tco at heatstroke onset (°C)
CONT	＞480	6/6	—	—
HS	36 ± 3	7/12	109 ± 8	43.5 ± 0.4
HS+Etanercept pretreatment	48 ± 4+	6/8	135 ± 8+	43.6 ± 0.7
HS+Etanercept co-treatment	36 ± 4	5/7	134 ± 5+	42.6 ± 0.3

MAP, Mean arterial pressure. Heat exposure in HS rats was withdrawn after 40 min exposure and then were allowed to recover at room temperature (25 °C) for 2 hrs. Survival rats/total rats, the ratio of survival rats to total rats from initiation of heatstroke to the 60-min observation period, CONT (n=6), rats with normothermic control; HS (n=12), rats subjected to heat stress (43 °C); HS+Etanercept pretreatment (n=8), rats subjected to heat stress with etanercept pretreatment 1 day before the start of heat stress; HS+Etancecept co-treatment (n=7), rats subjected to heat stress with etanercept immediately after the onset of heatstroke. Data are means ± SEM ; ***P ＜ 0.05 vs. CONT**; **＋ P ＜ 0.05 vs. HS**

the latent period until HS onset. In addition, both etanercept pretreatment and cotreatment significantly increased survival rate and MAP levels at HS onset. However, the Tco at heatstroke onset did not change with etanercept pretreatment or cotreatment in HS rats.

DISCUSSION

Cytokines are important regulators of the acute phase response to inflammation/injury and have been implicated as mediators of heatstroke. In the current study, all heatstroke rats displayed hypotension, hyperthermia and inflammation (evidenced by increased levels of TNF-α and IL-6 in plasma) during heatstroke. Both pretreatment and cotreatment with etanercept (a soluble fusion protein that binds specifically to human TNF) attenuated heatstroke-induced arterial hypotension and elevated plasma levels of TNF-α, as well as increased the survival rate. Etanercept pretreatment also enhanced the tolerance to hyperthermia as indicated by prolongation of the latent period and an increase in the magnitude of the blood pressure required to trigger heatstroke. Collectively, these results suggest that etanercept is a potential therapeutic agent for heatstroke.

The present study showed that plasma TNF-α levels increased significantly in HS rats in response to heat stress. The increase in circulating TNF-α is negatively correlated with the change in arterial blood pressure during the onset of heatstroke. Consistent with our observation, the plasma concentrations of inflammatory cytokines (including TNF-α and IL-1α are elevated in subjects with heatstroke[4-6, 15, 16]. Heatstroke has been documented to induce systemic and local (central nervous system) production of TNF-α and IL-1 in both rats and rabbits[7, 17]. The concentration of both TNF-α and IL-1α correlates well with the severity of heat stroke in previous studies[4, 16]. In fact, TNF-α, a proinflammatory cytokine, has been proposed to be involved in the pathogenesis of vascular dysfunction. In a study of cultured endothelial cells, TNF-α decreased the expression of endothelial NO synthase[18], which is the primary enzyme involved in NO production in the vasculature. Moreover, TNF-α can induce the activity of NAD(P)H oxidase[19], an enzyme that is a major source of superoxide anions in the vasculature[20]. Our present observations showed that etanercept pretreatment and cotreatment attenuated the heatstroke-induced elevation of plasma TNF-α level in rats. In addition, both arterial hypotension and survival rate were concomitantly improved, which may be attributable to reduction in the TNFα-induced production of reactive oxygen species (ROS) and suppression of TNF-α-induced NO production. These findings need to be further elucidated.

In the present study, the heatstroke-induced increase in plasma IL-6 was further enhanced after etanercept pretreatment and cotreatment. Elevated plasma levels of IL-6, a pleiotropic inflammatory cytokine produced by T cells, macrophages or monocytes[21], has been observed in patients with classic heat stroke[16]. In this study, it is possible that etanercept administration neutralized the circulating TNF-α protein and thus suppressed the action of TNF-α on tissues and organs. On the other hand, etanercept also diminishes the negative feedback signaling component of the regulation of TNF-α production, which could enhance local production of TNF-α and thus stimulate the release of IL-6, as shown in the present study. A previous study demonstrated that IL-6 knockout mice have higher heat stroke mortality than their littermates and also suggested that the importance of IL-6 synthesis, especially in liver, is its potential protective function in the liver and whole body via regulation of the acute phase response. Therefore, the elevation of circulating IL-6 is speculated to be an indirect mediator of the beneficial effect of etanercept in heatstroke treatment.

In conclusion, heatstroke significantly increased circulating TNF-α and IL-6, and attenuated arterial hypotension, hyperthermia and circulatory shock. Both etanercept pretreatment and cotreatment could attenuate the elevation of plasma TNF-α and heatstroke-induced arterial hypotension and improve the survival rate in HS rats. In addition, etanercept pretreatment could significantly enhance the tolerance to hyperthermia. Our observations imply that

etanercept is a potential therapeutic treatment for heatstroke.

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