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A potential for granulocyte-colony stimulating factor for use as a prophylactic agent for heatstroke in rats

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ABSTRACT

Heatstroke is a form of excessive hyperthermia associated with a systemic inflammatory response that leads to multi-organ dysfunction in which central nervous system disorders predominate. Herein we determined to ascertain whether heat-induced multi-organ dysfunction in rats could be attenuated by granulocyte-colony stimulating factor (G-CSF) preconditioning. Anesthetized rats were divided into 2 major groups and given vehicle solution (isotonic saline, 0.3 ml, subcutaneously) or G-CSF (50–200 μg/kg body weight in 0.3 ml normal saline, subcutaneously) daily and consecutively for 5 days before the start of thermal experiments. They were exposed to an ambient temperature of 43 °C for 68 min to induce heatstroke. G-CSF preconditioning significantly prolonged the survival time in heatstroke rats in a dose-related way (82–98 min vs 127–243 min). The non-preconditioning heatstroke animals showed hyperthermia, arterial hypotension, increased serum levels of systemic inflammatory response molecules, increased hypothalamic apoptotic cell numbers as well as neuronal damage scores, and increased serum levels of renal and hepatic dysfunction indicators. These heatstroke syndromes could be significantly reduced by G-CSF preconditioning. Thus our results revealed a potential for G-CSF used as a prophylactic agent for heatstroke in rats.

1. Introduction

Granulocyte colony-stimulating factor (G-CSF) is a polypeptide growth factor that stimulated the proliferation, survival and maturation of the neutrophilic granulocyte lineage (Xiao et al., 2007). G-CSF has been used for haematopoietic stem cell mobilization into the peripheral circulation (Lu and Xiao, 2006). It showed that G-CSF preconditioning decreased mortality rate, reduced infarction volume, and improved neurological behavior after cerebral ischemia in rats (Lu and Xiao, 2006).

Heatstroke is a form of excessive hyperthermia (core temperature rising above 40 °C) associated with a systemic inflammatory response that leads to multi-organ dysfunction in which central nervous system disorders predominate (Bouchama and Knochel, 2002; Chang et al., 2006). In an anesthetized rat model, the heatstroke animals display hyperthermia, hypotension, hypothalamic neuronal apoptosis and degeneration, up-regulation of systemic inflammation, and hepatic and renal dysfunction (Lin et al., 2009; Liu et al., 2009). However, it is unknown whether human recombinant G-CSF can be used as a prophylactic agent for experimental heatstroke.

To deal with the hypothesis, this study attempted to assess the temporal profiles of hyperthermia, hypotension, hypothalamic neuronal apoptosis and degeneration, hepatic and renal cell apoptosis, systemic inflammatory indicators, and bone marrow expression of endothelial progenitor cells (EPCs) during heatstroke in rats (Lin et al., 2009; Liu et al., 2009) with or without G-CSF preconditioning.

2. Materials and methods

2.1. Experimental animals

Adult male Sprague–Dawley rats (weight, 285–315 g) were obtained from the Animal Resource Center of the National Science Council of Republic of China. The animals were housed 4 in a cage at an ambient temperature of 22 ± 1 °C, with a 12-h light/dark cycle. Pellet rat

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chow and tap water were available *ad libitum*. The experimental protocol was approved by the Animal Ethics Committee of the Chi Mei Medical Center. Animal care and experiments were conducted according to the National Science Council Guidelines. They were allowed to become acclimated for ≥1 week. Adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes induced by tail pinching throughout all experiments (approximately 8 h) by an intraperitoneal dose of urethane (1.4 g/kg body weight). At the end of the experiments, control rats and any rats that had survived heatstroke were killed with an overdose of urethane.

The right femoral artery of rats was cannulated with polyethylene tubing (PE50), under urethane anesthesia, for blood pressure monitoring. Core temperature was monitored continuously by a thermocouple (DR130, Yokogawa, Yamanashiken, Japan) inserted into the rectum, while the mean arterial pressure and heart rate were continuously monitored with a pressure transducer and a chart recorder (2107, Gould, Valley view, OH, USA).

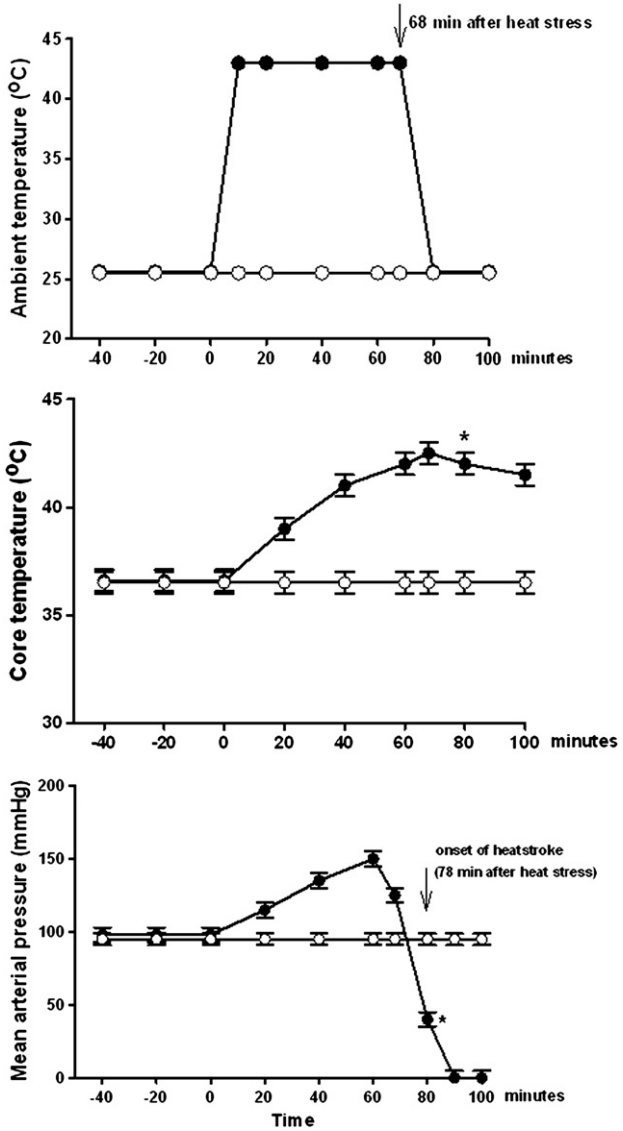

Fig. 1. Values of core temperature (Tco) and mean arterial pressure (MAP) for the normothermic rats exposed to an ambient temperature of 26 °C (○) and heatstroke rats exposed to an ambient temperature of 43 °C (●). Mean arterial pressure started to drop at 68 min and reached a value of about 45 mm Hg at 78 min. The time point of (78 min) was arbitrarily defined as the onset of heatstroke. Data were means ± S.D. of 6 animals per group. *P<0.05, in comparison with (●) group.

2.2. Induction of heatstroke

Before the induction of heat stress, the core temperature of the anesthetized animals was maintained at about 37 °C with a folded heating pad except in the heat stress experiment. Heatstroke was induced by patting the animals in a folded heating pad of 43 °C controlled by circulating hot water. As shown in Fig. 1, the time point (68 min) at which the mean arterial pressure dropped from the peak to a value of <50 mm Hg and core temperature over 42 °C was arbitrarily taken as the onset of heatstroke (Chen et al., 2005, 2007). Immediately after this time point (68 min), the heating pad was removed and the animals were allowed to recover at room temperature (26 °C). Seventy-eight minutes after the start of heat stress (or 10 min after the time point for the onset of heatstroke), the animals displayed both hyperthermia (~42 °C) and arterial hypotension (~48 mm Hg). The survival time was defined by the interval between the start of heat stress and the animal death.

Seventy-eight minutes after the start of heat stress for the heatstroke rats or equivalent time period for the normothermic rats, the samples were obtained for determination of neuronal damage score, number of apoptotic cells in multiple organs, serum levels of tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), and soluble intercellular adhesion molecule-1 (ICAM-1), and bone marrow levels of endothelial progenitor cells (EPCs).

2.3. Experimental groups

The animals were assigned randomly to one of four groups. The first group and the second group, respectively, treated with a subcutaneous (s.c.) dose of vehicle solution (1 mL normal saline per kilogram body weight) or human recombinant G-CSF (50–200 μg/kg body weight) daily and consecutively for 5 days, were exposed to an ambient temperature of 26 °C. These two groups were used as normothermic groups. The third group and the fourth group, respectively treated with an s.c. dose of vehicle solution or G-CSF daily and consecutively for 5 days, were exposed to an ambient temperature of 43 °C for exactly 68 min and were used as vehicle-treated heatstroke group or G-CSF-treated heatstroke group. Human G-CSF was supplied by the Kyowa Hakko Kogyo Co., Ltd. This G-CSF was highly purified (99%) and was endotoxin free as restrictively confirmed by Limulus amoebocyte lysate test. For injection, G-CSF was dissolved in sterile saline. Subcutaneous injection of G-CSF (or vehicle solution) was conducted 5 days before the start of thermal experiments.

2.4. Histological verification

At the end of the experiments, the animals were killed by an overdose of urethane and the brains were fixed *in situ* and left in the skull in 10% neutral buffered formalin for at least 24 h before removal from the skull. The brain was removed and embedded in paraffin blocks. Serial (10 μm) sections through the hypothalamus were stained with hematoxylin and eosin for microscopic evaluation. The extent of cerebral neuronal damage in hypothalamic section was scored on a scale of 0 to 3, modified from the grading system of Pulsinelli et al. (1982), in which 0 was normal, 1 indicated that approximately 30% of the neurons was damaged, 2 indicated that approximately 60% of the neurons was damaged, and 3 indicated that 100% of the neurons was damaged. Each hemisphere was evaluated independently without the examiner knowing the experimental conditions.

2.5. TUNEL assay for apoptotic cells

In situ apoptosis detection kit (Clontech, USA) was employed to assess apoptosis by using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) method.