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Protection in rats with heatstroke: Hyperbaric oxygen vs activated protein C therapy

Chao-Hung Yeh [a], Zhih-Cherng Chen [b,c], Chuan-Chih Hsu [a], Mao-Tsun Lin [d], Chien-Chang Chen [a,*]

[a] Department of Surgery Chi Mei Medical Center, Tainan, Taiwan
[b] Department of General Medicine, Chi Mei Medical Center, Tainan, Taiwan
[c] Chung Hwa University of Medical Technology, Tainan, Taiwan
[d] Department of Medical Research, Chi Mei Medical Center, Tainan, Taiwan

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ABSTRACT

The present study was attempted to evaluate the therapeutic effects of activated protein C and/or hyperbaric oxygen in an animal model of heatstroke. Sixty-eight minutes heat stress (43 °C) initiated, the anesthetized rats were randomized to several groups and administered: 1) no resuscitation (vehicle solution plus normabaric air, 2) intravenous activated protein C (1 mg in 1 ml of normal saline per kg of body weight), 3) hyperbaric oxygen (100% oxygen at 202 kpa for 17 min), and 4) intravenous activated protein C plus hyperbaric oxygen. Another group of rats exposed to room temperature (26 °C) was used as normothermic controls. Blood sampling was 0 min, 70 min, and 85 min after heat stress initiated. When the vehicle-treated rats underwent heat exposure, their survival time values found were to be 19–25 min. Resuscitation with activated protein C or hyperbaric oxygen significantly and equally improved survival during heatstroke (134–159 min). As compared with those of activated protein C or hyperbaric oxygen alone, combined activated protein C and hyperbaric oxygen significantly had higher survival time values (277–347 min). All vehicle-treated heatstroke animals displayed systemic response, hypercoagulable state, and hepatic and renal dysfunction. Combined activated protein C and hyperbaric oxygen therapy reduced these heatstroke reactions better than activated protein C or hyperbaric oxygen alone. The results indicate consequently, combined activated protein C and hyperbaric oxygen therapy heightens benefit in combating heatstroke reactions.

1. Introduction

Heatstroke is a life-threatening illness characterized by body hyperpyrexia (over 42 °C) and multiple organ dysfunction or failure (such as renal, cardiovascular, hematologic, hepatic, and brain disorders) (Simon 1993; Bouchama and Knochel, 2002; Chang et al., 2006). When the unanesthetized rodents were exposed to severe heat stress, they had hyperthermia (over 42 °C core temperature), hypotension (<60 mm Hg mean arterial pressure), intracranial hypertension, and central nervous system dysfunction such as delirium, convulsion, and coma (Shih et al., 1984). The time point in which the occurrence of hypotension (~60 mm Hg) was arbitrarily taken as the onset of heatstroke for the anesthetized animals. It was found that, at the time point of the onset of heatstroke, the anesthetized animals displayed systemic inflammation, activated coagulation or hypercoagulable state, and multiorgan dysfunction or failure (Chen et al., 2006b; Niu et al., 2007; Shen et al., 2008).

Recombinant human activated protein C is recommended for patients at high risk of death (septic shock, sepsis-induced acute respiratory distress syndrome, Acute Physiology and Chronic Evaluation II score ≥25, and sepsis-induced multiple organ failure) who have no absolute contraindication related to bleeding risk or relative contraindication outweighing the potential benefit (Fourrier 2004). Heatstroke patients display shock, acute respiratory distress syndrome, coma, and multiple organ failure) (Simon 1993; Bouthama and Knochel, 2002). Indeed, it has been shown that systemically delivering activated protein C beneficially improves heatstroke outcomes in both rats (Chen et al., 2006a) and humans (Brueckmann et al., 2006). Activated protein c inhibits clotting factors va and VIIIa, which are necessary for thrombin formation (Bernard et al., 2001). By introducing extrinsic activated protein C, the formation of thrombin is reduced, the clotting cascade is slowed, and finally the severity of disseminated intravascular coagulation (DIC) can be limited (Bernard et al., 2001). It is also shown that activated protein C has an anti-inflammatory property (Opal et al., 2002; Esmon 2001).

Hyperbaric oxygen therapy is noninvasive, non intrusive medically for a person to breathe 100% oxygen at a pressure greater than normal (Leifer 2001). This combined hyperoxia and hyperbaric pressure greatly enhances the quantity of oxygen both dissolved in the plasma, and physically dissolved in plasma at an extent of 0.003 mL·100 mL plasma^{-1}·1 mm Hg^{-1}. When the partial pressure of inspired oxygen increases from 110 mm Hg in air to 200 mm Hg, the hemoglobin

* Corresponding author. Department of Surgery, Chi Mei Medical Center, Tainan, 710, Taiwan. Tel./fax: +886 62 517850.
E-mail address: 891201@mail.chimei.org.tw (C.-C. Chen).

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oxygen saturation would increase from 97% to 100%. At 253 kpa, the quantity of oxygen dissolved in plasma reaches a value of 5.1 mL·100 mL plasma^{-1}·2.5 bar^{-1} (0.003 mL·100 mL plasma^{-1}·1 mm Hg^{-1}× [253 kpa×760 mm Hg/101 kpa]−200 mm Hg). Indeed, we have previously demonstrated that hyperbaric oxygen therapy decreasing multiple organ dysfunction may resuscitate heatstroke rats (Niu et al., 2007; Tsai et al., 2005). Additionally, hyperbaric oxygen therapy has been used successfully treating a heatstroke patient with multiple organ dysfunction (Niu et al., 2009).

The present work was to: assess the protective effects of activated protein C or hyperbaric oxygen therapy in heatstroke rats having hyperpyrexia, hypotension, activated inflammation, hypercoagulable state, and multiple organ dysfunction; ascertain whether combined activated protein C and hyperbaric oxygen therapy protects heatstroke rats more.

2. Materials and methods

2.1. Animals

Adult Sprague–Dawley rats (weight 276±17 g) were obtained from the Animal Resource center of the National Science Council of the Republic of China (Taipei, Taiwan). The animals were housed 4 in a group at an ambient temperature of 22±1 °C, with a 12-h light/dark cycle. Pellet rat chow and tap water were available *ad libitum*. All protocols were approved by the Animal Ethics committee of the Chi Mei Medical Center (Tainan, Taiwan) in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act. Adequate anesthesia was maintained to abolish the corneal reflex and Pain reflexes induced by tail pinching throughout all experiments (approximately 8 h) by a single 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.

2.2. Surgery and physiological parameter monitoring

The right femoral artery and vein of rats were cannulated with polyethylene tubing (PE50), under urethane anesthesia, for blood pressure monitoring and drug administration. Core temperature was monitored continuously by a thermocouple, while mean arterial pressure was continuously monitored with a pressure transducer.

2.3. Heatstroke induction

The core temperature of the anesthetized animals was maintained at about 36 °C with an infrared light lamp except the heat stress experiments. Heatstroke was animal placement-induced in a folded heating pad (43 °C controlled by circulating hot water). The instant in which the mean arterial pressure dropped to a value of about 60 mm Hg was taken as the heatstroke onset. After the onset the heating pad was removed, and the animals were allowed to recover at room temperature (26 °C). Our pilot results showed that the latency for heatstroke onset in vehicle-treated rats found was 70±2 min (n=8). Therefore, the following heatstroke groups were exposed to 43 °C for exactly 70 min and then allowed to recover at room temperature (26 °C).

2.4. Experimental group

Animals were assigned randomly to one of following 4 groups. The first group, treated with an i.v. vehicle (1 ml of 0.9% NaCl solution per kg of body weight), was exposed to an ambient temperature of 26 °C, and their physiological parameters were continuously recorded for up to 480 min (or at the experimental end). This group was as normothermic controls. The second group treated with an i.v. vehicle (0.3 ml normal saline) per rat immediately after heat exposure initiated (T=0), was as vehicle-treated heatstroke animals. The third groups was treated with i.v. recombinant human activated protein C (drotrecogin alfa, [activated]×igris; Eli Lilly, Vienna, Austraia; 10 mg in 1 ml of normal saline per kilogram of body weight) 70 min after the heat exposure onset (or the time point of the heatstroke onset). The fourth group was resuscitated directly after instrumentation with 100% oxygen at 202 kpa for 15 min. The chamber filled with pure oxygen (100%) was pressurized to 202 kpa at a rate of 40 kpa/min for 15 min and was terminated at the decompression rate of 20 kpa/min. The fifth group was resuscitated immediately after the heatstroke

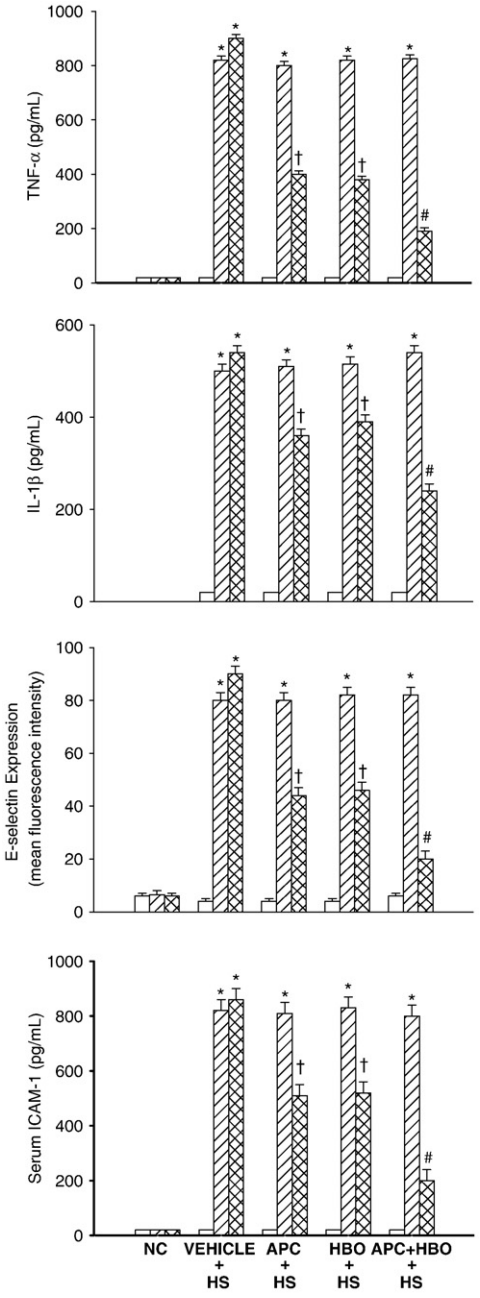

Fig. 1. The serum levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), E-selectin, and ICAM-1 0 (□), 70 (▨), and 85 (▩) min after the onset of heat exposure (43 °C) onset for vehicle-treated heatstroke (HS) rats (VEHICLE + HS; n=8), activated protein C (APC)-treated heatstroke (HS) rats (ACP + HS; n=8), hyperbaric oxygen (HBO)-treated heatstroke rats (APC + HS; n=8), and (APC + HBO)-treated heatstroke rats (APC + HBO + HS; n=8). The values of these parameters were obtained at equivalent time points for normothermic controls (NC; n=8). $^{*}P$<0.05 compared with NC; $^{\dagger}P$<0.05 compared with VEHICLE + HS group; and $^{\#}P$<0.05 compared with APC + HS group or HBO + HS group (Dunn's test-followed Kruskal–Wallis test).