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14. ABSTRACT

Novel therapeutic advances in the field of trauma management depend upon animal models that can accurately predict the clinical efficacy of interventions. In the battlefield, traumatic brain injury (TBI) and hemorrhagic shock often occur concomitantly due to multiple injuries. In this project, we determined the long-term effect of ghrelin, a 'gut-brain' hormone, in a highly military relevant experimental rat model of TBI combined with uncontrolled hemorrhagic shock. Our results showed that ghrelin improves long-term brain function, prevents weight loss and decreases mortality after traumatic brain injury and uncontrolled hemorrhagic shock. Thus, ghrelin can be further developed as a safe and effective resuscitation approach for the trauma victim with brain injury and severe blood loss.

15. SUBJECT TERMS
Traumatic brain injury; hemorrhagic shock; ghrelin; treatment

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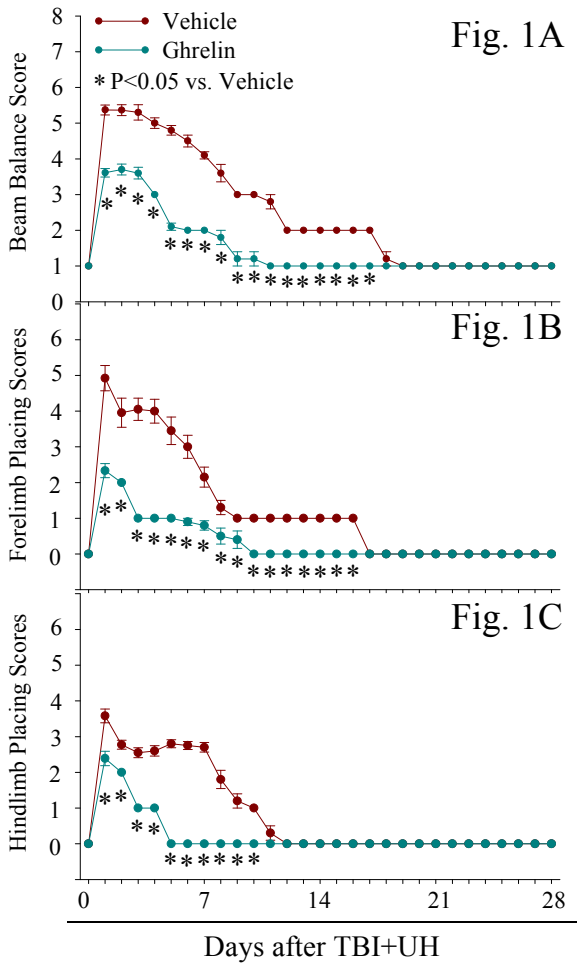
INTRODUCTION

Traumatic brain injury (TBI) and hemorrhagic shock often occur concomitantly in the battlefield due to multiple injuries (1,2). Hypotension markedly exacerbates secondary damage in the traumatically injured brain and doubles TBI mortality. TBI, on the other hand, impairs shock compensation. In this regard, a therapeutic intervention to treat posttraumatic hypotension and prevent secondary ischemia would be a powerful tool to improve outcome after brain injury. In this project, we determined the long-term effect of ghrelin, a 'gut-brain' hormone (3), in a highly military relevant experimental rat model of TBI combined with uncontrolled hemorrhagic shock.

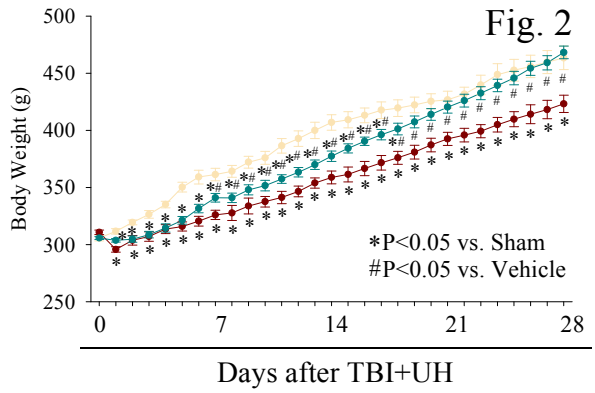
BODY

Long-term effects of ghrelin on sensorimotor and reflex function after traumatic brain injury and uncontrolled hemorrhagic shock: Traumatic brain injury (TBI) and hemorrhagic shock, the most common causes of trauma deaths, often occur concomitantly due to multiple injuries (1,2). In this regard, the rat model of TBI combined with uncontrolled hemorrhagic shock, as we developed last year, was used in this study. Briefly, brain injury was induced by dropping a 450 g weight from 1.5 m onto a steel helmet attached to the skull of male Sprague-Dawley rats weighing 325-375 g. Immediately after TBI, the rat was subjected to non-lethal uncontrolled hemorrhage (UH) induced by venous injury (4). Briefly, a midline laparotomy was performed and both lumbar veins were isolated and severed at the junction with the vena cava. The abdomen was kept open but covered with a saline wet gauze for 20 min, and it was closed in layers thereafter. At 45 min after TBI and UH, the animals were intravenously resuscitated with 1 ml normal saline (i.e., low volume resuscitation) with or without ghrelin (16 nmol/rat) over 10 min. In order to determine long-term effects of ghrelin on neurological damage following TBI and uncontrolled hemorrhage, ghrelin (16 nmol/rat) or vehicle (1 ml normal saline) was *sc*. administered daily for 10 days starting at 4 h after TBI+UH. Neurological functional impairments and improvements were measured daily for up to 28 days after TBI+UH with or without ghrelin treatment by scales of beam balance test, forelimb placing test and hindlimb placing test, indicating sensorimotor functions and reflex. As shown in **Figure 1A**, immediately after TBI and UH, beam balance scores increased markedly in both vehicle and ghrelin treated animals. However, ghrelin treated animals had much lower beam balance scores than vehicle treated animals. Moreover, it took 19 days for beam balance scores to return to normal in the vehicle-treated animals. Beam balance scores in ghrelin treated animals returned to normal in 11 days. As shown in **Figure 1B**, forelimb placing scores increased significantly after TBI and UH. Ghrelin treatment significantly reduced forelimb placing scores after TBI and UH as compared with vehicle treatment (**Fig. 1B**). Regarding the subtests of the forelimb placing tests (i.e., visual, tactile, and proprioceptive), enhanced recovery was seen on all subtests following ghrelin treatment (data not shown). Similarly, hindlimb placing scores increased significantly after TBI and UH (**Fig. 1C**). Ghrelin treated animals had significantly lower hindlimb placing scores than vehicle treated animals. Hindlimb placing scores also returned to normal quicker in ghrelin treated animals than vehicle treated animals (**Fig. 1C**).

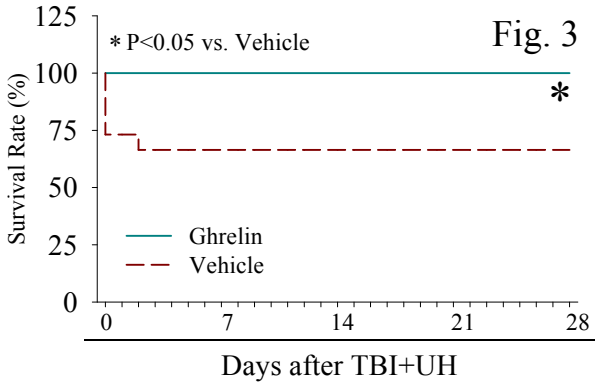


Effects of ghrelin on body weight gain after traumatic brain injury and uncontrolled hemorrhagic shock: Ghrelin plays an important role in the regulation of food intake. To determine the effects of ghrelin on body weight gain after TBI and UH, male adult rats were subjected to TBI and UH or sham operation. The TBI and UH animals were treated with ghrelin (16 nmol/rat) or vehicle (1 ml normal saline) daily for 10 days as described above. The animals

were allowed food and water *ad libitum* after TBI+UH and were monitored for 28 days to record body weight changes. As shown in **Figure 2**, sham operated rats gained 5.6±0.60 grams every day. A significant drop in body weight was observed at day 1 after TBI-UH in vehicle treated animals. Then, the animals started to gain body weight at day 2 after TBI-UH, however, at a slower pace (4.7±0.27 grams every day) than sham operated animals. At 28 days after TBI-UH, the body weight of vehicle treated animals was 8.7% lower than that of sham operated animals. A slight drop in body weight was observed at day 1 after TBI-UH in ghrelin treated animals. Moreover, ghrelin treated animals gained body weight at a quicker pace (6.1±0.42 grams every day) than vehicle treated animals after TBI and UH. At day 7 after TBI-UH, the body weight in ghrelin treated animals were significantly higher than that in vehicle treated animals. At day 28 after TBI-UH, the body weight in ghrelin treated animals was even slightly higher than that of sham operated animals.



Effects of ghrelin on survival after traumatic brain injury and uncontrolled hemorrhagic shock: To determine the effects of ghrelin on survival after TBI and UH, male adult rats were subjected to TBI and UH or sham operation. The TBI and UH animals were treated with ghrelin (16 nmol/rat) or vehicle (1 ml normal saline) daily for 10 days as described above. The animals were allowed food and water ad libitum after TBI+UH and were monitored for 28 days to record survival. As shown in **Figure 3**, the survival rate after TBI and UH was 73% at day 1 and reduced to 66% at days 3-28. On the other hand, there was no mortality in ghrelin treated TBI+UH animals during the 28 day observation period.



Two Tasks were proposed in the Statement of Work. The Tasks have not been modified. The above described accomplishments are associated with Task 2 (i.e., To determine the long-term effect of ghrelin on brain function following TBI and uncontrolled hemorrhage.).

KEY RESEARCH ACCOMPLISHMENTS

1. Ghrelin treatment improves long-term brain function after traumatic brain injury and uncontrolled hemorrhagic shock.

2. Ghrelin treatment prevents weight loss after traumatic brain injury and uncontrolled hemorrhagic shock.

3. Ghrelin treatment decreases mortality after traumatic brain injury and uncontrolled hemorrhagic shock.

REPORTABLE OUTCOMES

Based on the work supported by this DoD grant, we have published one abstract [*Qi L, Dong W, Nicastro J, Coppa GF, Wang P, Wu R: Ghrelin attenuates brain injury after traumatic brain injury and uncontrolled hemorrhagic shock in rats. Crit Care Med 38(12, Suppl):72, 2010.*], one review paper [*Qi L, Jacob A, Wang P, Wu R: Peroxisome proliferator activated receptor-γ and traumatic brain injury. Int J Clin Exp Med 3:278-287, 2010. (PMCID: PMC2971540)*], and obtained an R21 grant (*Ghrelin and Traumatic Brain Injury, 1R21 NS072608-01A1, PI: Wu, 05/01/11-04/30/13*).

CONCLUSION

Ghrelin improves long-term brain function, prevents weight loss and decreases mortality after traumatic brain injury and uncontrolled hemorrhagic shock. Thus, ghrelin can be further developed as a safe and effective resuscitation approach for the trauma victim with brain injury and severe blood loss, especially for the use in combat casualty care at the far-forward battlefield setting.

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