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Treatment of TBI and concomitant hemorrhage with ghrelin

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14. ABSTRACT TBI and hemorrhagic shock, the most common causes of trauma deaths, often occur concomitantly due to multiple injuries. Hemorrhagic shock markedly exacerbates secondary damage in the traumatically injured brain and doubles TBI mortality. Therefore, a therapeutic intervention to treat posttraumatic hypotension and prevent secondary ischemia would be a powerful tool to improve outcome after brain injury. Ghrelin is a 'gut-brain' hormone mostly produced by the stomach. In this project, we first established a highly military relevant experimental rat model of TBI combined with uncontrolled hemorrhagic shock, and then evaluated the efficacy of ghrelin using this model. Our results showed that ghrelin improves sensorimotor and reflex function, reduces cortical apoptosis and downregulates brain inflammation after traumatic brain injury and uncontrolled hemorrhagic shock. Thus, ghrelin can be further developed as an effective resuscitation approach for trauma victims 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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INTRODUCTION

Traumatic brain injury (TBI) and hemorrhagic shock, the most common causes of trauma deaths, often occur concomitantly due to multiple injuries [1,2]. Hemorrhagic shock markedly exacerbates secondary damage in the traumatically injured brain and doubles TBI mortality. Therefore, a therapeutic intervention to treat posttraumatic hypotension and prevent secondary ischemia would be a powerful tool to improve outcome after brain injury. Ghrelin is a 'gut-brain' hormone mostly produced by the stomach [3]. It was originally reported to induce growth hormone release through stimulation of ghrelin receptors in the central nervous system. However, a large body of evidence has indicated other physiological functions of ghrelin mediated by the central and peripheral ghrelin receptors. However, whether ghrelin plays any role in brain injury and functional recovery following TBI remains unknown. In this project, we first established a highly military relevant experimental model of TBI combined with uncontrolled hemorrhagic shock in rats, and then evaluated the efficacy of ghrelin using this model.

BODY

Establishment of the rat model of traumatic brain injury and uncontrolled hemorrhagic shock:

Novel therapeutic advances in the field of trauma management depend upon animal models that can accurately predict the clinical efficacy of interventions. Traumatic brain injury (TBI) and hemorrhagic shock, the most common causes of trauma deaths, often occur concomitantly due to multiple injuries [1,2]. Hemorrhagic shock markedly exacerbates secondary damage in the traumatically injured brain and doubles TBI mortality [1,4]. TBI, on the other hand, impairs shock compensation. In order to investigate the efficacy of ghrelin in brain trauma, we first established a highly military relevant model: TBI combined with uncontrolled hemorrhagic shock in rats. In the battlefield, TBI injuries occur as a direct result of blast waves (primary blast injury), impact from objects put in motion by the blast (secondary blast injury), and by people being forcefully put in motion by the blast (tertiary blast injury). The brain is clearly vulnerable to both secondary and tertiary blast injury. The most common type of TBI in the battlefield is diffuse axonal injury. In this regard, we chose to use Marmarou's acceleration impact model to induce head trauma. This model induces diffuse cellular and axonal injury in forebrain structures such as sensorimotor cortex and hippocampus but limited brainstem damage [5]. 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. Under combat conditions, most trauma victims are associated with uncontrolled hemorrhage.

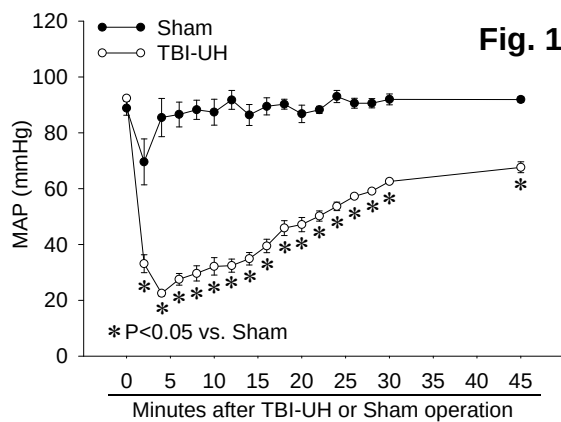


Fig. 1

Therefore, immediately after TBI, the rat was subjected to non-lethal uncontrolled hemorrhage (UH) induced by venous injury [6]. 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) over 10 min. As shown in **Figure 1**, mean arterial pressure (MAP) dropped from about 90 mmHg to below 40 mmHg in 2 min, and reached around 20 mmHg at 4 min after cutting the vein, then slowly went up to about 70 mmHg at 45 min after injury. The overall time stayed below 40 mmHg was about 15 min. The total bleedout volume in this model was 5.88 ± 0.21 ml/rat. To evaluate the neurological damage in this model, the beam balance test, forelimb placing test and hindlimb placing test were performed at 1.5 and 4 h after TBI. As shown in **Figure 2**, animals showed severe disturbances of sensorimotor and reflex function on all three behavioral tests at both 1.5 and 4 h after TBI and UH.

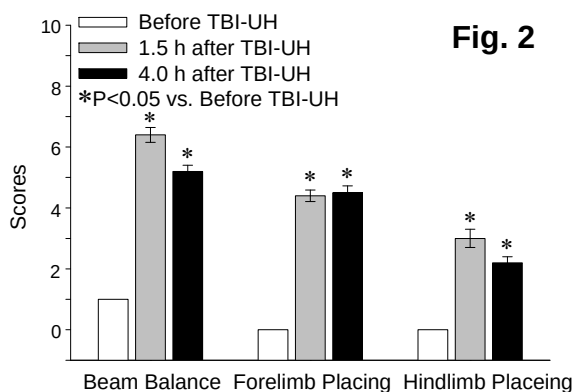
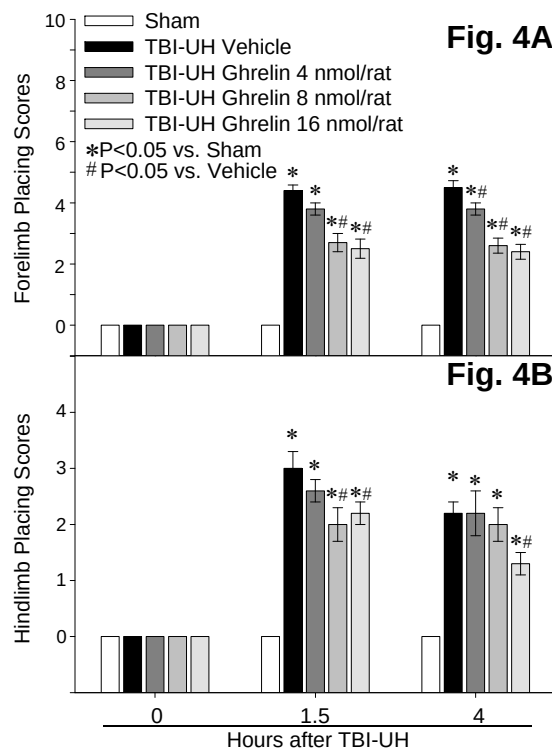
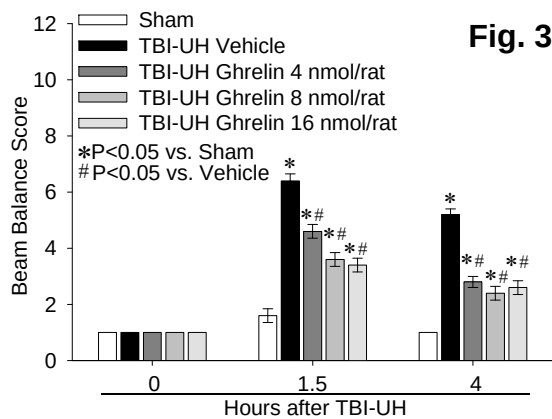


Fig. 2

Effects of ghrelin on sensorimotor and reflex function after traumatic brain injury and uncontrolled hemorrhagic shock:

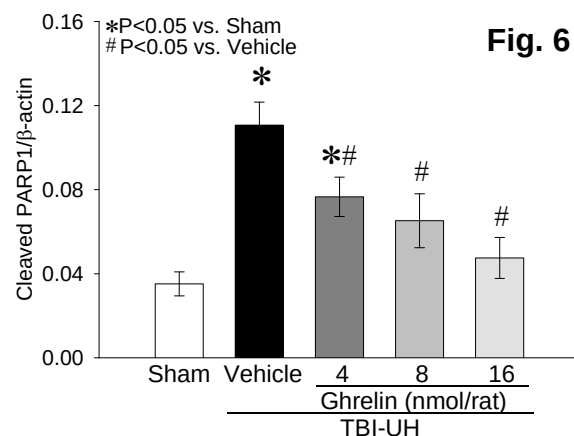
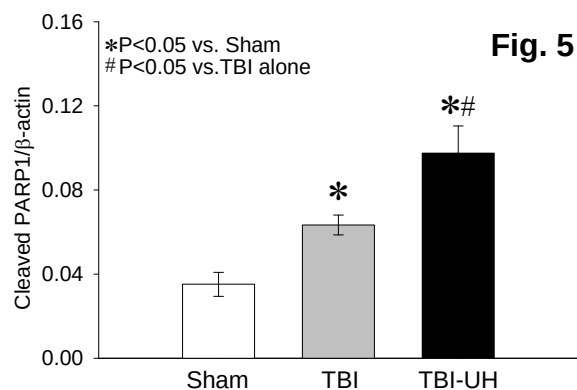
In order to determine the effect of ghrelin on neurological damage following TBI and uncontrolled hemorrhage, brain injury was induced by dropping a 450 g weight from 1.5 m onto a steel helmet attached to the skull of male Sprague-Dawley rats weighing 325-375 g. Immediately after TBI, the rat was subjected to non-lethal uncontrolled



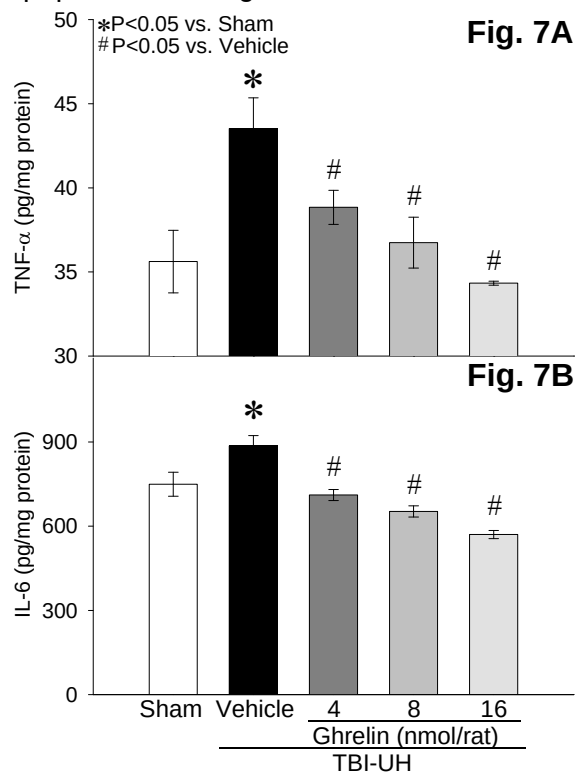
hemorrhage (UH) induced by venous injury as described above. At 45 min after TBI and UH, the animals were intravenously resuscitated with 1 ml normal saline (i.e., low volume resuscitation) with or without various doses of ghrelin over 10 min. At 1.5 and 4 h after TBI and UH, the above mentioned three behavioral tests (i.e., beam balance test, forelimb placing test and hindlimb placing test) were used to assess sensorimotor and reflex function. As shown in **Figure 3**, all three doses of ghrelin treatment significantly improved beam balance scores at both 1.5 h and 4 h after TBI. For the forelimb placing test, slight improvements were found in the 4 nmol/rat ghrelin-treated animals at both 1.5 and 4 h after TBI-UH (**Fig. 4A**). However, these improvements were not statistically significant at 1.5 h after TBI-UH. When 8 or 16 nmol/rat ghrelin was administered, all the improvements were statistically significant (**Fig. 4A**). 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). For the hindlimb placing test, on the other hand, only the 16 nmol/rat ghrelin group showed a significant improvement at both 1.5 and 4 h after TBI-UH (**Fig. 4B**).

Effects of ghrelin on cortical apoptosis after traumatic brain injury and uncontrolled hemorrhagic shock: Apoptosis is thought to play an important role in both acute and chronic brain injury [7]. Both animal and human studies have shown substantial evidence of neuronal apoptosis after TBI [8]. As shown in **Figure 5**,

the level of cleave PARP-1 in the cortex, an indicator of cortical apoptosis, increased significantly at 4 h after TBI as compared to sham-operated animals. A further significant increase was found in TBI plus UH animals, indicating synergistic effects of hemorrhage and



brain trauma on cortical apoptosis. Ghrelin has been shown to inhibit apoptosis in neuronal cells during oxygen-glucose deprivation [9], and protect cortical neuron against focal ischemia/reperfusion (I/R) in rats [10]. In order to determine the effect of ghrelin on cortical apoptosis following TBI and uncontrolled hemorrhage, cleaved PARP-1 levels were measured



by Western blot at 4 h after TBI plus UH with or without ghrelin treatment. As shown in **Figure 6**, ghrelin reduced cortical apoptosis at 4 h after TBI plus UH in a dose-dependent manner.

Effects of ghrelin on brain levels of inflammatory cytokines after traumatic brain injury and uncontrolled hemorrhagic shock: TBI initiates a cascade of inflammatory processes that can serve to exacerbate the initial injury [11]. Ghrelin is anti-inflammatory [12-15]. However, whether ghrelin inhibits inflammatory responses in the brain after TBI remained largely unknown. In order to determine the effect of ghrelin on brain inflammation following TBI and uncontrolled hemorrhage, brain levels of TNF-α and IL-6, two important pro-inflammatory cytokines, were measured by ELISA at 4 h after TBI plus UH with or without ghrelin treatment. As shown in **Figures 7A-B**, significantly increased brain levels of TNF-α and IL-6 were markedly decreased by all three doses of ghrelin treatment.

Two Tasks were proposed in the Statement of Work. The Tasks have not been modified. The above described accomplishments are associated with Task 1 (i.e., To determine the short-term effect of ghrelin on brain injury following TBI and uncontrolled hemorrhage). We are currently working on the experiments outlined in Task 2.

KEY RESEARCH ACCOMPLISHMENTS

1. Established the rat model of traumatic brain injury and uncontrolled hemorrhagic shock.
2. Ghrelin treatment improves sensorimotor and reflex function after traumatic brain injury and uncontrolled hemorrhagic shock.
3. Ghrelin treatment reduces cortical apoptosis after traumatic brain injury and uncontrolled hemorrhagic shock.
4. Ghrelin treatment downregulates brain inflammation after traumatic brain injury and uncontrolled hemorrhagic shock.

REPORTABLE OUTCOMES

We have successfully established the rat model of traumatic brain injury combined with uncontrolled hemorrhagic shock.

We submitted a review paper to *PPAR Research* (Qi L, Jacob A, Wang P, Wu R. Peroxisome proliferator activated receptor- γ and traumatic brain injury)

CONCLUSION

Ghrelin improves sensorimotor and reflex function, reduces cortical apoptosis and downregulates brain inflammation 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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