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UCH-L1 is a Poor Serum Biomarker of Murine Traumatic Brain Injury After Polytrauma

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Abstract

Background: Several serum biomarkers have been studied to diagnose incidence and severity of traumatic brain injury (TBI), but a reliable biomarker in TBI has yet to be identified. Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) has been proposed as a biomarker in clinical and preclinical studies, largely in the setting of isolated TBI or concussion. The aim of this study was to evaluate the performance of UCH-L1 as a serum biomarker in the setting of polytrauma and TBI.

Methods: Multiple variations of murine TBI and polytrauma models were used to evaluate serum biomarkers. The different models included TBI with and without hemorrhagic shock and resuscitation, isolated extremity vascular ligation, extremity ischemia/reperfusion, and blunt tail injury. Blood was drawn at intervals after injury, and serum levels of neuron-specific enolase, UCH-L1, creatine kinase, and syndecan-1 were evaluated by enzyme-linked immunosorbent assay.

Results: UCH-L1 levels were not significantly different between TBI, tail injury, and sham TBI. By contrast, neuron-specific enolase levels were increased in TBI mice compared with tail injury and sham TBI mice. UCH-L1 levels increased regardless of TBI status at 30 min and 4 h after hemorrhagic shock and resuscitation. In mice that underwent femoral artery cannulation followed by hemorrhagic shock/resuscitation, UCH-L1 levels were significantly elevated compared with shock sham mice at 4 h (3158 ± 2168 pg/mL, 4 h shock *versus* 0 ± 0 pg/mL, 4 h shock sham; $P < 0.01$) and at 24 h (3253 ± 2954 pg/mL, 24 h shock *versus* 324 ± 482 pg/mL, 24 h shock sham; $P = 0.03$). No differences were observed in UCH-L1 levels between the sham shock and the arterial ligation, vein ligation, or extremity ischemia/reperfusion groups at any time point. Similar to UCH-L1, creatine kinase was elevated only after shock compared with sham mice at 4, 24, and 72 h after injury.

Conclusions: Our study demonstrates that UCH-L1 is not a specific marker for TBI but is elevated in models that induce central and peripheral nerve ischemia. Given the increase in UCH-L1 levels observed after hemorrhagic shock, we propose that UCH-L1 may be a useful adjunct in quantifying severity of shock or global ischemia rather than as a specific marker of TBI.

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Disclosure

All authors have no conflicts of interest to disclose.

Keywords

UCH-L1; TBI; Hemorrhagic shock; Trauma

Introduction

Traumatic brain injury (TBI) is a significant burden on the American health care system and accounts for 2.5 million emergency department visits, 280,000 hospitalizations, and 56,000 deaths per year.¹ Historically, TBI has been classified as mild, moderate, or severe depending on the Glasgow Coma Score.² However, the heterogeneity of TBI within these generic categories demonstrates the need for improved methods to classify and differentiate TBI severity. Certain biomarkers such as myelin basic protein,^{3,4} neuron-specific enolase (NSE),^{5,6} S100 β ,⁷⁻⁹ glial fibrillary acidic protein (GFAP),^{10,11} and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) have potential use as prognostic indicators and therapeutic targets after TBI. Identification of a unique biomarker for TBI could allow future targeted treatment strategies tailored to specific injury patterns serve as a diagnostic adjunct to radiographic imaging and identify patients at risk for long-term cognitive effects.

UCH-L1 is present in neurons and nerve fibers of both the central and peripheral nervous systems.¹² Although originally investigated for its role in neurodegenerative disorders such as Alzheimer's and Parkinson's disease,¹³ UCH-L1 has recently been investigated as a biomarker in the setting of TBI. In both rat¹⁴ and human¹⁵⁻¹⁷ studies, there is evidence that UCH-L1 may be used as a biomarker in serum and cerebrospinal fluid to predict severity and outcomes in TBI.

Although recent studies have shown a significant increase in post-TBI UCH-L1 levels compared with uninjured controls, it remains unclear whether UCH-L1 is actually a specific biomarker for TBI or rather reflects injury to any part of the nervous system.¹⁴⁻¹⁷ For example, UCH-L1 serum levels were measured and shown to be no different in patients with imaging-negative mild TBI compared with patients admitted with acute orthopedic trauma.¹⁸ Therefore, we hypothesize that UCH-L1 represents an important biomarker of damage to either the central or peripheral nervous system. Furthermore, UCH-L1 may serve as an early marker of global neuronal ischemia from hypotension or mal-perfusion with other useful clinical applications.

Methods

Animal model

The Institutional Animal Care and Use Committee of the University of Cincinnati approved all murine experiments. All mice used were 8- to 10-week-old male C57BL6/J purchased from Jackson Laboratory. The mice were fed a standard laboratory diet, allowed free access to water, and acclimated for 1 wk before experiments in a controlled housing environment with 12-h light-dark cycle. Male mice were used in all experiments to demonstrate consistency and limit the neuroprotective effect of estrogen and progesterone as a confounding variable.¹⁹

TBI and tail injury model

To test the specificity of serum biomarkers for TBI compared with blunt trauma alone, mice underwent either moderate TBI, isolated tail injury, or anesthesia only (TBI sham). A moderate TBI was induced using a closed weight drop model as previously described.^{20–22} In brief, mice were anesthetized with 2% inhaled isoflurane in 100% oxygen at 1 L/min for 2 min. Once adequately anesthetized, they were placed in a prone position. The head was centered in both the lateral and rostral/caudal directions below the weight. A 400 g weight was dropped from 2.5 cm above the table surface. In the isolated tail injury group, mice were similarly anesthetized with 2% inhaled isoflurane in 100% oxygen at 1 L/min for 2 min. After appropriate anesthesia, the mice were placed prone on the closed weight drop model. Their tail was centered 2 cm from the base of the tail below the weight. A 400 g weight was dropped from 2.5 cm above the table surface, a height identical to the TBI model. The control anesthesia only (TBI sham) group was anesthetized with isoflurane as previously described but did not undergo TBI or tail injury. Mice were sacrificed at 4 h, 24 h, or 72 h after injury for serum analysis.

TBI, hemorrhagic shock, and resuscitation model

To further evaluate serum biomarkers in the setting of TBI with the addition of hemorrhagic shock, mice underwent TBI or sham TBI followed by shock and resuscitation with normal saline (shock + NS) or shock sham. TBI was induced as described previously. Ten minutes after TBI was induced, mice were anesthetized with intraperitoneal phenobarbital (0.1 mg/g body weight). The left groin was shaved, prepped in a sterile fashion and the femoral artery was identified and isolated. The femoral artery was cannulated with a polyethylene catheter and connected to a pressure transducer for continuous hemodynamic monitoring. The mice were given 10 min for acclimation and then blood was withdrawn over 3 min until a systolic blood pressure of 25 mmHg was achieved to establish a pressure-controlled model of hemorrhagic shock. Mice were maintained in this shock state for 60 min. In this model, mice were then resuscitated with 0.9% saline to a systolic blood pressure of 80 mmHg, which is commensurate with baseline blood pressure. Mice were monitored for 20 min after resuscitation. All femoral arteries were ligated proximal to the catheter insertion site after completion of resuscitation. TBI controls (TBI shams) underwent anesthesia without head injury; shock sham animals underwent femoral artery cannulation and ligation without hemorrhage or resuscitation. Mice were sacrificed at 30 min or 4 h after injury for serum analysis.

Isolated extremity vascular ligation models

To further investigate the use of serum biomarkers in hemorrhagic shock, mice underwent pentobarbital sedation (0.01 mL/g of body weight) and then were randomly assigned to one of five experimental groups to examine the effects of arterial ischemia, venous hypertension, or arterial injury with hypoperfusion. The groups included 60 min of hemorrhagic shock followed by resuscitation with shed blood (shock + blood), 4 h of ischemia induced with a femoral artery clamp followed by reperfusion, ligation of the femoral artery, and ligation of the femoral vein or femoral artery exposure alone (vessel exposure sham). Resuscitation with shed blood, rather than NS, was used to potentially ameliorate the effect of crystalloid

resuscitation for hemorrhagic shock. Artery ligation, vein ligation, and ischemia/reperfusion (I/R) models served as controls for the hemorrhagic shock model in which the femoral artery cannula is removed and the artery is ligated once resuscitation is complete. These controls served to differentiate changes caused by hemorrhagic shock from changes caused by the ligation of the femoral artery after hemorrhagic shock. Mice were sacrificed at 4 h, 24 h, or 72 h after operation, and blood was drawn via cardiac puncture for serum analysis.

Enzyme-linked immunosorbent assays

Whole blood was collected and placed in serum separator tubes (BD Bioscience, San Diego, California), centrifuged at 1,000 g for 10 min, and the serum was collected. Serum levels of UCH-L1, syndecan-1, creatine kinase (CK) and NSE were measured by enzyme-linked immunosorbent assays according to the manufacturer's protocols (MyBioSource Inc, San Diego, California). CK was used as a serum biomarker for muscle damage and ischemia, whereas syndecan-1 was assessed as a marker of the severity of shock-induced endotheliopathy.

Statistics

Prism 6 (GraphPad Software, La Jolla, California) was used for all statistical analyses. All data are presented as the mean $\pm$ the standard deviation. Student's *t*-test was used to compare continuous variables between two groups. ANOVA was used to compare continuous variables between three or more groups. A $P < 0.05$ was considered statistically significant.

Results

Neuron-specific enolase is more predictive of TBI than UCH-L1

At 4 h after injury, NSE levels were elevated in TBI mice compared with tail injury mice (40.8 ± 22.2 $\mu\text{g/mL}$, TBI *versus* 3.4 ± 0.6 $\mu\text{g/mL}$, tail injury; $P = 0.001$) and TBI sham mice (40.8 ± 22.2 $\mu\text{g/mL}$, TBI *versus* 2.2 ± 0.4 $\mu\text{g/mL}$, TBI sham; $P = 0.001$) (Fig. 1). NSE levels remained elevated in TBI mice compared with TBI sham mice at 24 h (10.6 ± 8.5 $\mu\text{g/mL}$, TBI *versus* 2.1 ± 0.2 $\mu\text{g/mL}$, TBI sham; $P < 0.05$). However, NSE levels in TBI mice normalized by 72 h and were similar to TBI sham mice at that time point. By contrast to NSE as a potential biomarker for TBI, no significant differences in UCH-L1 levels were demonstrated in mice that underwent TBI compared with tail injury mice at any time point.

UCH-L1 levels remain similar despite the presence of TBI in a polytrauma model

In mice that underwent shock sham, no significant differences in UCH-L1 levels were observed between the TBI and sham TBI models at any time point. Similarly, the addition of either TBI or TBI sham did not affect serum UCH-L1 levels in mice that also underwent hemorrhage with 0.9% saline resuscitation. UCH-L1 levels increased regardless of TBI status at 4 h after shock + NS (Fig. 2). Interestingly, UCH-L1 levels in TBI sham/shock + NS mice were not significantly increased compared with TBI sham/shock + NS sham mice at 30 min (983 ± 1082 pg/mL, shock sham *versus* 1986 ± 1212 pg/mL, shock + NS, $P = 0.3$). By 4 h, a difference in UCH-L1 levels between these groups developed (2678 ± 1938 pg/mL, shock sham *versus* 5243 ± 975 pg/mL, shock + NS; $P < 0.03$) (Fig. 3).

UCH-L1 and CK levels are acutely elevated following hemorrhagic shock

In mice that underwent hemorrhagic shock followed by resuscitation with blood, UCH-L1 levels were significantly elevated compared with vessel exposure sham mice at 4 h (Fig. 4A). UCH-L1 levels were not significantly different among time points in the shock + blood group, remaining elevated compared with vessel exposure sham mice through 72 h post-injury (Fig. 4B). No differences were observed in UCH-L1 levels between the vessel exposure sham and the arterial ligation, vein ligation, or I/R groups at any time point.

Similar to the UCH-L1 results, CK was also higher in the shock + blood group compared with vessel exposure sham mice at 4, 24, and 72 h after injury (Fig. 5). The CK levels were similar between the arterial ligation and vessel exposure sham groups at 4 and 24 h, but CK levels were higher at 72 h in the animals with arterial ligation ($3.9 \pm 1.2 \mu\text{g/mL}$, 72 h vessel exposure sham *versus* $13.1 \pm 4.2 \mu\text{g/mL}$, 72 h artery ligation; $P < 0.01$). No differences were observed in CK levels between the vessel exposure sham and vein ligation or I/R groups at any time point.

Syndecan 1 levels are acutely elevated after isolated lower extremity trauma

Syndecan-1 was used as a marker of endothelial glycocalyx injury. Endothelial glycocalyx injury has been associated with hemorrhagic shock, and syndecan-1 has been proposed as a serum biomarker to determine severity of shock and guide resuscitation.²³ Syndecan-1 levels were acutely elevated but then decreased by 72 h in both the vessel exposure sham and shock + blood groups ($12.2 \pm 5.4 \text{ pg/mL}$, 4 h vessel exposure sham *versus* $1.5 \pm 1.9 \text{ pg/mL}$, 72 h vessel exposure sham; $P = 0.004$) ($14.1 \pm 8.5 \text{ pg/mL}$, 4 h shock + blood *versus* $0.9 \pm 0.5 \text{ pg/mL}$, 72 h shock + blood; $P = 0.002$). In the artery ligation, vein ligation, and the I/R groups, syndecan-1 levels were similarly elevated at 4 h but normalized by 24 h ($12.1 \pm 7.8 \text{ pg/mL}$, 4 h artery ligation *versus* $1.1 \pm 0.9 \text{ pg/mL}$, 24 h artery ligation; $P = 0.03$) ($13.8 \pm 9.6 \text{ pg/mL}$, 4 h vein ligation *versus* $1.5 \pm 0.8 \text{ pg/mL}$, 24 h vein ligation; $P = 0.02$) ($10.4 \pm 7.2 \text{ pg/mL}$, 4 h I/R *versus* $0.6 \pm 0.1 \text{ pg/mL}$, 24 h I/R; $P = 0.002$). There were no differences in syndecan-1 levels among the injury groups compared with the vessel exposure sham group at any time point.

Discussion

TBI is a major public health problem and is cause of significant morbidity and mortality in the United States. Serum biomarkers, such as UCH-L1 and NSE, have been studied in hopes of identifying better methods to diagnose, classify, and prognosticate TBI. Using multiple murine models of polytrauma, we have demonstrated that UCH-L1 is not specific for TBI or central nervous system trauma. By contrast, TBI mice were observed to have significant increases in NSE levels compared with either tail injury or sham treatment. These findings suggest that NSE is a better marker for TBI than UCH-L1. Given elevations in UCH-L1 levels in models of systemic hypotension, UCH-L1 may be more accurately characterized as a biomarker of global neuronal ischemia.

In this study, serum biomarkers were measured using enzyme-linked immunosorbent assays to compare the effects of TBI in multiple murine polytrauma models. Our findings suggest

that UCH-L1 is not specific for TBI, especially in the setting of polytrauma. A recent meta-analysis evaluating traumatically injured patients for potential TBI concluded UCH-L1 is a reliable serum biomarker for brain injury.²⁴ However, the studies included did not compare patients with TBI to those without TBI, limiting the evaluation of UCH-L1 as a specific biomarker in the setting of TBI. Huang *et al.*,¹⁸ showed significant increases in UCH-L1 levels in rats that underwent TBI compared with sham. Although UCH-L1 levels were compared between TBI and sham mice, they did not study these comparisons in the model of polytrauma. By contrast, our findings showed that UCH-L1 was significantly increased in murine polytrauma models with or without TBI. Posti *et al.*,²² showed similar results in when they compared patients with CT-negative mild TBI *versus* patients with orthopedic trauma. They concluded that UCH-L1 was unable to distinguish between these groups of patients and thus support our findings.

In addition, our study found that UCH-L1 is significantly elevated in mice that underwent hemorrhagic shock and resuscitation compared with mice that underwent femoral artery cannulation without shock or femoral arterial ligation alone. To our knowledge, this is the first time that UCH-L1 has been investigated in these settings. Our hypothesis is that elevation of UCH-L1 is the result of neuronal ischemia throughout the entire nervous system during a state of global shock. Syndecan-1 has previously been described as a serum biomarker in the setting of shock. It is hypothesized that damage to the endothelial glycocalyx in the shock state causes release of syndecan-1 systemically. Previous studies have demonstrated increased mortality with elevated levels in trauma patients²⁵ and elevated levels in patients with severe sepsis.²⁶ In our study, UCH-L1 was significantly elevated at 4 h in the shock/resuscitation group compared with all other groups at 4 h. Syndecan-1 was elevated at 4 h compared with later time points, but this was not specific for the shock group. Therefore, our findings support UCH-L1 as a more specific marker of global ischemic injury such as after hemorrhagic shock than syndecan-1. However, we recognize that these findings are limited to preclinical, murine models and further studies are needed to extrapolate these data to human patients.

CK is an enzyme that catalyzes the reversible reaction of creatine and adenosine triphosphate forming phosphocreatine and adenosine diphosphate.²⁷ Consequently, it is found in cells that have high-energy requirements such as muscle and it is a widely used serum biomarker of muscle injury and breakdown. Valdez *et al.* showed an association between compartment syndrome and serum CK levels >4000 U/L.²⁸ Interestingly, we did not see an elevation in CK levels in the limb I/R group as compared with the vessel exposure sham group. However, serum CK in the group undergoing hemorrhagic shock was elevated at all time points compared with sham and in the group with arterial ligation compared with sham at 72 h. The late elevation in the arterial ligation likely demonstrates the significant cell breakdown developing over time and may suggest worsening muscle ischemia as seen clinically in compartment syndrome. The ideal biomarker would identify neuronal injury before irreversible damage is caused. To our knowledge, this is the first experience with evaluating serum CK levels in a murine isolated vascular ligation models. Our data do not support the use of serum CK as a biomarker in this murine vascular ligation or I/R model and future studies should take the present models and findings into consideration for study design.

NSE is another serum biomarker that has been proposed to assist in the diagnosis and treatment of TBI. Honda *et al.* found a significant increase in NSE level in patients with TBI compared with patients without TBI.²⁹ A meta-analysis that included 6 studies showed a correlation between NSE levels and unfavorable outcomes including mortality.³⁰ Murine models have shown elevations of serum NSE in stroke and encephalomyelitis.³¹ Our group has previously shown increased serum levels of NSE after TBI when followed by hypobaric/hypoxia exposure.³² In our TBI and tail injury model, we demonstrated a significant increase in NSE in mice that underwent TBI compared with blunt trauma as simulated by tail injury or compared with uninjured mice. These data further support the use of NSE as a specific biomarker for TBI.

There are limitations of our study that deserve attention. First, our isolated extremity trauma model did not involve a TBI or untouched sham model for comparison. Second, our TBI model does not perfectly reproduce human TBI. It lacks rotational and angular elements seen in the injury patterns sustained by human patients. Finally, this is only a moderate TBI model and cannot evaluate the specificity of these serum biomarkers in the setting of mild or severe TBI. Additional data are needed to evaluate various TBI biomarkers with varying severity of TBI.

In conclusion, our study demonstrates that UCH-L1 is not a specific marker for TBI but is likely elevated in situations of global hypoperfusion that may include peripheral and central nerve ischemia. Given the increase in UCH-L1 levels observed after hemorrhagic shock, we propose that UCH-L1 may be a useful adjunct in quantifying severity of shock and lasting tissue damage and merits further investigation.

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Authors' contributions: M.M., A.B., and M.G. contributed to study conception and design. M.M., A.B., R.V., and L.F. contributed to acquisition of data. M.M., A.B., and M.G. contributed to analysis and interpretation of data. M.M., A.B., and M.G. drafted the manuscript. M.M., A.B., G.N., F.K., T.P., A.M., and M.G. contributed to critical revision. Each author has made final approval of the manuscript. Furthermore, each author certifies that this material has not been and will not be published or submitted to any other publication before its appearance in *Journal of Surgical Research*.

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NSE levels at 4 hours following injury

Fig. 1 –.

NSE levels are elevated in the TBI group compared with both tail injury and TBI sham groups at 4 h after injury. $n = 5$ in each group. $*P < 0.05$.

Fig. 2 –.

UCH-L1 levels are elevated in shock + NS over time regardless of whether mice underwent TBI or not. $n = 5$ in each group. $*P < 0.05$.

Fig. 3 –.
UCH-L1 levels are elevated at 4 h after shock + NS compared with shock sham. $n = 5$ in each group. $*P < 0.05$.

Fig. 4 –.

(A) UCH-L1 levels are elevated in the shock + blood group compared with exposure sham at 4 h. All other groups were similar compared with sham. $n = 5$ in each group. (B) UCH-L1 levels are elevated in the shock + blood group compared with shock sham at 4, 24, and 72 h and remain similar throughout the entire study period in each injury group. $n = 5$ in each group. * $P < 0.05$.

Fig. 5 –.

CK levels are elevated in the shock + blood groups compared with shock sham at 4, 24, and 72 h $n = 5$ in each group. * $P < 0.05$.