Original Article
A rat model of concurrent combined injuries (polytrauma)

Robert M Akscyn¹, J Lee Franklin¹, Tatyana A Gavrikova¹, Martin G Schwacha²,³, Joseph L Messina¹,⁴

¹Department of Pathology, Division of Molecular and Cellular Pathology, University of Alabama at Birmingham, Birmingham, Alabama, 35294-0019; ²Department of Surgery, Division of Trauma and Emergency Surgery, University of Texas Health Science Center San Antonio, San Antonio, Texas, 78229-3900; ³US Army Institute of Surgical Research, Fort Sam Houston, Texas, 78234; ⁴Veterans Affairs Medical Center, Birmingham, AL 35233

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Abstract: Polytrauma, a combination of injuries to more than one body part or organ system, is common in modern warfare and in automobile and industrial accidents. The combination of injuries can include burn injury, fracture, hemorrhage, trauma to the extremities, and trauma to specific organ systems. To investigate the effects of combined injuries, we have developed a new and highly reproducible model of polytrauma. This model combines burn injury with soft tissue and gastrointestinal (GI) tract trauma. Male Sprague Dawley rats were subjected to a 15-20% total body surface area scald burn, or a single puncture of the cecum with a G30 needle, or the combination of both injuries (polytrauma). Unlike many ‘double hit’ models, the injuries in our model were performed simultaneously. We asked whether multiple minor injuries, when combined, would result in a distinct phenotype, different from single minor injuries or a more severe single injury. There were differences between the single injuries and polytrauma in the maintenance of blood glucose, body temperature, body weight, hepatic mRNA and circulating levels of TNF-α, IL-1β and IL-6, and hepatic ER-stress. It has been suggested that models utilizing combinatorial injuries may be needed to more accurately model the human condition. We believe our model is ideal for studying the complex sequelae of polytrauma, which differs from single injuries. Insights gained from this model may suggest better treatment options to improve patient outcomes.

Keywords: Burn, cecal ligation and puncture, ER-stress, hyperglycemia, hypoglycemia, hypothermia, proinflammatory

Introduction

There are various clinical metrics to define polytrauma [1-3], which can be defined most simply as multiple, simultaneous injuries to more than one body part or organ system. Polytrauma occurs often in modern warfare as well as in automobile and industrial accidents [4-6]. Polytrauma includes numerous types of injury including burn injury, fracture, hemorrhage, trauma to the extremities, and penetrating trauma to the gastrointestinal (GI) tract. A defining feature of polytrauma is the deleterious pathophysiology created by multiple injuries. Patient outcomes following multiple minor injuries are often worse than those with a single severe injury. Advances in battlefield medicine and emergency response strategies have increased initial survival following polytrauma, but later complications (multiple organ failure, metabolic dysfunction, severe infection) result in high mortality [7-9].

Polytrauma with burn injury, by its very name, represents a complex injury pattern. Not surprisingly, it is a more common injury pattern in military combatants than in civilian injuries. For instance, military patients had a higher incidence of associated non-burn injuries (37%) versus only (11%) in civilian patients [10]. In recent military conflicts, significant burn injury with concomitant penetrating injury (such as the bowel) were common in closed space explosion events, such as within vehicles [11-13].

The physiological and pathological consequences of this injury pattern are not well understood. In the current study, we have developed
A model of burn/CLP polytrauma

a rodent model of simultaneous combined burn and penetrating injury of the bowel to begin to characterize the pathophysiological and inflammatory aspects of this complex injury pattern (polytrauma). This model combined burn injury with cecal ligation and puncture (CLP). Cecal ligation and puncture can be viewed as polytrauma itself, as the laparotomy produces soft tissue trauma and the penetrating damage to the GI tract is a second injury. Cecal ligation and puncture has long been used to study sepsis and peritonitis. Use of CLP in this context often involves a more severe CLP than the CLP used in the present studies. For the purposes of these studies CLP has been utilized to mimic a penetrating, lower abdominal injury that nicks the bowel as often occurs in blast injuries, or in automobile or industrial accidents [4-6]. Combining CLP and burn injury resulted in two discrete injuries that when performed together represented a type of polytrauma observed on the battlefield and in civilian populations [4, 5, 14-18].

Performing these two well-established injury models at the same time resulted in a simple and reproducible polytrauma model. The crux of our model is the simultaneous performance of the injuries, which is distinct from ‘double hit’ models where CLP is performed one or more days after burn injury to model the infection often observed in burn patients [19, 20]. Additionally, both the burn and CLP injuries are less severe than those typically utilized in burn and sepsis studies [21-28]. In our model the burn injury was 15-20% of the total body surface area (TBSA). Usually 20-60% TBSA or more is burned in animal models of burn injury [21-24]. The CLP used in this study was a single puncture of the cecum with a small-bore G30 needle. Most groups performing CLP use one or more punctures of the cecum with larger gauge needles [25-28].

Recently, the ability of animal models to reflect the effects of injury in humans has been called into question and generated much controversy [29, 30]. Moreover, it has recently been suggested more severe injury models, including models of polytrauma, are needed to more accurately replicate the human response to severe trauma [31, 32]. The burn/CLP model of polytrauma described here resulted in systemic pathophysiology similar to, yet distinct from, either of the single injuries alone. To further demonstrate that the deleterious effects of this burn/CLP polytrauma model were unique to simultaneous, multiple, minor injuries and not produced by a more severe single injury, a second, more ‘traditional’ CLP alone group was generated with a double puncture of the cecum with a 18-gauge needle.

The present study is an attempt to determine the effects of combined minor injuries, and whether there is a discernable phenotype that differs from single minor or major injury. The data demonstrated that polytrauma resulted in a lessened ability to maintain normothermia and euglycemia compared to rats following a single injury. Additionally, rats that underwent polytrauma displayed a resistance to fasting induced weight loss. Injury-induced increases in proinflammatory gene expression were observed in liver, as well as increases in circulating levels of proinflammatory cytokines, with IL-6 increased to a much greater degree following polytrauma. Markers of endoplasmic reticulum stress (ER-stress) increased and were mostly specific to polytrauma.

Materials and Methods

Animal model of polytrauma (burn/CLP)

All animal procedures were carried out in accordance with the guidelines set forth in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (Bethesda, Maryland). The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham. This study conforms to the ARRIVE guidelines set forth for animal research. Male Sprague-Dawley rats, 12 weeks of age, with an average weight of 290 g (SD ± 10.3 g) were used throughout this study. Prior to experiments, rats were acclimatized for at least one week in a 12 h light/dark cycle with free access to food and water. Rats were not fasted prior to manipulation. Rats were anesthetized by brief inhalation of 2% isoflurane, followed by injection of sodium pentobarbital (60 mg/kg i.p.).

Burn injury

A well-established model of scald burn served as the basis of burn injury in this study [21].
Animals were placed in a supine position in a plastic template, which exposed the dorsum. A full-thickness skin scald burn was inflicted by immersing the back of the animal in 95°C water for 10 seconds. To calculate the percentage of total burn surface area (TBSA) the burn area was immediately traced, measured and TBSA of each burned animal was calculated using Meeh’s formula (Total Body Surface Area = kW\(^{0.667}\)) with a Meeh constant of 9.46 [33]. Based on these measurements an approximate 15-20% TBSA burn was generated. A sham burn was performed by exposing the rat dorsum to 37°C water for 10 seconds. Rats were quickly dried and while still under pentobarbital anesthesia, CLP or sham-CLP was performed.

**Cecal ligation and puncture (CLP)**

The CLP procedure used in this model was based on the methodology first described by Wichterman, et al. [25]. Following a 2-cm ventral midline laparotomy (soft tissue trauma), the cecum was exposed and ligated just distal to the ileocecal valve to avoid intestinal obstruction. A G30 or G18 needle was used to puncture the cecum once or twice, respectively, and a small amount of the bowel content was extruded to insure patency of the opening. The cecum was returned to the abdominal cavity and the incision closed in layers. Sham-operated rats underwent the same surgical procedure with the cecum being exposed except the cecum was neither ligated nor punctured. All animals received resuscitation fluid (0.9% sterile saline, 30 ml/kg, warmed to 37°C) via a s.c. injection in the nape of the neck with care not to disturb the burn area. Rats were returned to the cages and thermal support provided (heating pad set to 37°C) until consciousness and mobility were regained. Water was provided ad libitum. Chow was not provided to rats in the 6 h groups. Chow was provided to rats in the 24 h and 32 h groups until a fasting period prior to euthanasia of 20 h and 23 h respectively. Chow was weighed prior to being provided to animals and after removal. At the timed endpoints, rats were anesthetized with 2% isoflurane inhalation and the laparotomy was reopened. Livers were harvested and immediately snap frozen in liquid nitrogen. For euthanasia, the diaphragm was cut and the heart excised.

**Experimental groups**

This study used five treatment groups. To account for the effects of anesthesia and surgical manipulation a double sham group (sham/sham) was included: sham burn with sham CLP which served as the control group. To discern the effects of the single injuries, burn injury alone with a sham CLP (burn/sham) or CLP injury alone with a sham burn (sham/CLP; G30, 1×) was performed. To investigate the effects of polytrauma, a double injury, burn plus CLP (G30, 1×; burn/CLP), was performed. To compare the differences between polytrauma and a more typical, more severe CLP, a second sham/CLP group was generated at the 32 h timepoint for some measurements. The CLP in this group was performed with a G18 needle and a double puncture of the cecum (sham/CLP; G18, 2×).

**Total RNA extraction**

Livers were frozen in liquid nitrogen until extraction of total cellular RNA using the Aurum Total RNA Fatty and Fibrous Kit (Bio-Rad, Hercules, CA, USA) and treated with DNase I on the column according to the manufacturer’s recommendations. Briefly, 100 mg liver tissue samples were ground into a fine powder under liquid nitrogen and homogenized in PureZOL. Following centrifugation, ethanol was added to the supernatant and the lysate transferred to an RNA binding column. After a low-stringency wash, DNase I treatment, a high-stringency wash and an additional low-stringency wash, the RNA was eluted off the column. RNA concentration and quality was determined by spectrophotometry at 260/280 nm and samples were stored at -80°C.

**Quantitative real-time PCR**

RNA samples were used as a template for the reverse transcriptase reaction to generate cDNAs using the High Capacity Reverse Transcriptase Synthesis kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s protocol. RNA (1 μg) was reverse transcribed in 20 μl reaction volume containing Reaction Mix with a blend of oligo (dT) and random hexamer primers, and reverse transcriptase. cDNA samples were diluted 1:10 and 1 μl of diluted cDNA was used in each 25 μl real-
time PCR reaction, using the iQ Supermix (Bio-Rad) with the iQ5 Real Time PCR Detection System (Bio-Rad). The mRNA expression level of each gene was quantified using the oligonucleotide primers specially designed and optimized for this study using PrimerQuest (Integrated DNA Technologies, Coralville, IA). Each sample was run in triplicate, and the mean threshold cycle value of target genes was normalized to the expression of 18S rRNA in the corresponding sample. Fold expression of genes was calculated using the comparative \( C_T \) method (fold expression = \( 2^{-\Delta\Delta C_T} \)). The following primer sequences were used; 

- **TNF-α** Forward (5’-CGT AGC CCA CGT CGT AGC-3’), Reverse (5’-GTC CCT TGA AGA GAA CCT GGG AGT-3’);
- **IL-1β** Forward (5’-AAG AGC TTC AGG GCA GTGTC-3’), Reverse (5’-TGG GAA CAT CAC ACA CTA GCA GTG-3’);
- **IL-6** Forward (5’-AAC TCC ATC TGC CCT TCA GGA ACA-3’), Reverse (5’-AAG GCA GTG GCT GTC AAC ATC-3’);
- **GRP78** Forward (5’-GCC ACT AAT GGA GAC ACT CAT C-3’), Reverse (5’-CTG ACA TCT TTC CCA GTC TTC TT-3’);
- **CHOP** Forward (5’-GAG AGT GTG CCT GCA GGA AGT ATG-3’), Reverse (5’-ACT GTC ACT GAA GAC ACT CAT C-3’);
- **WFS1** Forward (5’-CTG ACA TCT TCC CCA GTC TTC TT-3’).

**Measurement of circulating proinflammatory cytokines, insulin and glucose**

Blood was collected from the inferior vena cava into microfuge tubes containing 15 units of heparin. Samples were immediately centrifuged for 10 m at 3,000 g. The plasma fraction was collected and stored at -80°C.

Plasma samples were sent to the University of Alabama at Birmingham Physiology and Metabolism Core for analysis. Levels of TNF-α, IL-1β and IL-6 were measured using a Meso Scale Discovery multiplex spot assay and analyzed with MSD Discovery Workbench software (Meso Scale Discovery, Gaithersburg, MD, USA). Insulin levels were measured in duplicate via a radioimmunoassay (EMD Millipore, Billerica, MA, USA).

Blood glucose was measured with a StatStrip Xpress glucometer (Nova Biomedical Corporation, Waltham, MA, USA). Starting blood glucose measurements were taken after anesthesia before any surgical manipulation. End blood glucose measurements were taken after anesthesia before proceeding with euthanasia. Measurements were taken in duplicate and averaged.

**Measurement of body temperature**

Rectal temperature was measured with a TH-5 Thermalert Monitoring Thermometer (Physitemp, Clifton, NJ, USA). Starting body temperatures were measured after anesthesia before any surgical manipulation. End body temperatures were measured after anesthesia before proceeding with euthanasia.

**Statistical analysis**

Data are presented as mean ± SEM. Data were analyzed using the InStat statistical program (GraphPad Software, Inc., San Diego, California). Differences between groups were determined using 1-way ANOVA (Tukey post-test) or Student’s \( t \) test (unpaired, Welch-corrected). Significant differences are denoted as a = \( P < .05 \) versus sham/sham, b = \( P < .05 \) versus burn/sham, c = \( P < .05 \) versus sham/CLP (G30, 1×) and d = \( P < .05 \) versus burn/CLP (G30, 1×).

**Results**

A prominent outcome of this model is the effects on survival rates following injury. As we have recently observed (manuscript undergoing minor revisions, which examined potential treatment options to reduce mortality), either burn or CLP (G30, 1×) injury alone was highly survivable, with approximately 10-20% mortality. However, when the injuries were combined, polytrauma resulted in greatly increased mortality of approximately 60% within the first 48 h following injury. Furthermore, the overwhelming majority of these mortalities occurred between 36 h and 48 h. Therefore an endpoint of 32 h was chosen as the latest endpoint for these studies, with only a 5% mortality rate by this timepoint. This data indicates the effects of polytrauma produced a deleterious synergy, leading to higher mortality than if the effects were merely additive. Additionally, the more severe CLP performed (G18 needle, double puncture of the cecum) resulted in 40% mortality. This data indicates that the pathogenic effects of the combined minor injuries were greater than that of a more severe single injury.
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To assess the effects of injury on body temperature, measurements were taken initially and at various times following injury. Six hours following injury both burn alone and CLP alone caused a small but significant reduction in body temperature versus sham/sham (Figure 1). The combination of burn and CLP resulted in a further reduction in body temperature that was significant versus sham/sham and both single injuries. At the 32 h timepoint burn injury alone and CLP alone were not significantly different compared to sham/sham, although the CLP alone, either (G30, 1×) or (G18, 2×), trended towards reduced body temperature. However, combined injuries resulted in body temperatures significantly lower than sham/sham, burn alone or either CLP injury alone, dropping to 27°C (Figure 1), indicating a severe thermo-dysregulation and hypothermia following polytrauma.

Polytrauma-induced thermo-dysregulation

Body-weight retention following polytrauma

To determine the effects of injury on fasting induced weight loss body weight was measured before any surgical manipulation and at the time of euthanasia. Rats in the 6 h group were not provided chow following manipulation and, thus, were fasted approximately six hours. At 6 h there was no change in body weight compared to the pre-injury weight except for burn/sham group which displayed a 5 g increase in body weight, which was significant versus sham/sham, sham/CLP and burn/CLP (Figure 2A). The increased body weight in the burn/sham group may be attributable to reduced excretion of resuscitation fluid or increased water intake. Water was provided ad libitum to all groups.

Rats in the 24 h group were provided chow until a fasting period of twenty hours. At the 24 h timepoint fasting resulted in approximately 23 g of weight loss in the sham/sham group (Figure 2A). Burn or CLP alone resulted in significantly less weight loss, approximately half of sham/sham. And, interestingly, burn/CLP animals did not lose any weight following the 20 h fast (Figure 2A).

Rats in the 32 h group were provided chow until a fasting period of twenty-three hours. The longer fasting period in the 32 h groups resulted in additional weight loss in all groups. However, all injured rats failed to lose as much weight as the sham/sham rats (although the burn injury alone was not significant). Burn/CLP rats retained significantly more weight versus all other groups including the more severe (G18, 2×) CLP.

Chow consumption was recorded at the 24 h and 32 h time points. At 32 h chow consumption was approximately 4.5 g in the sham/sham and less than one-half that in the burn/sham group (Figure 2B). Both sham/CLP groups (G30, 1×) or (G18, 2×) consumed significantly less chow, about the same as that of the combined injury group (Figure 2B).

Polytrauma-induced changes in glucose homeostasis

Blood glucose measurements were taken to assess the effects of polytrauma on glucose metabolism. At the 6 h timepoint the burn injury alone resulted in a significant decrease in

![Figure 1. Body temperature following polytrauma.](image-url)
blood glucose versus the sham/CLP and burn/CLP groups and polytrauma resulted in an increase in blood glucose which was higher than the sham/sham and the single injuries (Figure 2C). At the 32 h timepoint burn injury alone resulted in increased blood glucose levels that were significant versus all other groups and the CLP alone and the burn/CLP groups had blood glucose levels that were significantly lower (Figure 2C).

Circulating insulin levels were measured and 6 h following injury there were no differences between treatment groups. However, the low-
Hypoglycemia was observed at 6 h in the burn/CLP group, which also had significant hyperglycemia. At 32 h, insulin levels decreased due to the 23 h fasting period in all groups. The burn/CLP group trended towards an increase compared to the sham/sham, and was significantly elevated compared to the sham/CLP group, respectively (Figure 2D).

**Polytrauma-induced increases in circulating proinflammatory cytokines**

Circulating TNF-α levels were significantly increased at the 6 h timepoint in the sham/CLP and burn/CLP groups versus the sham/sham and burn/sham groups (Figure 3A). At the 32 h timepoint TNF-α was decreased slightly, but still significantly elevated in the sham/CLP group. However, TNF-α levels in the burn/CLP group increased further, and were significantly higher than all other groups (Figure 3A).

Circulating levels of IL-1β were significantly elevated by sham/CLP and burn/CLP versus sham/sham and burn/sham at the 6 h timepoint (Figure 3B). At the 32 h timepoint IL-1β remained significantly elevated by sham/CLP versus sham/sham and burn/sham. However, burn/CLP resulted in IL-1β levels that were significantly higher versus all other groups (Figure 3B).

Circulating levels of IL-6 displayed a pattern similar to that of TNF-α and IL-1β as IL-6 levels were significantly increased by sham/CLP and burn/CLP versus sham/sham and burn/sham.
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at the 6 h timepoint (Figure 3C). Again, at the 32 h timepoint circulating IL-6 was significantly elevated by sham/CLP, but the burn/CLP group had IL-6 levels that were significantly higher than all other groups (Figure 3C). Cumulatively, this data demonstrates increases in circulating proinflammatory cytokines at the early 6 h timepoint were primarily CLP-driven with the burn injury having little effect. However, at the 32 h timepoint increases in TNF-α, IL-1β and IL-6 were greater (TNF-α, IL-1β) to much greater (IL-6) following polytrauma compared to the sham/sham and either single injury group.

Altered hepatic proinflammatory gene expression following injury

TNF-α mRNA levels were measured at 6 h and 32 h following injury. Six hours following injury TNF-α mRNA levels were significantly elevated in sham/CLP and burn/CLP versus sham/sham and burn/sham (Figure 4A). At the 32 h timepoint TNF-α message levels remained elevated in sham/CLP and burn/CLP groups. Interestingly, a significant decrease of TNF-α mRNA was observed in the burn/sham group versus sham/sham at 32 h (Figure 4A).

IL-1β mRNA levels were measured at 6 h and 32 h following injury. At the 6 h timepoint both sham/CLP and burn/CLP resulted in a significant increase in IL-1β mRNA levels versus sham/sham and burn/sham. These increases diminished by 32 h and were no longer significant versus sham/sham (Figure 4B). At both timepoints IL-1β mRNA levels were reduced by burn injury alone versus sham/sham but this was only significant at 32 h (Figure 4B).
IL-6 mRNA levels were measured at 6 h and 32 h following injury. Six hours following injury both sham/CLP and burn/CLP resulted in a significant increase in IL-6 mRNA levels versus sham/sham and burn/sham (Figure 4C). At the 32 h timepoint IL-6 mRNA levels remained significantly higher in burn/CLP versus all other groups (Figure 4C), indicating a polytrauma-dependent effect.

**Polytrauma specific induction of ER-stress genes in liver**

We have recently observed that our model of polytrauma resulted in hepatic insulin resistance and ER-stress (manuscript submitted). In the present study we asked if a more severe single injury, sham/CLP (G18, 2×), would also result in hepatic ER-stress. To answer this question expression of the mRNAs for the ER-stress inducible genes glucose-regulated protein 78/Binding immunoglobulin Protein (GRP78/BiP), CCAAT-enhancer-binding protein homologous protein (CHOP), and Wolfram syndrome 1 (WFS1) were measured 32 h following injury. GRP78 mRNA levels were not significantly increased by burn/sham or sham/CLP (G30, 1×). The more severe CLP (G18, 2×) resulted in an approximate 3.5-fold increase in GRP78 mRNA levels which was significantly higher than sham/sham, but not quite significantly different from either of the single injuries (Figure 5A). A greater increase (5.5-fold) in GRP78 message levels was observed following burn/CLP which was significantly higher than all other groups (Figure 5A).
A significant increase in the expression of CHOP mRNA was observed following only burn/CLP (Figure 5B). Neither single injury alone, burn, CLP (G30, 1×), nor CLP (G18, 2×) resulted in a significant increase of CHOP mRNA levels compared with sham/sham. Similarly, WFS1 mRNA levels were significantly increased following only burn/CLP with none of the single injuries having an effect (Figure 5C). Collectively, this data demonstrates induction of ER-stress responsive genes in the liver was almost exclusively polytrauma-dependent, with little effect of the single injuries. Further, the ER-stress response did not depend on the severity of a single injury as the two types of CLP (G18, 2×) and (G30, 1×) produced similar results.

Discussion

Polytrauma is common on the battlefield and also in some civilian injuries. With the progression of global industrialization, a rise in polytrauma within the civilian population has been predicted [34]. Modeling the complex injury patterns of polytrauma is difficult and no animal model will be able to fully recapitulate the conditions of diverse polytrauma observed in humans. However, to begin to improve patient prognosis, the basic pathophysiology of polytrauma needs to be better understood. To achieve this goal reproducible animal models are required. Our burn/CLP model of polytrauma produces a multifaceted pathophysiology, yet is straightforward to perform and highly reproducible, making this an ideal model for studying the basic mechanisms of a wide range of pathogenic outcomes of polytrauma.

Injury and time-dependent effects on body temperature were observed in this study. Burn injury alone resulted in decreased body temperature at 6 h. Loss of thermoregulation following a 20-30% TBSA burn injury in rats have been reported with decreased temperature occurring 1-6 h following burn [35]. At the 32 h timepoint burn injury alone resulted in an insignificant change in body temperature. However, studies using greater TBSA burn injury have produced prolonged increases in body temperature, attributable to the hypermetabolic response to burn [36, 37]. This suggests that the smaller TBSA burn injury in the current study was insufficient to induce a hypermetabolic response. The reported effects of CLP on body temperature in rats are conflicting with hypothermia, hyperthermia and no effects all being observed [38-41]. Both CLPs (G30, 1× and G18, 2×) used in this study resulted in mild (but not significant) hypothermia. However, polytrauma resulted in a large decrease of body temperature versus all other groups at 6 h and particularly 32 h. This suggests a severe hypothermic phenotype specific to the combined injuries. Of note, a recent study in human patients with sepsis revealed those with hypothermia had increased mortality and organ failure [42]. Although the pathological effects of hypothermia are unclear, they may partially explain the dramatic increase in mortality observed following polytrauma.

Fasting induced weight loss varied, dependent on the injury. Burn injury alone resulted in less weight loss than the sham/sham rats animals at the 24 h and 32 h timepoints. Burn induced weight retention at early timepoints has been previously observed and is likely attributable to burn-induced edema [43]. The sham/CLP (G30, 1×) group lost significantly less weight than the sham/sham group at both the 24 h and 32 h timepoints. A more severe CLP (G18, 2×) also resulted in less weight loss compared with sham/sham at the 32 h timepoint. A possible explanation for these observations is reduced GI motility following CLP leading to less weight loss due to decreased defecation and/or decreased fluid loss due to reduced kidney function. In other studies, weight loss in the first 24-48 following CLP has been observed [39, 41]. The differences between these studies and ours is likely due to weight measurements taken after a fasting period in our study while rats were in the fed state in the mentioned studies. The burn/CLP group retained more weight than all other groups at the 24 h and 32 h timepoints. It is uncertain what caused weight retention following burn/CLP, but reduced urine production and reduced GI motility, and therefore defecation, are likely explanations. Notably, a study utilizing CLP in mice demonstrated that animals which lost weight in the first 24 h had a higher survival rate over the first 72 h [44]. Since polytrauma animals lost the least weight, this may help explain the higher mortality in the burn/CLP animals.

Chow consumption prior to fasting did not account for the differences in weight loss since the sham/sham group ate significantly more
than all injured groups and lost the most weight. Cecal ligation and puncture resulted in severely reduced chow consumption, which is in agreement with findings from other studies [39, 41].

Glucose homeostasis following burn injury and CLP is often compromised [45-49]. At the 6 h timepoint burn injury alone resulted in slightly lower blood glucose levels that transitioned to significantly elevated levels at the later timepoints. Hyperglycemia following burn injury was expected [39].

The typical hyper-to-hypometabolic shift associated with CLP [41] induced sepsis was also observed, even with the less severe CLP used in the present study. The combination of burn and CLP resulted in an exacerbated early hyperglycemia with glucose levels higher than all other groups. At the 32 h timepoint the most severe hypoglycemia was observed in the burn/CLP group and was likely CLP-driven. Hypoglycemia has been proposed as a survival marker in CLP sepsis [49, 50]. Interestingly, blood glucose levels at 32 h were not different between sham/CLP and burn/CLP, but the survival rate of burn/CLP animals is considerably lower than the sham/CLP group (manuscript submitted). This suggests that blood glucose levels alone are not predictive of mortality.

Examination of circulating insulin levels revealed no differences between groups at 6 h and a modest increase in polytrauma animals at 32 h. A lack of increased circulating insulin during hyperglycemia at 6 h may suggest a loss of glucose regulated insulin secretion following polytrauma. Conversely, elevated insulin levels at 32 h, with simultaneous hypoglycemia is suggestive of an insulin resistant state.

To assess the inflammatory response to polytrauma, hepatic mRNA and circulating levels of TNF-α, IL-1β and IL-6 were examined. Levels of hepatic TNF-α mRNA followed a CLP-dependent pattern and circulating TNF-α levels were also CLP-driven at the 6 h timepoint. However, by 32 h circulating levels of TNF-α doubled and were significantly higher in the burn/CLP group versus all other groups, while hepatic TNF-α mRNA levels did not differ between sham/CLP and polytrauma (burn/CLP). This suggests that extra-hepatic production of TNF-α is higher following polytrauma than following single injuries. Levels of IL-1β mRNA in liver peaked at 6 h and were likely attributable to CLP. Circulating IL-1β was highest at 32 h in the burn/CLP group. This timeframe discontinuity between hepatic mRNA and circulating IL-1β may be explained by protein translation lag and protein half-life as well as other tissues/cell types producing IL-1β. Interestingly, burn injury alone suppressed hepatic IL-1β mRNA levels at 6 h and significantly at 32 h. IL-1β is an ‘early response’ cytokine and measurement at 6 h may be too late to observe an early rise, with lower mRNA following as a potential physiological negative feedback after a relatively minor injury.

Hepatic mRNA and circulating levels of IL-6 were CLP-dependent at the 6 h timepoint, but at later timepoints burn/CLP resulted in higher levels than single injury alone. The prognostic usefulness of circulating IL-6 in a CLP model has previously been reported [44]. At the 32 h timepoint circulating levels of TNF-α, IL-1β and IL-6 were significantly higher in the burn/CLP group versus all other groups. Only IL-6 had a polytrauma-specific hepatic elevation in mRNA levels at this timepoint, indicating polytrauma elicited greater proinflammatory cytokine production from tissues/cells other than liver at the 32 h timepoint. The inflammatory response and ‘cytokine storm’ following major injury is complex and continues to be heavily studied. The three cytokines studied here demonstrate interesting polytrauma-dependent changes and warrants a more in-depth examination of the inflammatory repose to polytrauma in future studies. Additionally, future work may include measurements to determine if additional pro- and anti-inflammatory mediators and chemokines are altered following polytrauma. It would be interesting to examine whether any changes in a specific proinflammatory/anti-inflammatory cytokine correlates with mortality rates. The high mortality just prior to 36 h dictates using 32 h as the latest endpoint. Using 6 h as the earliest timepoint may preclude observation of early occurring polytrauma specific differences that influence the higher mortality rate. However, the presented data revealed no difference in circulating or hepatic mRNA levels of TNF-α, IL-1β and IL-6 between the CLP alone (sham/CLP) and polytrauma (burn/CLP) at 6 h despite very different mortality rates.

In this study we sought to answer whether intensified hepatic ER-stress is specific to the
combined injuries of polytrauma or if a more severe single injury, (G18, 2×) CLP, would produce similar results. Our data demonstrates that minor single injuries alone, or a more severe single injury, does not result in an amplified hepatic ER-stress response which occurs following polytrauma. The mechanism(s) leading to the increased hepatic ER-stress response following polytrauma are not yet known. However, it is clear by multiple measurements that ER-stress is significantly increased due to combined injuries (polytrauma). Therefore, understanding the causative factors of the hepatic ER-stress response and their contribution to liver damage, metabolic dysfunction, and higher mortality following polytrauma are of great interest and are currently being studied.

In summary, the presented studies describe a versatile model that can be utilized to study the effects of polytrauma on multiple organ systems at both the molecular and physiological level. Additionally, this model is highly reproducible in regards to survival outcome (previous manuscript) and the measures examined in this study. This is a straightforward model of polytrauma which is based on two very well established single injury models allowing for investigators to adapt this model for their research questions. This model concurrently combines two minor single injuries to produce injury patterns that are observed in modern warfare and civilian accidents [14-18]. Our findings demonstrate numerous pathophysiologic responses that differ or are exacerbated in response to polytrauma, compared to single minor injuries or a single, more severe injury. The effects of burn/CLP polytrauma likely mimic many of the human responses to complex injuries [7, 8, 9, 5]. We believe our model of burn/CLP polytrauma is a valuable tool that can be used to investigate a large breadth of areas in injury research.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Joseph L Messina, Department of Pathology, Division of Molecular and Cellular Pathology, The University of Alabama at Birmingham, Volker Hall, G019J, 1530 Third Ave S, Birmingham, AL35294-0019. Tel: 205-934-4921; Fax: 205-975-1126; E-mail: messinaj@uab.edu

References

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