Simulated Aeromedical Evacuation in a Polytrauma Rat Model

Françoise Arnaud; Georgina Pappas; Eric Maudlin-Jeronimo; Carl Goforth

BACKGROUND: Hemorrhage and traumatic brain injury can be lethal if left unattended. The transportation of severely wounded combat casualties from the battlefield to higher level of care via aeromedical evacuation (AE) may result in unintended complications. This could become a serious concern at the time of evacuation of mass casualties or for prolonged field care scenarios with limited resources.

METHODS: Following instrumentation (t1), anesthetized Sprague-Dawley rats were injured or not [75-kPa blast and 30% estimated blood-volume controlled hemorrhage] (t2). After 15 min, all rats were resuscitated with saline. During the simulated 3-h evacuation, 8000 ft (2440 m) vs. sea-level heart rate, temperature, and oxygenation (S_pO_2) were continuously recorded. One group of rats was euthanized immediately after evacuation (t3) and another after a 72-h recovery period (t4). Hematology and metabolic levels were measured at t1, t2, t3, and t4.

RESULTS: Survival was 100% in control-uninjured animals, 83% in injured animals under normobaria, and significantly reduced to 50% under hypobaria. This AE setting resulted in significantly lower hemodynamics, thermoregulation, and oxygenation parameters in the animals under hypobaria than those under normobaria. The initial lower mean arterial pressure (MAP) with the reduced oxygen level before AE were critical factors for the survival of injured animals. We observed a general increase of white blood cells and platelet ability to aggregate at t4 in all experimental groups.

CONCLUSION: Physiological parameters were affected during aeromedical evacuation in all groups. This was worsened for injured animals with MAP less than 60 mmHg associated with low S_pO_2 in a simulated aeromedical evacuation. This represented a high risk of mortality for severely polytraumatized animals.

KEYWORDS: altitude, aero-evacuation, blast, hemorrhage, hypobaria, trauma, resuscitation.

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Individual protective armor and early battlefield damage control strategies have largely contributed to lower mortality rates of wounded soldiers over different conflicts.[4] Early interventions for vascular injuries have benefitted both military and civilians due to key improvements in trauma care such as tourniquets, hemostatic bandages, and blood resuscitation strategies.[13,18,27] In addition, rapid medical evacuation via aeroevacuation (AE) to definitive care has become more effective since World War II and has become recognized as a desirable military capability to "clear the battlefield" and expedite casualty evacuation to hospitals.[4,11,19] However, AE is not recommended for all wounded and there have been anecdotal reports suggesting that air transport worsens complex trauma outcomes. For example, air filled organs, such as injured pulmonary or intestinal tissues, could suffer from additional trauma due to the rapid expansion of gases at higher altitudes.[24] Indeed, gas expansion resulting from hypobaria during AE, including decreasing partial pressure of oxygen, can aggravate conditions for hypoxic patients or for patients with cardiopulmonary disorders.[12,21,31] Specifically, poor outcomes have been reported in combined traumatic brain injury (TBI) and polytrauma

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patients.[17,21,31] In addition, coagulopathy due to severe trauma is often reported when injured service members finally arrive at continental U.S. military hospitals,[25] indicating physiological degradation related to the effects of AE.[33] The few preclinical small animal studies seem to indicate deleterious neurological and cognitive effects of AE.[12,19,21] Nonetheless, the impact of AE on polytrauma patients is still not well understood, particularly in the event of mass evacuation where a wide range of patients may require triage under austere conditions or extreme environments for the best usage of resources and limited AE availability.[8,23,35]

To address some of these questions regarding the transport of the wounded, we simulated an air evacuation setting using a polytrauma rat model including potential hypotensive resuscitation, hypoxemia, and hypothermia. We then hypothesized that an AE setting at a cabin pressure set at 8000 ft (2440 m) would worsen the hemodynamics and metabolism of the animals.

METHODS

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research. The experiments reported herein were conducted in compliance with the Animal Welfare Act and per the principles set forth in the "Guide for Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 2011.

Animals

Upon arrival, 10–12-wk-old male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA, USA) (387 ± 25 g; 335–468 g) were single-housed in a colony that was maintained on a 12:12 light-dark cycle (lights on from 06:00 to 18:00). A 5-d quarantine period allowed time for acclimation to the environment and handling by the investigators prior to any experimental manipulations. Rats were fasted overnight for ~16 h before the first experimental intervention with water provided ad libitum. During the postop recovery time, the animals were returned to their cages and monitored twice daily for adverse health effects.

Before instrumentation (catheter placement), the animals in all groups were initially anesthetized with a mixture of ketamine (70 mg · kg^{-1}) and acepromazine (3 mg · kg^{-1}) administered intraperitoneally (IP). Animals received an additional dose of ketamine (20 mg · kg^{-1} IP) 15 min later and subsequent doses (10 mg · kg^{-1} IP) as needed to maintain a surgical plane of anesthesia (no response to toe pinch for surgical manipulations). A final dose of ketamine (10 mg · kg^{-1} IP) was administered before transportation to the blast room. Buprenorphine (0.05 mg · kg^{-1} subcutaneously), a pre-emptive analgesia, was administered before instrumentation. During the entire procedure, the animals spontaneously breathed room air (F_IO_2 = 0.21); similarly the hypobaric chamber was continuously flushed with medical air. Animals were placed on a heating pad (Kent Scientific) to maintain normothermia at 37°C.

Using an aseptic cut-down surgical procedure, the femoral artery was catheterized using PE50 tubing (Intramedic 427,517; Becton Dickenson Primary Care Diagnostics, Sparks, MD) which was used to collect blood, infuse saline, and monitor blood pressure. After fluid administration subsequent to the shock period, the catheter was removed, the artery ligated, and the surgical incision closed prior to anesthetic recovery and simulated evacuation. Similarly a catheter was placed in the other leg after the 72-h recovery.

Procedure

Blast injury. The blast room was 5 min in walking distance from the surgery suite and the rats were hand transported to and from the blast room using their home cage. The blast injury (TBI) was produced by exposure to blast overpressure generated in a laboratory shock tube[6,7] (**Fig. A1** online, https://doi.org/10.3357/AMHP.5477sd.2019). The shock tube is a horizontally mounted, 12-inch diameter, circular, 19.5-ft long steel tube that is divided into a 2.5-ft compression chamber and a 17-ft expansion chamber where the animal is placed toward the distal end. The peak pressure generated by the shock tube depends on the thickness of the Mylar™ membranes placed between the compression and expansion chambers. When the compression pressure reaches a critical point, the membrane ruptures, releasing a pressure wave that propagates into the expansion chamber and impacts the animal. A 127-μm thick polyester Mylar™ membrane (500, Du Pont Co., Wilmington, DE, USA) sheet generates a peak pressure of 72–75 kPa pressure (equivalent of 10.4–10.9 psi) directed toward the rodent's head for 3–5 ms. The anesthetized animals were placed at the distal end of the expansion chamber into a plastic cone and lightly but securely restrained in a basket (one strap over the nose, one in the middle of the animal's body, and one around the back end of the animal) to prevent unwanted motion of the animal's body during the blast wave exposure. Our lab has demonstrated that a blast pressure of 72–75 kPa produces a mild TBI and abnormal postmortem brain histology.[7] Following the blast exposure, animals were returned to the surgical suite for the remainder of the experiment. Only rats assigned to the injury groups received the blast wave exposure; uninjured rats were transported to the blast room where they remained for an equivalent duration of time as the injured rats, but were not exposed to a blast wave (**Table AI** online, https://doi.org/10.3357/AMHP.5477sd.2019).

Hemorrhage. After the blast, anesthetized animals in the injury groups underwent a 30% of estimated blood volume controlled hemorrhage over a 5-min period. The last 2 ml of the hemorrhaged blood was used for laboratory analysis (blood gases, hematology, and clinical chemistry analysis). In the no-injury groups, 1 ml of blood was withdrawn for analysis.

Fluid administration. Animals in the no-injury groups received 3 ml of 0.9% sodium chloride IV immediately after

blood sample collection. The resuscitation of injured animals occurred 15 min after hemorrhage over a period of 5 min using 0.9% sodium chloride at twice the volume of blood loss (2:1 ratio) with an average volume of 17.9 ml given to the injury group. Sodium chloride was used as a conservative intravenous fluid for hypovolemia; it is used as a solution for the noncritically ill.[36] This could serve as basic bridge fluid after bleeding has stopped in case of mass casualty evacuation when blood components or other fluids are not available. Also, permissive hypotensive resuscitation has been shown acceptable for patients sustaining trauma when resources are limited as in prolonged field care.[2]

Simulated evacuation. Simulated evacuation occurred at either sea level [normobaric level, Silver Spring, MD: 300 ft (8 m)] or at an altitude of 2440 m (8000 ft) (hypobaria). Animals in the hypobaria groups (AE) were placed in a small cage inside the hypobaric chamber (Fig. A1). The flight chamber itself sits within the regular laboratory space. The simulated aeromedical evacuation consists of a 10–12 min depressurization step at a rate of approximately 300 $m \cdot min^{-1}$ (984 $ft \cdot min^{-1}$) to reach the pressure equivalent (430 mmHg) at an altitude of 2440 m. A vacuum pump draws the chamber gas to maintain the necessary pressure differential inside the chamber while a continuous in-flow of room air ensures air exchange. An altimeter is used to ensure desired altitude is achieved and maintained. The vacuum level is set using a servomechanism to maintain the required altitude. During the 3-h simulated evacuation, the vacuum and in flow of room air was continuously monitored to maintain the pressure. After 3 h, the chamber was repressurized to ambient level at the rate of 300 $m \cdot min^{-1}$ over 10 min. At the end of the 3-h simulated evacuation, some animals were euthanized (t3) and the other animals were recovered and returned to their cages. The chamber temperature was not controlled, but the animal cage in the chamber was sitting on a circulating water box connected to a regulated warming system. Ambient Po_2 and Pco_2 were measured from an air sample collected from the chamber that was measured at sea level outside the chamber. Therefore, the Po_2 and Pco_2 values were converted to the actual chamber pressure using the gas law equation: P (mmHg) = gas% $\times$ [3$\times$ 10^6 $\times$ 2 $-$ 0.0831*alt (m) + 760], with gas% representing the measured gas ratio. This setting simulated an aircraft environment where precise oxygen delivery cannot be controlled. Animals in the normobaric control groups and hypobaric groups were allowed to recover from anesthesia in the chamber at either the hypobaric pressure or ambient barometric pressure.

Physiological measurements. Mean arterial pressures (MAP) were continuously monitored and recorded every minute using a pressure monitoring device (BPA, Micro-Med Inc, Louisville, KY, USA) linked to the arterial catheter. Continuous MAP measurements were discontinued after resuscitation, but discrete measures were taken before euthanasia. Systemic parameters such as rectal temperature (Temp) were recorded via a rectal probe and pulse oximetry parameters, oxygen saturation (S_pO_2), and heart rate (HR) were continuously recorded every 30 s via a clip type sensor placed on the animal's paw (Physiosuite, Kent Scientific Corp., Litchfield, CT, USA) during the 3-h simulated AE transportation. This was discontinued when the animal recovered from anesthesia. HR, S_pO_2, and Temp measurements from the animals in the hypobaric chamber were read at sea level; however, the signal was transferred directly from the sensor (in the chamber) to the monitor and did not need correction.

Assays. Arterial blood samples were analyzed on an automated blood gas system (ABL 700, Radiometer, Copenhagen, Denmark) for pH, partial pressure of oxygen (P_aO_2), carbon dioxide (P_aCO_2), glucose, lactate, bicarbonate (HCO_3), and electrolytes. Blood samples were also obtained to measure complete blood count (CBC) [hematocrit (Hct), hemoglobin, red blood cell, white blood cell (WBC), neutrophil, and platelet counts] performed on a Hemavet (Drew Scientific, CT, USA). Coagulation parameters from fresh citrated blood thromboelastography (ROTEM delta, Tem Systems, Inc., Durham, NC, USA) included the clot formation time (Rotem-CFT), prothrombin time, partial thromboplastin time, and fibrinogen and thrombin (Diagnostica Stago, Parsippany, NJ, USA). Platelet aggregation (Multiplate, Diapharma, http://diapharma.com) was measured as the area under the curve. A standard clinical chemistry profile (including liver enzyme panel, creatinine, and creatine kinase) was performed on the Alera chemistry analyzer (AlfaWasserman, West Cadwell, NJ, USA). Plasma concentrations of IL-1α, IL-1β, IL-4, IL-6, IL-10, IL-18, GM-CSF, TNF-α, IFN-γ, Fractalkine, MCP-1, and Rantes were measured at t1, t2, t3, and t4 in EDTA samples stored frozen at −80°C. The cytokine assessment was performed with magnetic bead kits (RECYTMAG-65K, Millipore Sigma, Burlington, MA, USA) and analyzed with a Bio-Plex® 200 System (Bio-Rad, Hercules, CA, USA).

A schematic representation of the experimental design can be found in the auxiliary material online (**Fig. A2** online, https://doi.org/10.3357/AMHP.5477sd.2019). All animals were anesthetized prior to injury. The end of instrumentation (catheter and monitoring devices in place for the first blood collection) was designated as t1, the end of the injury phase (consisting of a blast and hemorrhage while under anesthesia) was designated as t2, after the 3-h post-trauma simulated transport (during which the animals were left to recover from anesthesia) was designated as t3, and finally the end of the longest post-transport observation period (the animals were recovered and observed for 72 h) was designated as t4. The animals were distributed into groups referring to treatment: instrumented sham, treated no-injury, treated injury; animals in all groups except the sham were subdivided into subgroups with different end-point times after simulated 3-h AE transportation, or after full recovery at 72 h. Therefore the number of animals differs in each subgroup. This resulted in the following distribution: instrumented sham (N = 10), no-injury (N = 64), injury (N = 80). Blood samples were collected at t1, t2, and t3 or t4 (Table AI online).

Data Analysis/Statistics

All rats were divided into subgroups by block randomization. Data were averaged for each group with standard deviation. Data obtained from the 72-h time points were compared with a Student's *t*-test for each individual group. Between group comparisons were performed using multiple analysis of variance (ANOVA) and/or logistic regression depending upon the nature of the outcome of interest (i.e., continuous or binary/categorical). The effects of the two independent variables (injury and transport) on the dependent physiological variables were analyzed using ANOVAs. Specifically, for time t2, the main effects of injury on physiological outcomes were analyzed using a 2-tailed ANOVA. Then, at time t3 and t4, a 2-way ANOVA was conducted to determine the effects of both injury and transport on dependent physiological measures (e.g., injury vs. no-injury, AE vs. no-AE). Based on the results from the ANOVAs across the time points, the dependent variables that were found to be significant were then further analyzed with Student's *t*-test to compare the outcome measures at specific times. The physiological measures that were gathered at t4 were from animals that survived 3 d post-AE. Continuous data were analyzed in a multiple ANOVA for mixed model (Groups × time). Fisher exact test was used for survival analysis. All statistical analyses were considered significant at the $P < 0.05$ level. The data was analyzed using SPSS version 22.0 software (IBM, Armonk, NY, USA).

RESULTS

A total of 144 animals were randomly distributed in different treatment groups. The cumulative mortality rate before the simulated evacuation was 16.3% after the polytrauma (13/80). Five animals did not survive after the blast and eight animals did not survive after hemorrhage following the blast, probably due to a combination of the injuries (polytrauma). Flying uninjured animals did not result in any loss. However, eight injured animals expired during the simulated evacuation or immediately after landing (one), incurring a 50% mortality (8/16) at t3 ($P < 0.01$) (**Fig. 1**). These injured animals did not survive between 42 and 150 min of hypobaric evacuation (average: 101 ± 34 min) out of 180 min flight. Animals that survived the simulated AE survived to 72 h. One rat expired in the normobaria uninjured group 18 h after the recovery from simulated transport for reasons probably unrelated to the procedure.

In the injured group, 11 animals showed pulmonary contusions as gross visual observations. The five animals that did not recover from blast exposure showed pulmonary contusions. At t1, all animals had a MAP of 91.2 ± 8.7 mmHg and HR of 361 ± 48 bpm. The controlled hemorrhage (32.9 ± 0.9% estimated blood volume) was similar in all injured groups, causing MAP and HR to decrease sharply to 25.7 ± 7.6 mmHg and 294 ± 81 bpm, respectively ($P < 0.01$), and these parameters rebounded after fluid resuscitation to 65.7 ± 15.1 mmHg and 345 ± 58 bpm without reaching baseline levels ($P < 0.01$; **Fig. 2A**). In contrast, uninjured animals' MAP was 85.4 ± 14.3 mmHg and HR 391 ± 20 bpm before simulated transportation without significant difference to baseline level. S_pO_2 remained stable during the pre-evacuation phase. All animals were within the normothermic range at the beginning of the experimental procedure and after the instrumentation (37.2 ± 0.2°C and 36.9 ± 0.4°C, respectively) although core temperature is known to decrease under anesthesia.

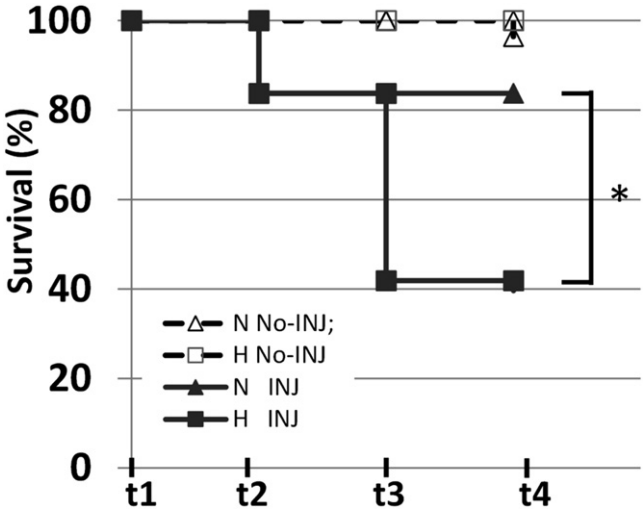

Fig. 1. Kaplan Meyer chart representing survival for animal groups: no-injury (- - -), injury (——), normobaria (▲), and hypobaria (■). N: normobaria, H: hypobaria, No-INJ: no-injury, INJ: injury. *$P < 0.05$ between t3 and t4 for the hypobaric injury group (Fisher exact). t1: following instrumentation; t2: after injury; t3: immediately after evacuation; and t4 after 72 h recovery.

The target altitude of 2440 m (8000 ft) was reached within 10 min and was maintained at 2465 ± 25 m during the 3-h simulated hypobaric evacuation (2401–2596 m min-max). Inspired oxygen and carbon dioxide levels were 20.7 ± 0.4% (20.0–21.8% O_2 min-max) and 3–5% CO_2. The surface equivalents of these partial pressures were 15.9% O_2 and 3.1% CO_2 as at 2440 m; the partial pressure of O_2 and CO_2 were 120 mmHg and 23 mmHg, respectively. Descent time at the end of the flight was 8 min. The temperature of the chamber averaged 20.5 ± 0.8°C. Some animals recovered from anesthesia in the chamber between 135–165 min during the aero-evacuation. In comparison, animals being evacuated at sea level had a faster recovery (90–120 min; $P < 0.05$).

At the beginning of the simulated transportation temperature and S_pO_2 were comparable in all animals (Fig. 2). During simulated evacuation the animal temperature steadily increased under normobaria and reached 38.5 ± 0.4°C for uninjured animals; temperature was slightly lower after injury (37.9 ± 0.6°C, $P < 0.01$). During the simulated hypobaric evacuation, body temperature decreased initially (36.4 ± 0.4°C, $P < 0.01$) and increased thereafter if there was no injury (37.8 ± 0.4°C), but after injury post-trauma the temperature remained lower (37.1 ± 0.5°C) during the course of AE. With a similar pattern, HR increased under normobaria, with a slight lower rate after injury (not significantly different), reaching 475 ± 45 bpm. During the simulated hypobaric evacuation, HR decreased

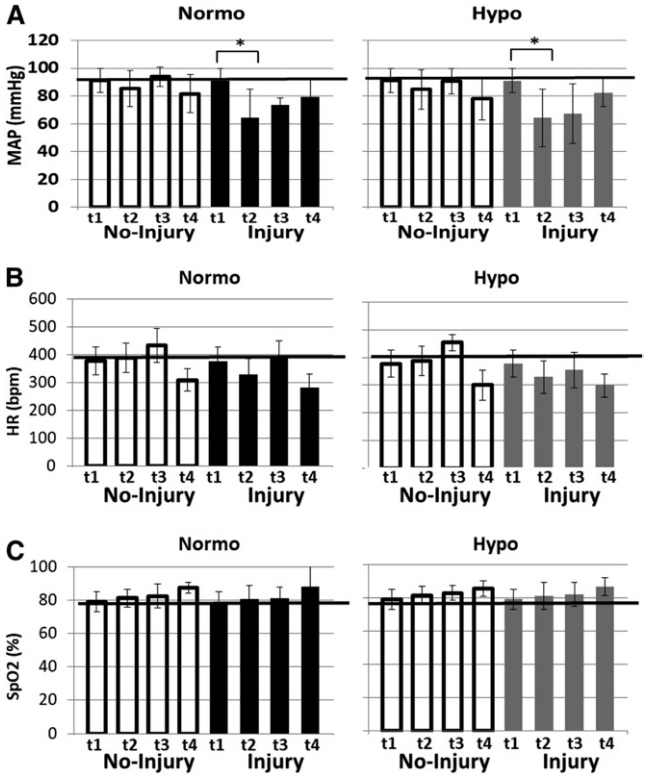

Fig. 2. A) mean arterial pressure (MAP, mmHg); B) heart rate (HR, bpm); and C) oxygen saturation (S_pO_2, %) at t1, t2, t3, and t4 in no-injury (white bars) and injury (black bars) groups during normo- and hypobaria. The line at the top of the graph represents the value for the sham animals. t1: following instrumentation; t2: after injury; t3: immediately after evacuation; and t4 after 72 h recovery. Mean and standard deviation; *$P < 0.01$.

initially in uninjured animals but was significantly lower after injury (346 ± 54 bpm vs. 293 ± 24 bpm, $P < 0.01$ for uninjured vs. injured animals). HR increased thereafter but was lower compared to normobaric evacuation if there was no injury, but after injury HR remained lower (340 ± 50 bpm, $P < 0.01$). Under normobaric evacuation, S_pO_2 remained stable. However, under hypobaria, S_pO_2 decreased significantly upon reaching altitude (87.4 ± 2.4% vs. 71.7 ± 2.5%, $P < 0.01$). Once the animals started recovering from anesthesia and resumed normal movement, temperature and HR tended to vary among animals. Overall, HR, S_pO_2, and temperature remained lower under hypobaria compared to normobaria ($P < 0.01$) (**Fig. 3**).

Hct, WBC, neutrophils, and platelets are presented as part of the CBC parameters (**Fig. 4**). Hct was slightly reduced at t2 (not significantly) but was lower at t3 and t4 compared to t1 in non-injury groups ($P < 0.05$). This could be due to the fluid administered to this control group. However, this was significantly lower at t3 and t4 in the injury group due to hemorrhage compared to no-injury groups ($P < 0.02$). WBC was similar at t1, t2, and t3 in all groups, but showed a significant increase in injured animals at t4 ($P < 0.01$). There was a higher level of neutrophils at t3 compared to t1 ($P < 0.01$) that declined by t4 if there was no injury, but remained slightly higher after injury ($P < 0.05$). Among the serum cytokine panel that was examined, the data showed high variability and thus no significant

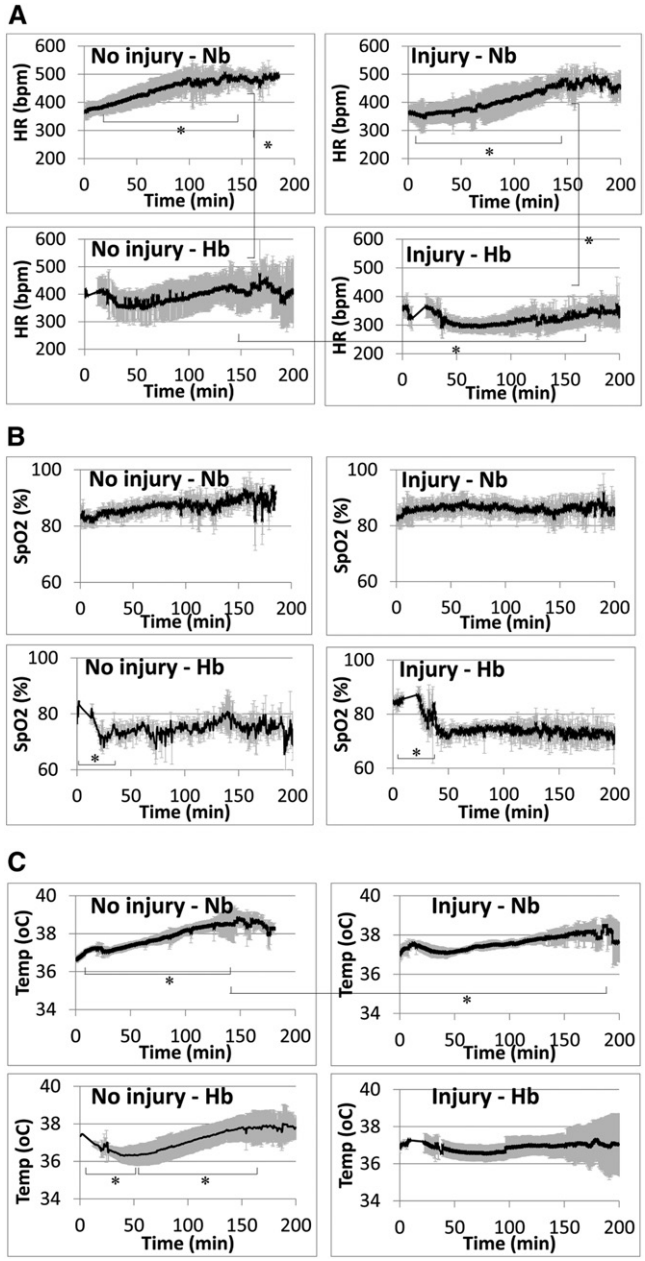

Fig. 3. Continuous recording during simulated evacuation until t3: A) HR, B) S_pO_2, and C) temperature in no-injury and injury groups under normobaria (Nb) or hypobaria (Hb). At the end of transport (t3), awakening of the animals occurring before the end of t3 caused the loss of the monitoring attachment and therefore from the data recording. Mean and standard deviation; *$P < 0.01$.

differences were detected between the various experimental groups at the different time points. TNF-α, IL-1β, and IL-4 fell below the lower level of detection; only a few cytokines indicated some variation in the course of the experiment. The initial level of MCP-1 (monocyte chemotractant), Rantes (leukocyte chemotractant), IFN-γ (for the early anti-inflammatory response), IL-6 (proiflammatory), and IL-10 (anti-inflamatory) were 264 ± 94 pg · ml^{-1}, 125 ± 73 pg · ml^{-1}, 60 ± 34 pg · ml^{-1}, 116 ± 90 pg · ml^{-1}, and 36 ± 14 pg · ml^{-1}, respectively.[29] Chemotractant Rantes and MCP-1 showed a trend of a higher expression at t3 and t4 after injury (**Table AII** online,

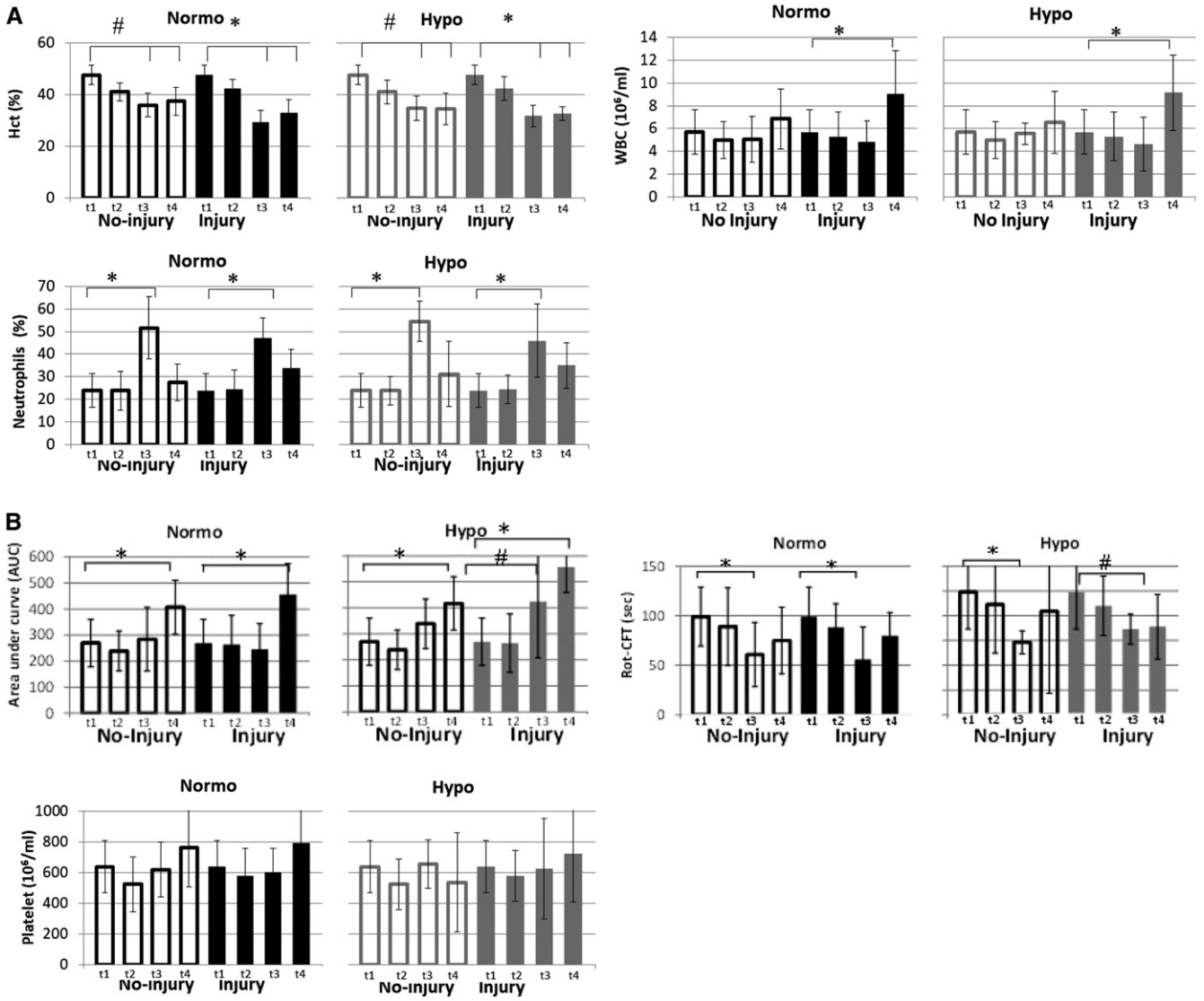

Fig. 4. Hematology variables for all rat groups at t1, t2, t3, and t4 for no-injury (white bar) and injury (black bar) groups under normo- and hypobaria. Mean and standard deviation. A) CBC panel with Hematocrit (Hct), white blood cell (WBC), and neutrophils. B) Thrombosis panel with aggregation (AUC; area under the curve), Rotem clotting formation time (CFT), and platelet count. t1: following instrumentation; t2: after injury; t3: immediately after evacuation; and t4 after 72 h recovery. Mean and standard deviation; $^{\#}P < 0.05$; $^{*}P < 0.01$.

https://doi.org/10.3357/AMHP./5477sd.2019). The platelet count was variable among animals, but was not significantly altered with injury or hypobaria; the slight increase of platelets at t4 was not significant. However, platelet aggregation, as measured by the area under the curve, increased significantly at t4 in the normobaria groups ($P < 0.01$) and this increase started to occur at t3 in the hypobaria groups ($P < 0.05$). Rotem-CFT, illustrating the time for platelet and fibrin to interact, was shortened at t3 in all groups ($P < 0.01$) and by t4 it began to rebound, but remained lower than the baseline (Fig. 4). The maximum clot formation Rotem-MCF was also elevated at t4 in all groups, prothrombin time was similar in all groups, and ATIII was elevated at t4 in all groups (data not shown). There were no significant differences in the hematology parameters for CBC, coagulation, or platelet aggregation prior to the AE that could separate survivors from nonsurvivors.

Blood gases (P_aO_2, P_aCO_2) and pH were measured at sea level at t1, t2, t3, and t4, but could not be measured while the animals were in the hypobaric chamber as blood sampling was not available. These values remained within normal ranges at each time point of the experiment (105.8 ± 37.8 mmHg, 43.8 ± 7.9 mmHg, and 7.38 ± 0.08, for P_aO_2, P_aCO_2, and pH) (**Table I**). Creatinine and electrolyte (data not shown) were within normal range and showed no significant differences among the groups at all time points. AST and ALT values were highly variable at t3 and t4 and tended to be elevated, but without statistical significance in the normo- vs. hypobaria injury groups. Glucose and lactate were similar and unremarkable in all groups.

Interestingly, the hemodynamics from nonsurviving animals departed from the surviving animals. There was a different physiological pattern in the injured animals that did not survive the hypobaric evacuation compared to survivors. Initially MAP

Table I. Chemistry and Metabolism.

NO-INJURY								
	Po_2 mmHg	Pco_2 mmHg	pH	Creat mM	AST mM	ALT mM	GLU mM	LAC mM
Sham	110 ± 44	40 ± 9	7.42 ± 0.06	0.3 ± 0.1	43 ± 10	32 ± 5	9 ± 0.9	1.4 ± 0.4
Normo t1	102 ± 44	44 ± 5	7.4 ± 0.07	0.3 ± 0.1	45 ± 11	30 ± 6	8.7 ± 1.5	1 ± 0.4
Normo t2	100 ± 23	44 ± 5	7.37 ± 0.04	0.3 ± 0.1	45 ± 18	23 ± 6	8.8 ± 1.4	1 ± 0.4
Normo t3	117 ± 37	37 ± 2	7.4 ± 0.05	0.4 ± 0.0	109 ± 83	38 ± 14	11.1 ± 2.1	1.2 ± 0.4
Hypo t3	117 ± 34	40 ± 7	7.41 ± 0.04	0.3 ± 0.1	98 ± 85	31 ± 12	8.6 ± 1.2	0.8 ± 0.1
Normo t4	110 ± 25	36 ± 6	7.45 ± 0.05	0.4 ± 0.1	109 ± 99	28 ± 7	9.7 ± 1.5	1.7 ± 0.8
Hypo t4	114 ± 32	43 ± 16	7.39 ± 0.13	0.3 ± 0.1	247 ± 211	59 ± 32	10.1 ± 2	2.3 ± 1.2
INJURY								
	Po_2 mmHg	Pco_2 mmHg	pH	Creat mM	AST mM	ALT mM	GLU mM	LAC mM
Normo t2	104 ± 40	47 ± 8	7.34 ± 0.1	0.3 ± 0.1	67 ± 92	36 ± 47	8.9 ± 1.7	1.2 ± 0.5
Normo t3	106 ± 24	43 ± 6	7.35 ± 0.07	0.4 ± 0	89 ± 42	25 ± 8	9.4 ± 1.5	1 ± 0.5
Hypo t3	125 ± 40	50 ± 15	7.35 ± 0.07	0.4 ± 0.3	213 ± 302	119 ± 169	7.2 ± 2.1	1.7 ± 1.4
Normo t4	95 ± 15	38 ± 3	7.44 ± 0.05	0.3 ± 0.1	155 ± 250	41 ± 41	8.7 ± 0.9	1.7 ± 0.6
Hypo t4	122 ± 34	37 ± 3	7.44 ± 0.05	0.3 ± 0.1	526 ± 467	105 ± 66	9 ± 1.2	1.7 ± 0.5

Chemistry and metabolism patterns in rats at t1, t2, t3, and t4 in the normo- and hypobaria groups and no-injury and injury animal groups. Mean and standard deviation. There were no significant differences between groups.
Creat: creatinine; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GLU: glucose; LAC: lactate.

was similar for the nonsurviving and the surviving injured rats up to t2; however, the following shock period had a more drastic effect on the nonsurvivors as MAP did not rebound toward the baseline before aero-evacuation (45.5 ± 14.4 mmHg vs. 66.5 ± 17.9 mmHg for surviving vs. nonsurviving; $P < 0.01$). In contrast, at the beginning of the evacuation, the injured animals that had similar low MAP (~50 mmHg) under normobaria survived and recovered. Temperature, HR, and S_pO_2 were within normal ranges at the start of simulated evacuation for survivors and nonsurvivors; however, the injured animals could not increase their temperature at the end of AE and even though there was an attempt of compensation for HR this could not be sustained. There was no difference in the metabolic and gas exchange pattern between survivors and nonsurvivors before evacuation. Once in the chamber, S_pO_2 level dropped similarly for the survivors and nonsurvivors, but levels were lower than 70% for the nonsurvivors in contrast to survivors, which remained at the 70% mark ($P < 0.05$). (**Fig. 5**).

DISCUSSION

Multiple investigators have reported that complications of wounded service members occur shortly after AE evacuation with potential long-term consequences, particularly for patients with traumatic brain injury.[22,31] In the current study, we addressed these complications using a rodent (rat) model of polytrauma (with blast and hemorrhage) and simulated evacuation at an altitude of 2440 m (8000 ft) for 3 h. Hypobaric conditions in the current study had no significant effect on uninjured rats as the animals had normal metabolic parameters, and they survived the simulated AE as well as the 72-h observation period following evacuation. In contrast, our observations indicated that the hypobaric environment influenced several physiological measures following the mild/moderate polytrauma (injured by blast and hemorrhage) and this simulated hypobaric evacuation reduced the physiological function of injured animals, which contributed to 50% mortality within the first 150 min of the evacuation under hypobaric conditions. Interestingly, the combination of two nonlethal mild injuries produced severe effects that reduced the survival rate. Similar low survival rate was reported by Fiskum et al.[16] in polytrauma involving cortical impact and hemorrhage. We hypothesize that hemorrhage was the primary influence of the loss of compensation as in our study both S_pO_2 and HR remained lower under hypobaric compared to normobaric conditions. While hypobaria and the resulting hypoxemia were tolerated by the uninjured animals, polytraumatized animals, in particular those being hypotensive with a MAP less than ~60 mmHg prior to AE, did not perform well in flight. Such hypobaric conditions challenged the hemodynamic compensation of these injured animals, which, therefore, did not recover. At altitude, as pressure is reduced (hypobaria), oxygen concentration (21%) remains the same at any elevation, but at 2440 m, the oxygen partial pressure is lowered by 24% from 159 to 120 mmHg, which causes hypoxemia. Therefore, at such altitudes, the animals inhaled a surface equivalent of 15.9% concentration of inspired oxygen (causing hypoxemia), which could explain the lower S_pO_2 that was recorded (Fig. 3), and this lower pressure gradient across the pulmonary alveoli can influence physiology. This hypoxic phase could have been a factor that was compromising the physiology of the injured rats. In addition, we observed that during the first hour in the simulated AE, rectal temperature decreased, but with time, the uninjured animals resumed a homeothermic body temperature, whereas the injured animals maintained a lower temperature during AE. Although the temperature of the animals was monitored and controlled, it tended to decreased initially (at t2), most likely due to anesthesia, and during the transportation of the animal to different experimental stations. Nonetheless, given that all animals (from both the injured and uninjured groups) were able to return to their baseline temperature before undergoing the simulated AE, it is likely that the injury influenced the animal's thermoregulation under hypobaria. For injured animals with a low MAP and HR

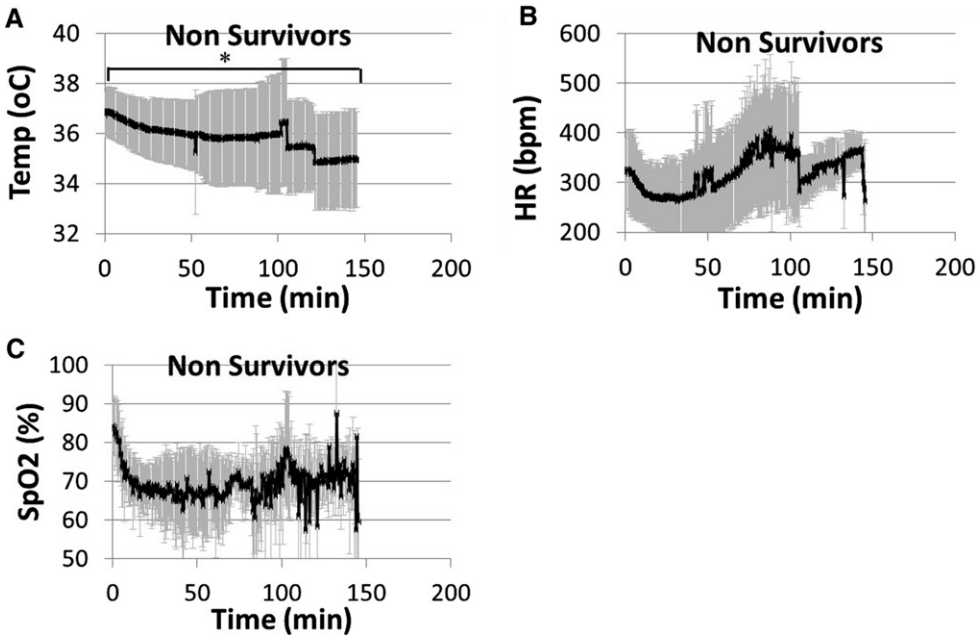

Fig. 5. Continuous physiology traces for nonsurviving injured animals during hypobaria. A) Temperature (°C), B) heart rate (HR, bpm), and C) oxygen saturation (S_pO_2, %). Mean and standard deviation; *$P < 0.01$ for initial to end point temperature.

before AE, blood flow is likely to be reduced, thus contributing to reduced body temperature. This is indicative of more powerful and immediate consequences of the hemorrhagic injury compared to the brain injury from the blast. However, interestingly, all surviving animals recovering from anesthesia in the flight chamber had transiently increased temperature toward the end of the simulated AE, which seems to indicate a regulated reaction from the brain that was absent in the severely injured animals (blast and hypoxia). Temperature is also regulated by the hypothalamus and hyperthermia is often observed clinically after TBI, hence suggesting potential damage to the hypothalamic-brain stem axis as a result of the blast.[10,28,30] In the current study, the hypothalamus could have been affected by the blast injury, and was perhaps the origin of the impaired thermoregulation.[32,34] Also, rapid breathing, bradycardia, and hypotension are frequently observed immediately after blast exposure due to vagal response, and hence are evidence of a regulated brain reaction.[5,20,26] Lungs are sensitive to barometric changes and pulmonary lesions during blasts have been reported previously from exposure to a moderate level of blast overpressure.[15] While low pressure did not impact the lungs dramatically, the addition of hemorrhage and hypoxia could have caused greater pulmonary damage than could be observed at the end of the AE.[6,7]

The hematological and metabolic profiles of the surviving animals were generally within normal range and the effect of hypobaria was not remarkable. The increase of neutrophils at t3 may have been caused by an acute response to the surgical procedure and hence triggered the increase of WBC at t4 in the injured animals. As an acute response to trauma, the recruitment of neutrophils, monocytes, and macrophages may have been initiated by the inflammatory response. It is noteworthy that anesthesia present at t1, t2, and t3 for the animals that were still under sedation might have contributed to the modulation of the WBC and the expression of inflammatory markers; however, serum cytokine levels were not significantly different, but trending toward an elevation between t3–t4. Although platelet count did not change, their activity was increased at t4. The cause of the elevated platelet aggregation (measured as the area under the curve) at t4 in all groups that paralleled Rotem-MCF and the higher ATIII is unclear. Also, reduced Rotem-CFT at t3 indicated a similar response. Platelets are known to be responders to damage-associated molecular patterns and could also associate with leukocytes and monocytes at the time of injury.[9] Instrumentation could have produced the equivalent of surgical stress, which could explain the responses at t3 and t4.[1,3,14] An increase of corticosterone at the end of AE was also observed in a previous experiment and could be related to a delayed response from the animals after the surgical intervention.[3] Finally, the level of liver enzymes at t3 and t4 in the animals in the hypobaria-injury group suggests a trend for delayed response to altitude.

Limitations of this study were as follows. Supplemental oxygenation would have been necessary to maintain oxygen surface equivalents of 20.7% O_2 at 2440 m (8000 ft), but the hypobaric chamber used for this protocol simulated situations in aircrafts with limited oxygen control or rotary wings. Additionally, lower Po_2 challenges this polytrauma model by producing conditions that are survivable, but are also restrictive in critically wounded individuals, which simulates actual AE scenarios. Implementation of F_IO_2 of 0.3 should be considered for future experiments. Normal saline was used as an emergency fluid in case of mass casualty where balanced crystalloid fluids or colloids may have contributed to better outcomes; nonetheless, this model is indicative of a minimum threshold when all resources are limited. Also, the 72-h observation period may not have been long enough to contribute to significant physiological changes after hypobaric exposure, or most importantly, to substantiate any behavioral deficits related to motor and cognitive effects.

In conclusion, overall, AE altered the physiology of the animals and, in particular, the combination of injury and hypobaria significantly affected survival in a rat injury model after a 3-h simulated AE. Surviving animals exhibited similar metabolic changes despite whether or not they were injured. There were no robust differences for the outcomes between groups of

surviving animals whereas nonsurvivors exhibited poorer control of their hemodynamics and homeostasis. These findings warrant further investigation into more specific effects of brain injury, including metabolic markers and cognitive behavior.

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