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Persistent injury-associated anemia in aged rats☆

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ABSTRACT

Background: Hypercatecholaminemia and bone marrow dysfunction have been implicated in the pathophysiology of persistent-injury associated anemia. The elderly may be vulnerable to this phenomenon due to high basal and peak catecholamine levels, impaired erythroid progenitor growth, and baseline anemia. We hypothesized that aged F344-BN rats subjected to severe trauma and chronic stress would have persistent injury-associated anemia.

Methods: Male F344-BN rats age 25 months were randomly allocated to: naïve (n = 8), lung contusion (LC, n = 9), LC followed by daily chronic restraint stress (LC/CS, n = 9), LC followed immediately by hemorrhagic shock (LCHS, n = 8), and LCHS followed by daily CS (LCHS/CS, n = 8). Urine norepinephrine was measured on days one and seven. Locomotor testing was performed on day five. Bone marrow cellularity, hematopoietic progenitor growth, and peripheral blood hemoglobin levels were assessed at sacrifice on day seven. Data are presented as mean ± standard deviation, $*p < 0.05$ vs. naïve.

Results: Norepinephrine levels (ng/mL) were significantly elevated one day after LCHS (420 ± 239* vs. naïve: 97 ± 71) and LCHS/CS (375 ± 185*), and remained significantly elevated on day seven for LCHS/CS (359 ± 99*), but not LCHS (212 ± 130). On locomotor testing, groups subjected to CS traveled shorter distances at lower velocities and spent less time in the center of the cage. Colony forming units-erythroid (colonies/plate), representing late erythroid progenitors, were significantly decreased after LC/CS (40 ± 1* vs. naïve: 47 ± 4), LCHS (40 ± 1*), and LCHS/CS (38 ± 3*). LCHS/CS animals had significantly lower hemoglobin (g/dL) than naïve animals (13.3 ± 1.3* vs. naïve: 15.2 ± 0.9).

Conclusions: Persistent injury-associated anemia occurs in aged rats. Further research is needed to determine whether the pathophysiology of this phenomenon differs from that of younger rats, and to translate these findings to elderly trauma patients.

1. Introduction

Anemia is common with increased morbidity among the elderly. Anemia affects approximately 10% of subjects age ≥ 65, approximately 20% of subjects age ≥ 85, and is associated with two-fold increased all-cause mortality among the elderly (Patel, 2008; Izaks et al., 1999). Anemia and associated complications may be magnified in the setting of traumatic injury, which is often accompanied by acute blood loss and high levels of circulating catecholamines and inflammatory cytokines, which appear to inhibit erythropoiesis in the bone marrow, leading to persistent injury-associated anemia (Livingston et al., 2003; Bible et al., 2014; Pirie et al., 2016; Robinson et al., 2006; Rodriguez et al., 2001). The elderly may be especially vulnerable to post-injury hypercatecholaminemia and bone marrow dysfunction. Basal and peak plasma norepinephrine levels rise with increasing age (Ziegler et al., 1976; Rowe and Troen, 1980; McCarty, 1986; Barnes et al., 1982), and aging has been associated with reduced hematopoietic stem cell proliferative and regenerative capacity (Dumble et al., 2007; Rossi et al., 2007; Stelzer

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et al., 2010).

Preclinical investigation of persistent injury-associated anemia has been performed using young (age 8–9 week) Sprague-Dawley rats (Bible et al., 2015a; Beiermeister et al., 2010; Hannoush et al., 2011). Like many strains, Sprague-Dawley rats are not suitable for aging studies because they are afflicted by age-dependent renal insufficiency, and typically do not survive to old age (Erdely et al., 2003). In contrast, Fischer-Brown Norway (F344-BN) rats do not appear to be afflicted by age-dependent renal insufficiency, and are less affected by age-related cardiac and hematopoietic dysfunction than other commonly used rat strains (Moningka et al., 2011; Lipman et al., 1996; Walker Jr et al., 2006). Importantly, under normal physiologic conditions, F344-BN rats maintain the capacity for erythropoiesis throughout their lifespan, with average hemoglobin 15.3 g/dL at 30 months of age (Smith, 1992). We chose to use 25 month-old F344-BN rats in these experiments, roughly corresponding to 65 year-old humans (Sengupta, 2013).

The purpose of this study was to determine whether persistent injury-associated anemia occurs in aged rats. We hypothesized that aged F344-BN rats would have post-injury hypercatecholaminemia that would be sustained one week after injury by daily restraint stress, and that the combination of severe injury and chronic stress would be associated with bone marrow dysfunction and anemia.

2. Materials and methods

2.1. Animals

Male F344-BN rats age 25 months and weighing 450–600 g were housed in pairs and fed ad lib with Teklad Diet #7912 (Harlan Laboratories Inc., Tampa, FL) and water during a one week acclimation period. Light and dark cycles were 12 h each throughout acclamation and experimental periods. Male rats were used to avoid the potentially confounding effects of estrogens on the physiologic response to hemorrhagic shock. F344-BN rats were generously donated by the National Institute of Aging. Young naïve Sprague-Dawley rats age 8–9 weeks weighing 300–400 g (Charles River, Raleigh, NC) were used for purposes of comparison to aged naïve F344-BN rats as young F344-BN rats were not available. All animal care was conducted in accordance with University of Florida Institutional Animal Care and Use Committee standards. Animals were randomly allocated to one of six groups: naïve (n = 8), lung contusion (LC, n = 9), LC followed by daily CS (LC/CS, n = 9), LC followed immediately by hemorrhagic shock (LCHS, n = 8), and LCHS followed by daily CS (LCHS/CS, n = 8). Chronic restraint stress was incorporated into the injury models to simulate stressors associated with the intensive care unit environment. Animals were sacrificed on post-injury day seven by cardiac puncture following intraperitoneal injection of ketamine (80–100 mg/kg) and xylazine (5–10 mg/kg) on day seven.

2.2. Lung contusion

Prior to LC, animals were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). LC was performed by applying a percussive staple gun (PowerShot Model 5700 M, Saddle Brook, NJ) to a 12 mm metal plate applied to the right lateral chest wall 1–2 cm below the axillary crease. This model has been shown to produce a clinically significant and reproducible pulmonary contusion based on histologic findings (Bible et al., 2015b; Gore et al., 2015; Shah et al., 2009).

2.3. Hemorrhagic shock

Rats that would also undergo HS were then placed on a heating pad, and PE-50 tubing was inserted into the right internal jugular vein and right femoral artery under direct visualization. The arterial catheter was applied to a BP-2 Digital Blood Pressure Monitor (Columbus Instruments, Columbus, OH) for continuous blood pressure monitoring. Blood was withdrawn through the venous catheter into a heparinized syringe until a mean arterial pressure of 30–35 mm Hg was reached. This blood pressure range was maintained for 45 min by withdrawing or reinfusing blood as needed. After 45 min, shed blood was reinfused at 1 mL/min.

2.4. Chronic stress

CS was performed by placing animals in a restraint cylinder (Kent Scientific, Torrington, CT) for 2 h per day. In the LC/CS and LCHS/CS groups, CS began on post-injury day 1. To prevent acclimation, the cylinders were rotated 180° every 30 min, and alarms (80 dB) were transmitted by speakers placed immediately adjacent to the cylinders for 2 min each time the cylinders were rotated. Because animals undergoing CS had no access to food or water in the restraint cylinder, all other groups were subjected to a two hour daily fast while CS was being performed.

2.5. Locomotor testing

On post-injury day five, all F344-BN groups were subject to locomotor testing, performed prior to daily CS or fasting. Spontaneous activity was measured using locomotor activity chambers (Med Associates, Fairfax, VT), Plexiglas® and metal cubicles (17 × 17 × 12 in.) with arrays of infrared emitters and receivers, which allow the automatic recording of an animal's position and movement in the chamber. We recorded distance moved (cm), time spent moving (seconds), and time spent in the center of the cage (seconds) during a three-minute period.

2.6. Norepinephrine

Norepinephrine levels were measured in the urine as a surrogate for catecholamine levels in the blood because blood levels tend to be erratic with labile peaks and troughs, whereas urinary concentrations of norepinephrine are a more stable representation of circulating norepinephrine levels over time. Norepinephrine was measured rather than epinephrine because previous work has demonstrated that post-injury anemia is primarily mediated by norepinephrine (Fonseca et al., 2004). On post-injury days one, spontaneous urine samples were obtained during daily handling and weighing for non-CS groups, and were obtained during CS for CS groups. Spontaneous urine samples were also obtained on post-injury day seven prior to sacrifice. Samples were stored at −80 °C. Urine norepinephrine was measured by enzyme linked immunosorbent assay (Labor Diagnostika Nord, Nordhorn, Germany) according to manufacturer instructions.

2.7. Bone marrow cellularity and progenitor growth

Bone marrow samples were obtained at the time of sacrifice by removing the left femoral epiphysis and flushing the femur with a 5 mL syringe containing Iscove's modified Dulbecco's Medium and 10% fetal bovine serum. The resulting suspension was passed through a 40 μm sterile nylon strainer, stained with 0.4% Trypan blue, and placed on a hemocytometer plate. Total viable cell counts were assessed by light microscopy.

Bone marrow cells were plated and incubated with a stock solution as previously described (Mohr et al., 2011). Growth of hematopoietic progenitor cells was assessed at three stages: colony forming unit – granulocyte, erythrocyte, monocyte, megakaryocyte (GEMM, counted fourteen day after plating), blast forming unit – erythroid (BFU-E, counted fourteen days after plating), and colony forming unit – erythroid (CFU-E, counted seven days after plating). Hematopoietic progenitor cell growth was assessed by light microscopy to identify colonies by their characteristic morphologic patterns.