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The effects of propranolol and clonidine on bone marrow expression of hematopoietic cytokines following trauma and chronic stress

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ABSTRACT

Background: Attenuating post-injury neuroendocrine stress abrogates persistent injury-associated anemia. Our objective was to examine the mechanisms by which propranolol and clonidine modulate this process. We hypothesized that propranolol and clonidine would decrease bone marrow expression of high-mobility group box-1 (HMGB1) and increase expression of stem cell factor (SCF) and B-cell lymphoma-extra large (Bcl-xL).

Methods: Male Sprague-Dawley rats were allocated to naïve control, lung contusion followed by hemorrhagic shock (LCHS), or LCHS plus daily chronic restraint stress (LCHS/CS) ±propranolol, ±clonidine. Day seven bone marrow expression of HMGB1, SCF, and Bcl-xL was assessed by polymerase chain reaction.

Results: Following LCHS, HMGB1 was decreased by propranolol (49% decrease, $p = 0.012$) and clonidine (54% decrease, $p < 0.010$). SCF was decreased following LCHS/CS, and was increased by propranolol (629% increase, $p < 0.001$) and clonidine (468% increase, $p < 0.001$). Bcl-xL was decreased following LCHS/CS, and was increased by propranolol (59% increase, $p = 0.006$) and clonidine (77% increase, $p < 0.001$).

Conclusions: Following severe trauma, propranolol and clonidine abrogate persistent injury-associated anemia by modulating bone marrow cytokines, favoring effective erythropoiesis.

Introduction

Anemia affects nearly all patients who remain in an intensive care unit (ICU) for three or more days.[1,2] Trauma patients are often subjected to acute blood loss at the time of injury, making them immediately vulnerable to anemia and associated complications. Management of severe anemia often involves red blood cell transfusion, which is associated with immune suppression, infectious complications, and increased mortality.[3–5] More than half of all critically ill trauma patients receive a blood transfusion during their ICU stay, and about half of all transfusions are given after four days in the ICU, underscoring the clinical importance of persistent anemia following severe trauma.[6] The pathophysiology of post-injury anemia is multifactorial, involving reduced iron bioavailability, hemodilution, blood loss due to phlebotomy and procedural interventions, and bone marrow suppression.[7,8] These are challenging therapeutic targets. Iron supplementation and epoietin alpha administration have inconsistently demonstrated efficacy in treating post-injury anemia, hemodilution is often unavoidable during the acute resuscitation phase, and efforts to limit operative and phlebotomy blood loss are largely dependent on technical prowess and the necessity for laboratory measurements.[9,10] However, improved bone marrow function may be a viable therapeutic target.

Following severe traumatic injury, elevated levels of circulating catecholamines and inflammatory mediators inhibit bone marrow function and erythropoiesis in a process that is conserved between rodents and humans.[11–13] Blocking the neuroendocrine stress response with propranolol, a non-selective beta adrenergic receptor blocker, and clonidine, which decreases central sympathetic outflow by stimulating alpha-2 adrenergic receptors in the brain

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stem, has demonstrated efficacy in abrogating persistent injury-associated anemia.[14–16] In a rat model of severe blunt trauma and hemorrhagic shock, administration of propranolol immediately following resuscitation increased erythroid progenitor cell growth and hemoglobin levels.[14] In subsequent experiments, severe blunt trauma and hemorrhagic shock were followed by daily neuroendocrine activation via placement in a restraint cylinder.[15] Under these conditions, administering clonidine increased erythroid progenitor growth and hemoglobin levels. Using these experimental data to generate hypotheses for translational investigations, a retrospective review of severely injured trauma patients found that patients receiving beta blockade or clonidine had favorable hemoglobin trends compared with patients who did not receive beta blockade or clonidine.[16]

However, the mechanisms by which propranolol and clonidine promote erythropoiesis following severe trauma remain unclear. Several hematopoietic cytokines, which impact bone marrow function and erythropoiesis, may play and important role in this process.[17] Previous studies suggest that erythropoiesis may be promoted by interleukin 1 (IL-1), interleukin 10 (IL-10), stem cell factor (SCF), and B-cell lymphoma-extra large (Bcl-xL), and inhibited by high mobility group box 1 (HMGB1).[18–23] Recent work suggests that trauma and stress are associated with increased bone marrow expression of HMGB1, equivocal effects on Bcl-xL, and decreased bone marrow expression of IL-1, IL-10, and SCF.[17] We hypothesized that propranolol and clonidine would decrease bone marrow expression of HMGB1 and increase bone marrow expression of IL-1, IL-10, SCF, and Bcl-xL following severe trauma and chronic stress.

Methods

Animals

Eight week-old male Sprague-Dawley rats (Charles River, Raleigh, NC) weighing 300–400 g were housed in pairs and fed *ad lib* with Teklad Diet #7912 (Harlan Laboratories Inc., Tampa, FL) and water during 12 h light and dark cycles during a one week acclimation period. Animals were randomly allocated to seven groups (n = 6–7 per group): 1) naïve control, 2) lung contusion followed by hemorrhagic shock (LCHS), 3) LCHS plus propranolol, 4) LCHS plus clonidine, 5) lung contusion followed by hemorrhagic shock and daily restraint stress (LCHS/CS), 6) LCHS/CS plus propranolol, 17) LCHS/CS plus clonidine. The University of Florida Institutional Animal Care and Use Committee approved this study.

Lung contusion

Prior to injury, animals were anesthetized by intraperitoneal (IP) 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 cm below the axillary crease. This model has been shown to produce a clinically significant and reproducible pulmonary contusion.[24–26]

Hemorrhagic shock

The right internal jugular vein and right femoral artery were cannulated under direct visualization. Arterial blood pressure was monitored on a BP-2 Digital Blood Pressure Monitor (Columbus Instruments, Columbus, OH). Blood was withdrawn through the venous catheter into a heparinized syringe until a mean arterial pressure of 30–35 mm Hg was reached and maintained for 45 min by withdrawing or reinfusing blood as necessary. After 45 min, all blood was reinfused at 1 mL/min.

Chronic stress

In the LCHS/CS groups, CS began one day after LCHS. Animals were placed in a restraint cylinder (Kent Scientific Corporation, Torrington, CT) for two hours daily. The cylinders were rotated 180° every 30 min, and alarms and sirens (80 dB) were transmitted by speakers placed immediately adjacent to the cylinders for two minutes each time the cylinders were rotated. Non-CS groups were subjected to a two hour daily fast while CS was performed.

Propranolol and clonidine

Propranolol and clonidine were administered by intraperitoneal injection ten minutes following resuscitation from hemorrhagic shock and again following each episode of CS or daily fasting. Propranolol and clonidine were dosed at 10 mg/kg and 75 μg/kg, respectively, based on previous work.[15,24] Propranolol and clonidine were selected for use in this study because differences in mechanism of action between these medications may indicate the relative importance of central sympathetic tone and peripheral adrenergic receptors in the pathophysiology of persistent injury-associated anemia. Propranolol is a non-selective beta-adrenergic receptor antagonist. Pre-clinical studies using selective beta-blockers have demonstrated that bone marrow protection is mediated through β-2 and β-3 receptors.[27] Clonidine is a centrally acting alpha 2-adrenergic receptor agonist that inhibits norepinephrine release, which inhibits erythroid progenitor grown in a dose-dependent fashion.[28,29]

Bone marrow procurement

Animals were sacrificed by cardiac puncture following intraperitoneal injection of ketamine (80–100 mg/kg) and xylazine (5–10 mg/kg) seven days after initial injury. Bone marrow was collected immediately following sacrifice. This time interval was selected to allow for assessment of changes in bone marrow physiology due to chronic restraint stress based on previous work. The left femur was flushed with Iscove Modified Dulbecco Medium (Lonza, Walkersville, MD) to collect bone marrow, which was placed immediately on dry ice and then stored at −80 °C.

Bone marrow cytokine measurement

Bone marrow cytokine expression levels were assessed by endpoint polymerase chain reaction. The following primers were selected: IL-1a: forward 5′ gacaagcctgtgttgctgaa and reverse 5′ tggttccactaggctttgct (product region: 665–1046, product size: 381 bp); IL-1b: forward 5′ aggacccaagcaccttcttt and reverse 5′ gggattttgtcgttgcttgt (product region: 316–574, product size: 257 bp); IL-10: forward 5′ gaattccctgggagagaagc and reverse 5′ caaggagttgctcccgttag (product region: 328–624, product size: 295 bp); SCF: forward 5′ ggcctacaatggacagcaat and reverse 5′ ttcagtgcaggtttcacagc (product region: 724–1113, product size: 388 bp); HMGB1: forward 5′ gttctgagtaccgcccaaaa and reverse 5′ ttcatcctcctcgtcgtctt (product region: 374–639, product size: 264 bp); and Bcl-xL: forward 5′ aggatacagctggagtcag and reverse 5’ tctccttgtctacgctttcc (product region: 237–653, product size: 416 bp). Amplifications were performed using a SimpliAmp thermal cycler (Applied Biosystems, Carlsbad, CA). Products were separated on 1.5% agarose gel stained with Ethidium Bromide (Invitrogen, Carlsbad, CA), allowing visualization of Ethidium Bromide fluorescence from primer-bound complimentary DNA.

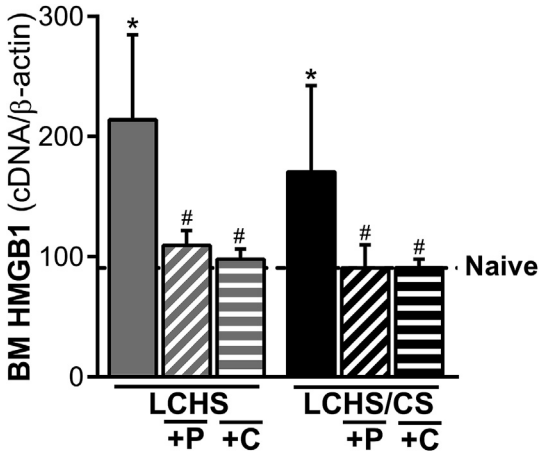

Fig. 1. Propranolol (P) and clonidine (C) significantly decreased bone marrow expression of high mobility group box 1 (HMGB1) seven days following lung contusion and hemorrhagic shock (LCHS) as well as LCHS followed by daily chronic stress (LCHS/CS). $^{*}p < 0.05$ vs. Naive, $^{\#}p < 0.05$ vs. untreated counterpart.

Analysis

Statistical analysis and figure production were performed using GraphPad Prism (version 6.05, GraphPad Software, La Jolla, CA). Bone marrow cytokine expression levels were compared by one-way analysis of variance. Data were illustrated and reported as mean ± standard deviation with α set at 0.05.

Results

High mobility group box 1

Bone marrow expression of HMGB1 seven days after traumatic injury is illustrated in Fig. 1. Compared to naive animals, HMGB1 expression was significantly increased following LCHS (136% increase, $p = 0.002$) and LCHS/CS (89% increase, $p = 0.021$). Propranolol and clonidine significantly decreased HMGB1 expression following LCHS (49% decrease, $p = 0.012$; 54% decrease, $p = 0.007$, respectively). Similar effects were observed for propranolol and clonidine administration following LCHS/CS (47% decrease, $p = 0.040$; 47% decrease, $p = 0.037$, respectively).

Interleukin 1

Bone marrow expression of IL-1 seven days after traumatic injury is illustrated in Fig. 2. Compared to naive animals, IL-1a expression was significantly decreased following LCHS (96% decrease, $p < 0.001$) and LCHS/CS (96% decrease, $p < 0.001$). Similar decreases were observed for IL-1b following LCHS (95% decrease, $p < 0.001$) and LCHS/CS (99% decrease, $p < 0.001$). Propranolol and clonidine significantly increased IL-1a expression following LCHS (5409% increase, $p < 0.001$; 4882% increase, $p < 0.001$, respectively) and LCHS/CS (2833% increase, $p < 0.001$; 1842% increase, $p < 0.001$, respectively). Similar patterns were observed for IL-1b. Propranolol and clonidine significantly increased IL-1b expression following LCHS (2636% increase, $p < 0.001$; 1477% increase, $p < 0.001$, respectively) and LCHS/CS (18,654% increase, $p < 0.001$; 10,073% increase, $p < 0.001$, respectively).

Interleukin 10

Bone marrow expression of IL-10 seven days after traumatic injury is illustrated in Fig. 3. Compared to naive animals, IL-10 expression was significantly decreased following LCHS (47% decrease, $p = 0.043$) and LCHS/CS (65% decrease, $p = 0.018$). Propranolol and clonidine increased IL-10 expression following LCHS, though the differences were not statistically significant (48% increase, $p = 0.342$; 76% increase, $p = 0.151$, respectively). Following LCHS/CS, propranolol and clonidine exhibited minimal effects (8% increase, $p = 0.843$; 25% increase, $p = 0.587$, respectively).

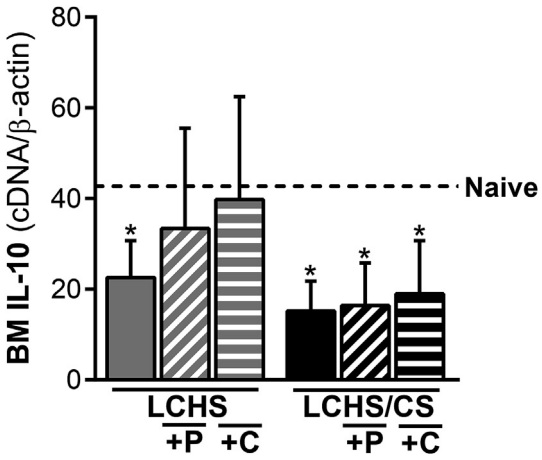

Fig. 3. Propranolol (P) and clonidine (C) slightly increased bone marrow expression of interleukin 10 (IL-10) seven days following lung contusion and hemorrhagic shock (LCHS), but did not significantly affect bone marrow expression of IL-10 after LCHS followed by daily chronic stress (LCHS/CS). $^{*}p < 0.05$ vs. Naive.

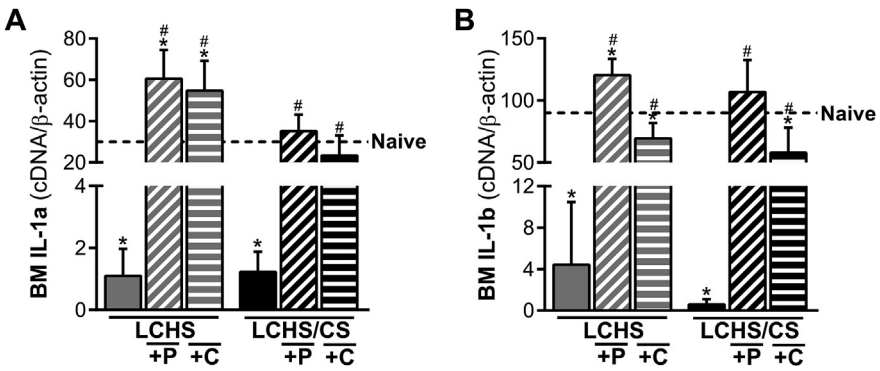

Fig. 2. Propranolol (P) and clonidine (C) significantly increased bone marrow expression of interleukin (IL) 1a (Figure A) and 1b (Figure B) seven days following lung contusion and hemorrhagic shock (LCHS) as well as LCHS followed by daily chronic stress (LCHS/CS). $^{*}p < 0.05$ vs. Naive, $^{\#}p < 0.05$ vs. untreated counterpart.

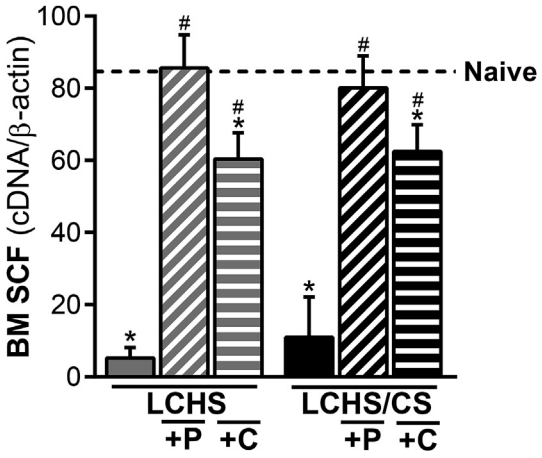

Fig. 4. Propranolol (P) and clonidine (C) significantly increased bone marrow expression of stem cell factor (SCF) seven days following lung contusion and hemorrhagic shock (LCHS) as well as LCHS followed by daily chronic stress (LCHS/CS). $^{*}p < 0.05$ vs. Naive, $^{\#}p < 0.05$ vs. untreated counterpart.

Stem cell factor

Bone marrow expression of SCF seven days after traumatic injury is illustrated in Fig. 4. Compared to naive animals, SCF expression was significantly decreased following LCHS (94% decrease, $p < 0.001$) and LCHS/CS (87% decrease, $p < 0.001$). Propranolol and clonidine significantly increased SCF expression following LCHS (1548% increase, $p < 0.001$; 1062% increase, $p < 0.001$, respectively). Similar effects were observed for propranolol and clonidine administration following LCHS/CS (629% increase, $p < 0.001$; 468% increase, $p < 0.001$, respectively).

B-cell lymphoma-extra large

Bone marrow expression of Bcl-xL seven days after traumatic injury is illustrated in Fig. 5. Compared to naive animals, Bcl-xL expression was not significantly affected by LCHS (2% decrease, $p = 0.930$) and LCHS/CS (4% decrease, $p = 0.375$). Following LCHS, Bcl-xL expression was significantly increased by propranolol (57% increase, $p = 0.016$), but not clonidine (24% increase, $p = 0.114$). Following LCHS/CS, Bcl-xL expression was significantly increased by propranolol (59% increase, $p = 0.006$) and clonidine (77% increase, $p < 0.001$). Because Bcl-xL promotes replication of hematopoietic progenitor cells and shifts myeloid lineage toward erythropoiesis, the observation that Bcl-xL expression was not significantly impacted by severe trauma and chronic stress but was increased by attenuating the neuroendocrine stress response with propranolol or clonidine suggests that Bcl-xL may be a therapeutic target despite a lack of clear evidence that it contributes to the pathophysiology of persistent injury-associated anemia.

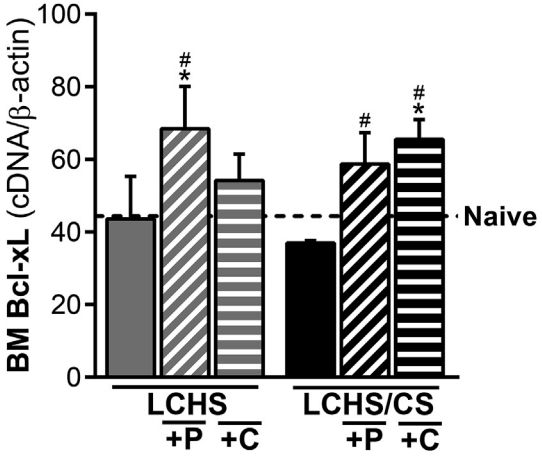

Fig. 5. Propranolol (P) significantly increased bone marrow expression of B-cell lymphoma-extra large (Bcl-xL) seven days following lung contusion and hemorrhagic shock (LCHS). Both propranolol (P) and clonidine (C) significantly increased bone marrow expression Bcl-xL seven days following LCHS followed by daily chronic stress (LCHS/CS). $^{*}p < 0.05$ vs. Naive, $^{\#}p < 0.05$ vs. untreated counterpart.

Discussion

Our objective was to examine the mechanisms by which propranolol and clonidine modulate bone marrow cytokines to abrogate persistent injury-associated anemia. Our findings suggest that attenuating the neuroendocrine stress response with propranolol and clonidine may modulate the bone marrow cytokine response to severe trauma and chronic stress, favoring effective erythropoiesis. Chronic restraint stress activates both central and peripheral arms of the neuroendocrine stress response, allowing for differential assessment of clonidine, which attenuates central sympathetic outflow, and propranolol, which acts peripherally on hematopoietic progenitors. Trauma and stress-induced increases in bone marrow expression of HMGB1 and decreases in IL-1, IL-10, and SCF and were reversed by propranolol and clonidine, and Bcl-xL expression was increased by propranolol and clonidine. Each of these phenomena may promote erythropoiesis, indicating that changes in bone marrow cytokine expression may play an important role in the pathophysiology of persistent injury-associated anemia. Our results suggest that multiple bone marrow cytokines are important in this process, and that central blockade of sympathetic tone may not be necessary because peripheral beta-adrenergic receptor blockade effectively induced bone marrow cytokine changes that favor effective erythropoiesis. These findings also support the hypothesis that propranolol and clonidine may have therapeutic value for severely injured trauma patients.

HMGB1 is a pro-inflammatory cytokine that may potentiate bone marrow suppression and anemia. Following septic insult, exogenous HMGB1 administration has been associated with anemia in a process that is reversed by HMGB1 blockade.[30] However, HMGB1 has also been shown to increase SCF expression and induce stress erythropoiesis.[31] It remains plausible that some degree of HMGB1 induction is helpful to trigger stress erythropoiesis, whereas excessively high levels may suppress erythropoiesis. The effects of HMGB1 may also be dependent upon experimental conditions. Seven days following severe trauma and chronic stress, bone marrow HMGB1 expression is persistently increased, and animals remain persistently anemic under these conditions.[17,32] We observed that propranolol and clonidine administration significantly decreased bone marrow HMB1 expression, resulting in expression levels similar to that of naive animals. Further investigation is needed to assess whether HMGB1 has a causal role in modulating erythropoiesis following an acute physiologic insult, and to identify the ideal range of HMGB1 for promoting stress erythropoiesis.

Persistent elevation of IL-1 has been associated with decreased hematopoietic stem cell maturation and self-renewal in a process that is reversible by IL-1 withdrawal.[33] IL-1 induces production of hematopoietic colony stimulating factors by bone marrow stromal fibroblasts, independent of other pro-inflammatory cytokines.[34] Therefore, decreases in bone marrow IL-1 following severe trauma and stress may contribute to decreased hematopoietic progenitor growth and anemia. The observation that propranolol

and clonidine administration significantly increased bone marrow IL-1 expression supports the hypothesis that targeting the neuroendocrine stress response, independent of the systemic inflammatory response, may be an effective therapeutic approach to persistent injury-associated anemia.

Similar to IL1, IL-10 promotes hematopoietic progenitor cell proliferation.[35] However, the effects of IL-10 on hematopoiesis appear to be indirect.[36] We observed that bone marrow IL-10 expression was significantly decreased following severe trauma, and further reduced by the combination of severe trauma and chronic stress. However, propranolol and clonidine were only moderately effective in increasing IL-10 expression following trauma alone, and did not significantly increase IL-10 expression when trauma was followed by chronic stress. Therefore, although IL-10 suppression may contribute to bone marrow dysfunction following severe trauma, it seems unlikely that bone marrow IL-10 modulation is a mechanism by which propranolol and clonidine abrogate persistent injury-associated anemia.

SCF promotes stress erythropoiesis.[37] During homeostasis and following pathophysiologic insult, SCF promotes proliferation and differentiation of hematopoietic stem and progenitor cells.[38] Our observation that bone marrow SCF expression was significantly decreased following severe trauma is consistent with previous observations that severely injured trauma patients have decreased hematopoietic progenitor growth.[11] Propranolol and clonidine significantly increased bone marrow SCF expression. Propranolol was more effective than clonidine in producing this effect, restoring SCF expression levels to that of naive animals, whereas SCF remained significantly lower than naive expression levels following clonidine administration. However, the magnitude of increase compared to untreated counterparts was substantial for both propranolol and clonidine. Upregulation of SCF following severe trauma may have considerable therapeutic value, without clinically significant untoward effects. Although exogenous SCF is associated with mast cell proliferation and activation, this is unlikely to occur in the absence of supra-physiologic levels of SCF.[39,40]

Bcl-xL promotes replication of hematopoietic progenitor cells and shifts myeloid lineage toward erythropoiesis.[41,42] Therefore, upregulation of Bcl-xL has the potential to abrogate persistent-injury associated anemia. However, bone marrow Bcl-xL expression was unchanged following severe trauma compared to naive animals, and was slightly decreased following the combination of severe trauma and chronic stress. Propranolol significantly increased Bcl-xL expression following injury alone and following injury plus chronic stress, and clonidine increased Bcl-xL expression following the combination of injury and stress. Therefore, attenuating the neuroendocrine stress response may abrogate persistent injury-associated anemia by upregulating Bcl-xL.

This study is limited by the absence of human data and lack of causal relationships. A dose-response study may suggest causality and further elucidate the mechanisms by which attenuating the neuroendocrine stress response abrogates persistent-injury associated anemia. To strengthen associations and assess the relative importance of persistent neuroendocrine activation at central and peripheral sites, injury models were performed with and without chronic stress, and were performed with and without propranolol and clonidine, which were chosen for their disparate mechanisms of action. Propranolol was somewhat more effective in restoring bone marrow expression of IL-1 and SCF compared with clonidine and was as effective as clonidine in modulating other bone marrow cytokines, suggesting that central sympathetic blockade may not be necessary for bone marrow protection following severe injury and chronic stress, consistent with other experimental studies suggesting that peripheral effects of beta-adrenergic receptor blockade on erythroid progenitor cells play an important role in abrogating persistent-injury associated anemia. This observation is fortuitous, as beta blockers are commercially available in formulas with short durations of action, whereas clonidine may propagate hypotension for up to ten hours, limiting its application to critically ill trauma patients with ongoing resuscitation requirements. Finally, this study was limited by the exclusive use of male rats, which were used to avoid the potentially confounding effects of female hormones on the physiologic response to hemorrhagic shock. However, there may be important differences between male and female rats in the bone marrow response to severe injury and chronic stress, and further research in this area is needed. Future research should also clarify the role of HMGB1 in persistent-injury associated anemia and assess the therapeutic value of neuroendocrine modulation to prevent persistent injury-associated anemia among severely injured trauma patients.

Conclusions

Attenuating the neuroendocrine stress response with propranolol and clonidine may modulate the bone marrow cytokine response to severe trauma and chronic stress, favoring effective erythropoiesis. Increases in bone marrow expression of HMGB1 and decreases in expression of IL-1, IL-10, and SCF and were reversed by propranolol and clonidine, and Bcl-xL expression was increased by propranolol and clonidine. Future research should assess the therapeutic value of neuroendocrine modulation to prevent persistent injury-associated anemia among critically ill trauma patients.

Acknowledgements

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References

1. Corwin HL, Gettinger A, Pearl RG, et al. The CRIT Study: anemia and blood transfusion in the critically ill–current clinical practice in the United States. *Crit Care Med*. 2004 Jan;32(1):39–52.
2. Rodriguez RM, Corwin HL, Gettinger A, et al. Nutritional deficiencies and blunted erythropoietin response as causes of the anemia of critical illness. *J Crit Care*. 2001 Mar;16(1):36–41.
3. Vincent JL, Baron JF, Reinhart K, et al. Anemia and blood transfusion in critically ill patients. *J Am Med Assoc*. 2002 Sep 25;288(12):1499–1507.
4. Charles A, Shaikh AA, Walters M, et al. Blood transfusion is an independent predictor of mortality after blunt trauma. *Am Surg*. 2007 Jan;73(1):1–5.
5. Malone DL, Dunne J, Tracy JK, et al. Blood transfusion, independent of shock severity, is associated with worse outcome in trauma. *J Trauma*. 2003 May;54(5):898–905. discussion -7.
6. Shapiro MJ, Gettinger A, Corwin HL, et al. Anemia and blood transfusion in trauma patients admitted to the intensive care unit. *J Trauma*. 2003 Aug;55(2):269–273. discussion 73-4.
7. Napolitano LM. Epoetin alfa in the critically ill: what dose? Which route? *Crit Care Med*. 2009 Apr;37(4):1501–1503.
8. Chant C, Wilson G, Friedrich JO. Anemia, transfusion, and phlebotomy practices in critically ill patients with prolonged ICU length of stay: a cohort study. *Crit Care*. 2006;10(5):R140.
9. Lin DM, Lin ES, Tran MH. Efficacy and safety of erythropoietin and intravenous iron in perioperative blood management: a systematic review. *Transfus Med Rev*. 2013 Oct;27(4):221–234.
10. Corwin HL, Gettinger A, Fabian TC, et al. Efficacy and safety of epoetin alfa in critically ill patients. *N Engl J Med*. 2007 Sep 6;357(10):965–976.
11. Livingston DH, Anjaria D, Wu J, et al. Bone marrow failure following severe injury in humans. *Ann Surg*. 2003 Nov;238(5):748–753.
12. Robinson Y, Hostmann A, Matenov A, et al. Erythropoiesis in multiply injured patients. *J Trauma*. 2006 Nov;61(5):1285–1291.
13. Loftus TJ, Mira JC, Miller ES, et al. The postinjury inflammatory state and the

bone marrow response to anemia. *Am J Respir Crit Care Med*. 2018 Sep 1;198(5):629–638.
14. Mohr AM, ElHassan IO, Hannoush EJ, et al. Does beta blockade postinjury prevent bone marrow suppression? *J Trauma*. 2011 May;70(5):1043–1049. discussion 9-50.
15. Alamo IG, Kannan KB, Ramos H, et al. Clonidine reduces norepinephrine and improves bone marrow function in a rodent model of lung contusion, hemorrhagic shock, and chronic stress. *Surgery*. 2017 Mar;161(3):795–802.
16. Loftus TJ, Rosenthal MD, Croft CA, et al. The effects of beta blockade and clonidine on persistent injury-associated anemia. *J Surg Res*. 2018 Oct;230:175–180.
17. Millar JK, Kannan KB, Loftus TJ, et al. Persistent injury-associated anemia: the role of the bone marrow microenvironment. *J Surg Res*. 2017 Jun 15;214:240–246.
18. Pietras EM, Mirantes-Barbeito C, Fong S, et al. Chronic interleukin-1 exposure drive hematopoietic stem cells towards percocious myeloid differentiation at the expense of self-renewal. *Nature Cell Biology*. 2016;18(6):607–619.
19. Valasselaer V, Falla N, Heuvel RvD, et al. Interleukin-10 stimulates hematopoiesis in murine osteogenic stroma. *Clin. Orthop. Related Res.*. 1995;(313):103–114.
20. Boulais PE, Frenette PS. Making sense of hematopoietic stem cell niches. *Blood*. 2015;125:2621–2629.
21. Metcalf D. Hematopoietic cytokines. *Blood*. 2008;111(2):485–491.
22. Haughn L, Hawley RG, Morrison DK, et al. BCL-2 and BCL-XL REstrict lineage choice during hematopoietic differentiation. *J Biol Chem*. 2003;278:25158–25165.
23. Valdes-Ferrer S, Papoin J, Dancho ME, et al. HMGB1 mediates anemia of inflammation in murine sepsis survivors. *Mol Med*. 2015;21(1):951–958.
24. Bible LE, Pasupuleti LV, Gore AV, et al. Daily propranolol prevents prolonged mobilization of hematopoietic progenitor cells in a rat model of lung contusion, hemorrhagic shock, and chronic stress. *Surgery*. 2015 Sep;158(3):595–601.
25. Gore AV, Bible LE, Livingston DH, et al. Can mesenchymal stem cells reverse chronic stress-induced impairment of lung healing following traumatic injury? *J. Trauma Acute Care Surg.* 2015 Apr;78(4):767–772.
26. Shah S, Ulm J, Sifri ZC, et al. Mobilization of bone marrow cells to the site of injury is necessary for wound healing. *J Trauma*. 2009 Aug;67(2):315–321. discussion 21-2.
27. Beiermeister KA, Keck BM, Sifri ZC, et al. Hematopoietic progenitor cell mobilization is mediated through beta-2 and beta-3 receptors after injury. *J Trauma*. 2010 Aug;69(2):338–343.
28. Sullivan PA, De Quattro V, Foti A, Curzon G. Effects of clonidine on central and peripheral nerve tone in primary hypertension. *Hypertension*. 1986 Jul;8(7):611–617.
29. Fonseca RB, Mohr AM, Wang L, et al. Adrenergic modulation of erythropoiesis following severe injury is mediated through bone marrow stroma. *Surg Infect*. 2004;5(4):385–393. Winter.
30. Valdes-Ferrer SI, Papoin J, Dancho ME, et al. HMGB1 mediates anemia of inflammation in murine sepsis survivors. *Mol Med*. 2016 May;21(1):951–958.
31. Kim TS, Hanak M, Trampont PC, Braciale TJ. Stress-associated erythropoiesis initiation is regulated by type 1 conventional dendritic cells. *J Clin Invest*. 2015 Oct 01;125(10):3965–3980.
32. Bible LE, Pasupuleti LV, Gore AV, et al. Chronic restraint stress after injury and shock is associated with persistent anemia despite prolonged elevation in erythropoietin levels. *J. Trauma Acute Care Surg.* 2015 Jul;79(1):91–96. discussion 6-7.
33. Pietras EM, Mirantes-Barbeito C, Fong S, et al. Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. *Nat Cell Biol*. 2016 Jun;18(6):607–618.
34. Schaafsma MR, Fibbe WE, Van Damme J, et al. Interleukin-6 is not involved in the interleukin-1-induced production of colony-stimulating factors by human bone marrow stromal cells and fibroblasts. *Blood*. 1989 Dec;74(8):2619–2623.
35. Kang YJ, Yang SJ, Park G, et al. A novel function of interleukin-10 promoting self-renewal of hematopoietic stem cells. *Stem Cell*. 2007 Jul;25(7):1814–1822.
36. Van Vlasselaer P, Falla N, Van Den Heuvel R, et al. Interleukin-10 stimulates hematopoiesis in murine osteogenic stroma. *Clin Orthop Relat Res*. 1995 Apr;(313):103–114.
37. Perry JM, Harandi OF, Paulson RF. BMP4, SCF, and hypoxia cooperatively regulate the expansion of murine stress erythroid progenitors. *Blood*. 2007 May 15;109(10):4494–4502.
38. Hassan HT, Zander A. Stem cell factor as a survival and growth factor in human normal and malignant hematopoiesis. *Acta Haematol*. 1996;95(3-4):257–262.
39. Galli SJ, Iemura A, Garlick DS, et al. Reversible expansion of primate mast cell populations in vivo by stem cell factor. *J Clin Invest*. 1993 Jan;91(1):148–152.
40. Tsai M, Shih LS, Newlands GF, et al. The rat c-kit ligand, stem cell factor, induces the development of connective tissue-type and mucosal mast cells in vivo. Analysis by anatomical distribution, histochemistry, and protease phenotype. *J Exp Med*. 1991 Jul 01;174(1):125–131.
41. Haughn L, Hawley RG, Morrison DK, et al. BCL-2 and BCL-XL restrict lineage choice during hematopoietic differentiation. *J Biol Chem*. 2003 Jul 04;278(27):25158–25165.
42. Hirose S, Takayama N, Nakamura S, et al. Immortalization of erythroblasts by c-MYC and BCL-XL enables large-scale erythrocyte production from human pluripotent stem cells. *Stem Cell Reports*. 2013;1(6):499–508.