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Clonidine restores vascular endothelial growth factor expression and improves tissue repair following severe trauma

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ABSTRACT

Background: We hypothesized that clonidine and propranolol would increase VEGF and VEGF-receptor expression and promote lung healing following severe trauma and chronic stress.

Methods: Sprague-Dawley rats were subjected to lung contusion (LC), lung contusion/hemorrhagic shock (LCHS), or lung contusion/hemorrhagic shock/daily restraint stress (LCHS/CS). Clonidine and propranolol were administered daily. On day seven, lung VEGF, VEGFR-1, VEGFR-2, and HMGB1 were assessed by PCR. Lung injury was assessed by light microscopy (*$p < 0.05$).

Results: Clonidine increased VEGF expression following LCHS (43%*) and LCHS/CS (46%*). Clonidine increased VEGFR-1 and R-2 expression following LCHS/CS (203%* and 47%*, respectively). Clonidine decreased HMGB1 and TNF-alpha expression following LCHS/CS (22%* and 58%*, respectively.) Clonidine decreased inflammatory cell infiltration and total Lung Injury Score following LCHS/CS. Propranolol minimally affected VEGF and did not improve lung healing.

Conclusions: Clonidine increased VEGF and VEGF-receptor expression, decreased HMGB1 expression, decreased lung inflammation, and improved lung tissue repair.

1. Introduction

Vascular endothelial growth factor (VEGF) plays an important role in restoring homeostasis following lung injury by propagating angiogenesis, potentiating pulmonary endothelial cell growth and survival, and stimulating type II alveolar cell surfactant production.[1,2] Animal studies have shown that following traumatic lung injury, VEGF and VEGF receptor expression increase over the course of seven days, and there is no histologic evidence of lung injury one week after injury.[3] However, when lung contusion (LC) is followed by hemorrhagic shock (HS), VEGF and VEGF receptor expression are decreased, and lung tissue does not heal.[3] The addition of chronic stress (CS) to LC and HS further suppresses VEGFR-1, and is associated with greater lung injury. These findings in animal studies are similar to data from human Acute Respiratory Distress Syndrome (ARDS) patients, for whom increased VEGF levels have been associated with resolution of lung injury.[4] Therefore, restoration of normal VEGF and VEGF receptor expression may be therapeutic for severely injured blunt trauma patients who are subjected to uncontrolled hemorrhage and the daily stressors of the intensive care unit environment.

The purpose of this study was to investigate the effects of two medications that modulate the neuroendocrine stress response,

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clonidine and propranolol, on VEGF and VEGF receptor expression and lung tissue repair following LC, HS and CS. Propranolol blocks the MAPK, JNK, and NF-κB stress response pathways occurring downstream of the NE-beta adrenergic receptor complex, and improves burn wound healing.[5–8] Clonidine is also known to attenuate the inflammatory response following surgery and experimental sepsis, though it has not been studied extensively in would healing and tissue repair.[9,10] Differences in mechanism of action between these medications may also elucidate the relative importance of central sympathetic tone and peripheral adrenergic receptors in stress-mediated would healing dysfunction. Clonidine reduces central sympathetic tone by stimulating alpha-2 adrenergic receptors in the medulla oblongata and reduces NE release, whereas propranolol has the ability to competitively block the actions of epinephrine and norepinephrine at peripheral beta-adrenergic receptors.[11,12] We hypothesized that daily clonidine or propranolol administration in a rodent model of lung contusion/hemorrhagic shock with chronic restraint stress would increase VEGF and VEGF receptor expression, and that these effects would be associated with lung tissue healing.

2. Methods

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 for a one week acclimation period. Dark and light cycles were 12 h each during acclimation and experimental periods. All animal care was conducted in accordance with the Institutional Animal Care and Use Committee standards. Animals were randomly allocated ten different groups (n = 6–8 per group): 1) naïve control, 2) lung contusion (LC), 3) LC with clonidine, 4) LC with propranolol, 5) lung contusion followed by hemorrhagic shock (LCHS), 6) LCHS with clonidine, 7) LCHS with propranolol, 8) lung contusion followed by hemorrhagic shock and daily restraint stress (LCHS/CS), 9) LCHS/CS with clonidine, 10) LCHS/CS with propranolol. Prior to the initial 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 5700M, Saddle Brook, NJ) to a 12 mm copper plate applied to the right lateral chest wall 1 cm below the axillary crease. This model has previously been shown to produce a clinically significant and reproducible pulmonary contusion.[13–15]

Rats allocated to HS groups were then placed on a heating pad, and the right internal jugular vein and right femoral artery were cannulated under direct visualization. Continuous blood pressure monitoring was performed by securing the arterial catheter to a BP-2 Digital Blood Pressure Monitor (Columbus Instruments, Columbus, OH). Blood was then withdrawn through the venous catheter into a heparinized syringe until a mean arterial pressure of 30–35 mm Hg was obtained. This blood pressure was maintained for a 45-min period by withdrawing or reinfusing blood as necessary. After 45 min of hemorrhagic shock, blood was reinfused at 1 mL/min. Animals did not receive intravenous or subcutaneous fluids at any point.

CS was performed by placing animals in a restraint cylinder (Kent Scientific Corporation, Torrington, CT) for two hours daily. CS began one day after LCHS in the LCHS/CS group. In order to prevent acclimation to the restraint cylinder, 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. All non-CS groups were subjected to a two hour daily fast while CS was administered.

Clonidine and propranolol were administered by intraperitoneal injection 10 min following resuscitation from hemorrhagic shock, and then daily following CS or daily handling. Clonidine and propranolol doses were 75 μg/kg and 10 mg/kg, respectively, based on previous work demonstrating the safety and efficacy of these doses in reducing heart rate by 10–20% without causing significant hypotension.[13,16] Propranolol and clonidine were administered once daily rather than more frequent dosing because the goal was to attenuate the neuroendocrine stress response following injury and daily restraint stress rather than to maintain a steady state of pharmacotherapy. Because norepinephrine has a short half-life, a single dose of propranolol or clonidine following resuscitation from hemorrhagic shock or cessation of restraint stress was given.

Animals were sacrificed by cardiac puncture following IP injection of ketamine (80–100 mg/kg) and xylazine (5–10 mg/kg) on day seven. Right lung and plasma specimens were collected. Lung specimens were initially placed in phosphate buffered saline (PBS). One portion of the contused right lung was placed in formalin for hematoxylin and eosin staining and histologic analysis by light microscopy, and another portion was placed immediately in dry ice and then stored at −80 °C. Plasma samples were obtained during cardiac puncture by withdrawing 7–10 mL of blood into a heparinized syringe. Blood was then centrifuged at 500G for 5 min and plasma was aliquoted, placed immediately in dry ice, and then stored at −80 °C.

Lung VEGF, VEGFR-1, VEGFR-2, high mobility group box 1 (HMGB1), and tumor necrosis factor alpha (TNF-alpha) expression were assessed by endpoint polymerase chain reaction. HMGB1 expression was measured as a molecular indicator of lung inflammation to correspond with histological findings. The following primers were selected: VEGF forward 5′ gtggacttgagttgggagga and reverse 5′ caaacagacttcggcctctc (product region: 2135–2228, product size: 147 bp), VEGFR-1 forward 5′ agtggctccacgaccttaga and reverse 5′ gaagaccgcttcagttttcgt (product region: 2258–2575, product size: 317 bp), VEGFR-2: forward 5′ acagcatcaccagcagtcag and reverse 5′ ccaagaactccatgccctta (product region: 3127–3274, product size: 147 bp), HMGB1 forward 5′ gttctgagtaccgcccaaaa and reverse 5′ ttcatcctcctcgtcgtctt (product region: 374–639, product size: 264 bp), TNF-alpha forward 5′ gaaacacacgagacgctgaa and reverse 5′ ccagatggggatagctggta (product region: 1034–1485, product size: 452 bp). Amplifications were performed using a SimpliAmp thermal cycler (Applied Biosystems, Carlsbad, CA) with an initial 4 min denaturation phase at 95 °C, followed by 32 cycles with denaturation at 95 °C, annealing at 60 °C, and extension at 72 °C for 45 s each. Products were separated on 1.5% agarose gel stained with Ethidium Bromide (Invitrogen, Carlsbad, CA). TNF-alpha and HMGB1 were measured on day seven to quantify the persistent inflammatory state following severe trauma and chronic stress to assess if chronic stress was associated with persistent inflammation and impaired wound healing.

Lung injury was assessed by a board certified pathologist using light microscopy to visualize portions of the contused right lung stained with hematoxylin and eosin. The pathologist was blinded to experimental group allocation. The severity of lung injury was analyzed according to the LIS scoring system described in Table 1, adapted from Claridge et al.[17] and Matute-Bello et al.[18]

Statistical analysis was performed using GraphPad Prism (version 6.05, GraphPad Software, La Jolla, CA) to calculate one-way analysis of variance. Data were reported as mean ± standard deviation with $\alpha = 0.05$.