Optimized induction of beta common receptor enhances the neuroprotective function of erythropoietin in spinal cord ischemic injury

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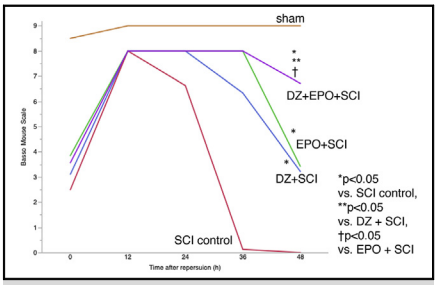
ABSTRACT

Background: Paraplegia remains the most feared complication of complex thoracoabdominal aortic intervention. Although erythropoietin (EPO) has demonstrated neuroprotective effects in spinal cord ischemia, it does not work until expression of the beta common receptor subunit of the EPO receptor (βcR) is induced by ischemia. We hypothesized that the βcR can be induced by diazoxide (DZ), amplifying the neuroprotective effects of EPO in spinal cord ischemia-reperfusion injury.

Methods: For the DZ time trial, adult male C57/BL6 mice received DZ (20 mg/kg) by oral gavage. Spinal cords were harvested after 0, 12, 24, 36, and 48 hours of administration. To evaluate optimal dosing, DZ was administered at 0, 5, 10, 20, and 40 mg/kg. The expression of βcR was assessed by Western blot analysis. Five groups were studied: PBS (pretreatment)+PBS (immediately before), PBS+EPO, DZ+PBS, DZ+EPO, and sham (without cross-clamping). Spinal cord ischemia was induced by 4 minutes of thoracic aortic cross-clamping. Functional scoring (Basso Mouse Score) was done at 12-hour intervals for 48 hours, and spinal cords were harvested for histological analysis.

Results: Western blot analysis demonstrated that optimal βcR up-regulation occurred at 36 hours after DZ administration, and the optimal DZ dosage for βcR induction was 20 mg/kg. Motor function at 48 hours after treatment was significantly better preserved in the DZ+EPO group compared with all other groups, and was significantly better preserved in the DZ only and EPO only groups compared with control (PBS+PBS).

Conclusions: Pharmacologic up-regulation of βcR with DZ can increase the efficacy of EPO in preventing spinal cord ischemia and reperfusion injury. Improved understanding of this synergetic mechanism may serve to further prevent ischemic complications for high-risk aortic intervention. (J Thorac Cardiovasc Surg 2018;■:1-12)



Postoperative motor function in each group.

Central Message

Treatment of mice subjected to spinal cord ischemia-reperfusion with diazoxide (DZ) plus erythropoietin (EPO) provides dramatically better preservation of motor function compared with either DZ or EPO alone.

Perspective

EPO and DZ are clinically available. Although EPO has shown a neuroprotective effect in the spinal cord, EPO does not work until the βcR subunit is induced by metabolic stress. Pretreatment of DZ up-regulated βcR expression, and treatment with the combination of DZ and EPO significantly preserved motor function in a murine model of SCI. This novel strategy may prevent paraplegia in aortic interventions.

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Although the outcomes of thoracoabdominal aortic intervention have improved steadily in recent years, paraplegia after thoracoabdominal aortic repair remains a devastating postoperative complication. The number of patients put at risk by undergoing these operations is also increasing.[1] Although the extent of repair plays a major role, the

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Abbreviations and Acronyms

AKT = serine/threonine-specific kinase
BCL-2 = B cell lymphoma 2
βcR = beta common receptor
BMS = Basso Mouse Scale
CREB = adenosine 3′,5′-cyclic monophosphate response element-binding protein
DZ = diazoxide
EPO = erythropoietin
EPOR = erythropoietin receptor
pAKT = phosphorylated serine/threonine-specific kinase
PBS = phosphate-buffered saline
pCREB = phosphorylated adenosine 3′,5′-cyclic monophosphate response element-binding protein
PI3K = phosphatidylinositol 3-kinase
pSTAT3 = phosphorylated signaling transducer and activator of transcription 3
SCI = spinal cord ischemia-reperfusion injury
STAT3 = signaling transducer and activator of transcription 3

incidence of postoperative spinal cord complications can be between 10% and 15% in the best centers. Despite ongoing efforts to provide protection from this injury, a universally accepted pharmacologic therapy remains to be established.[2]

Diazoxide (DZ; Proglycem) is an oral medication that can be used to manage symptomatic hypoglycemia. An ATP-dependent potassium channel opener, DZ has been shown to mediate the neuroprotective preconditioning effects against an ischemic insult.[3] Erythropoietin (EPO) is widely recognized as an essential hormone for its role in hematopoiesis. Recent studies have shown that EPO has protective effects in a variety of tissues, including the kidneys, heart, brain, and spinal cord.[4-6] Furthermore, we have reported on the protective mechanism of EPO in the mouse model of spinal cord ischemia-reperfusion injury (SCI).[7]

Recent investigations into the tissue protective effects of EPO have led to the identification of a unique receptor, distinct from the EPO receptor (EPOR), which mediates hematopoiesis. This unique tissue protective receptor is termed the beta common receptor (βcR) subunit.[8] EPOR and βcR subunit expression is up-regulated in response to hypoxia and inflammatory cytokines, such as tumor necrosis factor-α and interleukin (IL)-1β.[9,10] However, the molecular mechanisms of βcR subunit regulation are incompletely understood.[9] Although neuroprotective effects of EPO have been implicated in the spinal cord, these effects require βcR subunit up-regulation, which under physiological conditions requires an ischemic insult.[8]

TABLE 1. The Basso Mouse Scale for locomotion

Score	Characteristics
0	No ankle movement
1	Slight ankle movement
2	Extensive ankle movement
3	Plantar placing of the paw with or without weight support, or occasional, frequent -OR - consistent dorsal stepping but no plantar stepping
4	Occasional plantar stepping
5	Frequent or consistent plantar stepping, no coordination -OR- frequent or consistent plantar stepping, some coordination, paws rotated at initial contact and lift off
6	Frequent or consistent plantar stepping, some coordination, paws parallel at initial contact -OR- frequent or consistent plantar stepping, mostly coordinated, paws rotated at initial contact and lift off
7	Frequent or consistent plantar stepping, mostly coordinated, paws parallel at initial contact and rotated at lift off (P/R) -OR- frequent or consistent plantar stepping, mostly coordinated, paws parallel at initial contact and lift off (P/P), and severe trunk instability
8	Frequent or consistent plantar stepping, mostly coordinated, paws parallel at initial contact and lift off (P/P), and mild trunk instability -OR- frequent or consistent plantar stepping, mostly coordinated, paws parallel at initial contact and lift off (P/P), and normal trunk stability and tail down or up and down
9	Frequent or consistent plantar stepping, mostly coordinated, paws parallel at initial contact and lift off (P/P), and normal trunk stability and tail always up

Thus, in the present study, we aimed to pharmacologically induce βcR subunit up-regulation before ischemia to optimize the neuroprotective effect of EPO. We hypothesized that βcR subunit up-regulation by DZ before ischemia amplifies the neuroprotective effects of EPO in mice with SCI.

MATERIALS AND METHODS

Animals

Adult male C57BL/6 mice (age 12-20 weeks; Charles River Laboratories, Wilmington, Mass) were used for all experiments. This study was approved by the Animal Care and Use Committee at the University of Colorado at Anschutz Medical Campus, and all procedures conformed to the US National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Materials

DZ was purchased from Teva Pharmaceutical (North Wales, Pa). Recombinant erythropoietin from mouse was purchased from Sigma-Aldrich (St. Louis, Mo). Anti-βcR and anti-EPOR were purchased from Santa Cruz