Spinal cord protection via alpha-2 agonist-mediated increase in glial cell-line–derived neurotrophic factor

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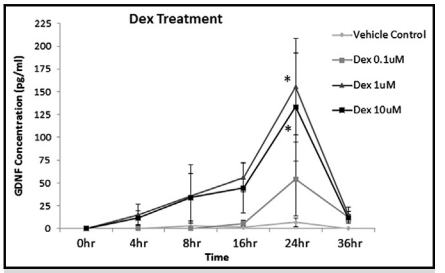
ABSTRACT

Objectives: Delayed paraplegia secondary to ischemia–reperfusion injury is a devastating complication of thoracoabdominal aortic surgery. Alpha-2 agonists have been shown to attenuate ischemia–reperfusion injury, but the mechanism for protection has yet to be elucidated. A growing body of evidence suggests that astrocytes play a critical role in neuroprotection by release of neurotrophins. We hypothesize that alpha-2 agonism with dexmedetomidine increases glial cell-line–derived neurotrophic factor in spinal cord astrocytes to provide spinal cord protection.

Methods: Spinal cords were isolated en bloc from C57BL/6 mice, and primary spinal cord astrocytes and neurons were selected for and grown separately in culture. Astrocytes were treated with dexmedetomidine, and glial cell-line–derived neurotrophic factor was tested for by enzyme-linked immunosorbent assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was used to assess neuronal viability.

Results: Spinal cord primary astrocytes treated with dexmedetomidine at 1 μmol/L and 10 μmol/L had significantly increased glial cell-line–derived neurotrophic factor production compared with control ($P < .05$). Neurons subjected to oxygen glucose deprivation had significant preservation ($P < .05$) of viability with use of dexmedetomidine-treated astrocyte media. Glial cell-line–derived neurotrophic factor neutralizing antibody eliminated the protective effects of the dexmedetomidine-treated astrocyte media ($P < .05$).

Conclusions: Astrocytes have been shown to preserve neuronal viability via release of neurotrophic factors. Dexmedetomidine increases glial cell–derived neurotrophic factor from spinal cord astrocytes via the alpha-2 receptor. Treatment with alpha-2 agonist dexmedetomidine may be a clinical tool for use in spinal cord protection in aortic surgery. (J Thorac Cardiovasc Surg 2015;149:578-86)



Astrocyte production of GDNF.

Central Message

Alpha-2 agonism with dexmedetomidine protected the spinal cord from injury by increasing glial cell–derived neurotrophic factor in astrocytes.

Clinical Relevance

These in vitro studies propose a novel strategy to protect the spinal cord from injury. Dexmedetomidine, a clinically available sedative, is an agonist of neuronal α2-adrenergic receptors. This agent stimulated spinal cord astrocytes to increase glial cell-line–derived neurotrophic factor, a potent neuroprotective factor for motor neurons. This new approach may prevent paraplegia during aortic surgery.

See Editorial Commentary pages 586-7.

ET/BS

Delayed paraplegia secondary to ischemia–reperfusion (IR) injury remains a devastating complication of thoracoabdominal aortic surgery. Advances have been made in adjuvant protective techniques, including hypothermic circulatory arrest and other adjuncts, but paraplegia continues to be a postoperative setback.[1] Ischemia and the inflammatory responses of reperfusion are both known to contribute to neuronal degeneration resulting in spinal cord dysfunction.[2]

Alpha-2 agonists have been shown to attenuate IR injury, but the mechanism for protection has yet to be elucidated. Cerebral,[3,4] cardiac,[5] lung,[6] and renal[7] models have all shown attenuation of IR injury by alpha-2 agonists, and the highly selective alpha-2a agonist dexmedetomidine has been shown to provide functional attenuation of spinal cord IR injury in our in vivo murine model.[8] The method by which dexmedetomidine protected the mice in our model has not been elucidated.

Astrocytes are the support cells of the central nervous system. Research has demonstrated that astrocytes play a

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Abbreviations and Acronyms

ELISA = enzyme-linked immunosorbent assay
GDNF = glial cell line–derived neurotrophic factor
IR = ischemia–reperfusion
MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OGD = oxygen glucose deprivation
SEM = standard error of the mean

critical role in neuroprotection.[9-12] In particular, they are critical in IR injury of neurons for a variety of reasons. Astrocytes are the most abundant cell type in the nervous system[13] with significant interconnections through gap junction channels.[14] They play a vital role in coupling neuronal activity blood flow that underlies the hemodynamic responses.[15] Astrocytes are also central to neuroprotection by release of neurotrophins.[16-18]

Specifically, astrocytes are the major source of a glial cell line–derived neurotrophic factor (GDNF).[19] GDNF has been shown to be a potent neurotrophic factor for motor neurons and to modulate neuronal death,[20] as well as to support neuronal survival and regeneration.[21] GDNF has yet to be examined in spinal cord IR injury; however, GDNF has shown functional recovery in a model of traumatic spinal cord injury.[22] An ongoing question is how to enhance the astrocyte production of GDNF. Significantly, the alpha-2 agonist dexmedetomidine has been linked to increased production of GDNF.[23] We hypothesize that alpha-2 agonism with dexmedetomidine increases GDNF production in spinal cord astrocytes to provide spinal cord protection after IR injury.

MATERIALS AND METHODS

Materials

Dexmedetomidine and atipamezole were purchased from Tocris Bioscience (Ellisville, Mo). Anti-GDNF neutralizing antibody was purchased from Abcam (Cambridge, Mass).

Animals

The Animal Care and Use Committee at the University of Colorado at Denver Health Sciences Center approved all experiments. This investigation conformed to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health publication No. 85-23, National Academy Press, Washington, DC, revised 1996). C57BL/6 mice aged postnatal day 2 to 3 from Jackson Laboratories (Bar Harbor, Maine) were used for all experiments. Each litter in total was considered n = 1.

Cell Culture

Primary spinal cord neuron cultures and primary astrocyte cultures were obtained from mice aged 2 to 3 days. Briefly, the mice were euthanized with isoflurane and then decapitated. The vertebral column was dissected out, and the spinal cord was removed en bloc via injection of cold phosphate-buffered saline (pH 7.4) through the spinal canal. The spinal cord tissue was minced and then digested in a solution of Hibernate-A (Invitrogen, Carlsbad, Calif) with Papain (Worthington, Lakewood, NJ). Neurons and astrocytes were isolated using an OptiPrep (Sigma-Aldrich, St Louis, Mo) density gradient adapted from Brewer and Torricelli.[24]

Neurons were plated on plates coated with Poly-D-Lysine (Sigma-Aldrich) at approximately 300,000 cells/well on a 24-well plate in 1 mL culture media of Neurobasal-A (Invitrogen), B27 (Invitrogen), GlutaMAX (Invitrogen), and penicillin/streptomycin (Gibco, New York, NY). On in vitro day 3, AraC (Sigma-Aldrich) was added to prevent astrocyte replication. Cell cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37°C and underwent half media change every 3 days. The cultures were greater than 90% neurons as seen by morphology on light microscope and confirmed with microtubule-associated protein 2–positive neuronal staining. Cells were used at in vitro days 7 to 10 for experimentation, which is considered mature for neuronal cultures.

Astrocytes were placed in culture flasks or plated on 12-well plates in culture media of Neurobasal-A (Invitrogen), B27 (Invitrogen), GlutaMAX (Invitrogen), fetal bovine serum (Gibco), and penicillin/streptomycin (Gibco). Astrocytes were maintained in culture and allowed to grow to confluence before experimentation. Astrocyte medium used for experimentation was collected and stored in a −80°C refrigerator until use.

Oxygen Glucose Deprivation

On the day of the experiment, the experimental medium of Dulbecco's Modified Eagle's Medium without glucose (Gibco) was placed in the Ruskinn Bug Box Plus (Ruskinn Technology Ltd, Bridgend, South Wales, UK) humidified airtight hypoxic chamber for 2 hours. The Ruskinn Bug Box Plus was used per the manufacturer's protocol to maintain an environment of 95%N_2/5%CO_2 at 37°C. A hypoxic environment was verified with Anaerobic Indicator Strips (Oxoid Ltd, Basingstoke, Hants, UK) before placement of media in the chamber, and hypoxia of the media after 2 hours was tested with an indicator strip. The neuronal maintenance culture medium was then removed, the cells were washed with phosphate-buffered saline, and 1 mL of glucose-deprived medium previously treated in the hypoxic chamber was added to each of the cell culture wells on a 24-well plate. Oxygen glucose deprivation (OGD) was induced by placing the plates in the hypoxic chamber for the experimental time periods. A hypoxic environment in the chamber was tested with an indicator strip before placement of cells into the chamber and on the completion of experimentation time to confirm a hypoxic environment. After OGD was completed, neurons were returned to a normal incubator for reperfusion and OGD media were replaced with normal maintenance neuronal media versus experimental astrocyte media.

Viability Studies

For viability studies, neuronal cells were cultured on 24-well plates. Cell viability was determined with the MTT Cell Proliferation Kit (Roche Diagnostics, Indianapolis, Ind) according to the procedure provided by the manufacturer. Briefly, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well at a concentration of 0.5 mg/mL and incubated for 4 hours at 37°C, and then dimethyl sulfoxide solution was added to the wells. The absorbance at 630 nm was measured on a BioTek Synergy H1 Hybrid microplate reader (BioTek Instruments Inc, Winooski, Vt). Cell viability is presented as the percentage of absorbance relative to nonischemic control.

Enzyme-Linked Immunosorbent Assay

After the experiments were conducted, cell culture supernatants were collected. A standard enzyme-linked immunosorbent assay (ELISA) was performed using these supernatants. A GDNF (Abcam) ELISA was performed according to the procedure provided by the manufacturer, and the absorbance at 450 nm was measured on a BioTek microplate reader. Data are presented as concentration in mean picograms/milliliter ± standard error of the mean.