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Expression of Antiapoptotic Survivin and Aven Genes in Rat Heart Tissue After Traumatic Brain Injury

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ABSTRACT

We have recently shown that experimental traumatic brain injury (TBI) results in ultrastructural damage in heart tissue. The aim of this study was to determine the two antiapoptotic signals "survivin" and "aven" in rat heart tissue following TBI, and comparing the effects of erythropoietin (EPO) and methylprednisolone (MPS). Thirty-six Wistar-Albino female rats weighing 190 to 230 g were randomly allocated into six groups: group 1 underwent head trauma with no treatment; group 2 and group 3, head trauma and intraperitoneally delivered EPO (1000 IU/kg) and MPS (30 mg/kg), respectively; group 4 (vehicle), head trauma and intraperitoneal albumin (0.4 mL/rat); groups 5 and 6, control and sham-operated groups, respectively. Three-hundred g-cm impact trauma was produced by the method of weight-drop. Real-time quantitative polymerase chain reactions were used to estimate survivin and aven gene expression at the total RNA level. Both survivin and aven were higher among the treatment than the trauma group ($P = .0006$, .0001 and $P = .0038$, .0033, respectively). Comparing survivin and aven between EPO and MPS treatment groups showed no significance ($P = .3027$, .2171, respectively). Also, both survivin and aven were significantly higher among the treatment than the vehicle, the control, or the sham-operated groups. These findings suggested that both EPO and MPS may play important roles in the expression of antiapoptotic survivin and aven genes in heart tissue after TBI.

DONOR hearts for cardiac transplantation are from brain-dead patients who have suffered extensive central nervous system damage caused by subarachnoidal hemorrhage or traumatic brain injury (TBI).[1] Studies in potential clinical donors and in experimental animals have suggested that brain injury can have major histopathological and functional effects on the myocardium.[2] In our previous study, we demonstrated a time-dependent increase in malondialdehyde content and gradual damage to the ultrastructure of rat cardiac myocytes after TBI.[3]

A member of the inhibitor of apoptosis (IAP) gene family,[4] "survivin," also known as *Birc5*, has been demonstrated to have a unique, dual role in the regulation of cell proliferation and cell death. Survivin, which prevents apoptosis by blocking caspase activity, is expressed in the G_2/M phase of the cell cycle.[5,6] Recent studies have suggested a potential beneficial role of survivin to prevent endothelial apoptosis in myocardial ischemia, pressure overload cardiac remodeling, and heart failure.[7,8]

A new intracellular membrane protein, "aven" (from Aventine, a Roman stronghold), has now been shown to bind both bcl-xL and Apaf-1. Aven is broadly expressed and conserved in mammalian species. It suppresses apoptosis-induced Apaf-1 and caspase-9. Thus, aven represents a new class of cell-death regulators.[9]

The pathophysiology of cardiac myocyte injury after severe TBI needs to be understood, particularly apoptotic processes. The ability of potential therapeutic agents to induce the expression of survivin and even proteins may provide a new avenue for therapy to prevent cardiac myocyte damage after TBI. Therefore, the aim of our study was to evaulate the two antiapoptotic signals survivin and aven in rat heart tissue following TBI.

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MATERIALS AND METHODS

The local Institutional Animal Care Committee approved protocols used in this study. The rats were randomly allocated into six groups, and all groups contained six rats: Trauma group underwent TBI of 300 g-cm; Erythropoietin group: TBI of 300 g-cm and r-Hu-EPO (erythropoietin) (1000 IU/kg; Eprex, Cilag AG, Zug, Switzerland) administered intraperitoneally immediately than after; Methylprednisolone (MPS) group: TBI of 300 g-cm and MPS (30 mg/kg) administered intraperitoneally immediately thereafter; Vehicle group: TBI of 300 g-cm and 0.4 mL albumin (contains 2.50 mg human serum albumin) administered intraperitoneally immediately thereafter; Control group: Tissue samples were obtained immediately after midline sternotomy with no head surgery; and Sham-operated group: Scalp closed after craniotomy and no trauma induced. Tissue samples were obtained 24 hours after induced trauma in all groups, except the control group.

Surgical Procedure

Wistar-Albino rats weighing 190 to 230 g were anesthetized with 10 mg/kg xylazine and 60 mg/kg ketamine hydrochloride. They were then pinned in the prone position, and their scalps were shaved and cleaned with 10% polyvinyl-pyrrolidone/iodine. After a midline scalp incision, a craniectomy (5 mm diameter) was performed with a dental drill system on the right parietal bone of all rats under microscopic visualization. The dura mater was kept intact during this procedure. Trauma was induced according to a study recently performed by our research team.[3] The force was applied via a stainless steel rod (3 mm diameter, weighing 30 g) that had a rounded surface. The injury apparatus was a 10-cm guide tube that had a 4-mm inner diameter with holes that allowed air to escape without making additional pressure effects. It was positioned perpendicular to the burr hole. The weight dropped vertically through the tube from a height of 10 cm onto the exposed dura. Then the scalp was sutured with 3/0 silk. Body temperature continuously monitored during the procedure with a rectal thermometer was maintained at 37°C using a heating pad and an overhead lamp. Rats were neither intubated nor ventilated between brain injury and heart sampling. None of the animals died during the study period. But severely injured animals that had no treatment were sick and hypoactive at the end of the 24 hours.

Sample Obtained From the Heart

Twenty-four hours after sham, trauma, or treatment, rats were re-anesthetized with the combination of ketamine and xylazine. They were killed by decapitation under general anesthesia. Samples for survivin and aven gene expression were obtained from the left ventricle of the heart. Heart samples were collected in randomly numbered containers, which were analyzed by blinded observers. After evaluating the numbered tissues, results were collected in the appropriate group lists.

Isolation of RNA and Synthesis of cDNA

Samples were immediately frozen in liquid nitrogen at −80°C. Total RNA of each heart tissue was isolated using a HighPure RNA tissue kit (Roche Diagnostics, Mannheim, Germany). Samples were assessed for RNA integrity, electrophoretically verified with ethidium bromide staining, and documented to show an OD_{260}/OD_{280} nm absorption ratio >1.95. One microgram of total RNA was used for cDNA synthesis using a first-strand cDNA synthesis kit for RT-PCR (AMV); (Roche Diagnostics) according to the manufacturer's protocol.

Quantitative Real-Time PCR Analysis

Real-time quantitative PCR (polymerase chain reaction) was performed to assess transcripts of aven and survivin relative to the housekeeping gene β-actin. The cDNA was used for quantitative real-time PCR amplification with SYBR Green I chemistry (Roche Applied Sciences, Mannheim, Germany). Primers were designed using Primer Premier 5 software (Premier Biosoft International, Palo Alto, Calif, USA). Aven primers were F 5′GACTTCAGTGTCCTCTTGAG3′ and R 5′ CCTTGCCATCATCGTTTCTC3′ (GenBank Acc. no. XM230438), survivin primers were F 5′GCCACTTGTCCCAGCTTTCC3′ and R 5′ GTCACAATAGAGCAAAGCCACA 3′ (GenBank Acc. no. AF276775), and β-actin primers were 5′ TCTTTAATGTCACGCACGATT 3′ and 5′ TCACCCACACTGTGCCCAT 3′ (GenBank Acc. no. XM230438). The real-time PCR reactions were performed in a total volume of 10 μL with 0.5 μmol/L of each primer and $MgCl_2$ 4 mmol/L using FastStart DNA Master SYBR Green I kit (Roche Applied Sciences). The β-actin mRNA was quantified to adjust the amount of mRNA in each sample with β-actin primer set.

The cycling parameters were 10 minutes at 95°C for activating hot-start Taq polymerase, 45 cycles of 10 seconds at 95°C, 5 seconds at 60°C for amplification and quantification, 10 seconds at 72°C, and 0 seconds at 80°C for extension. Fluorescence readings were performed at 84°C every cycle to prevent fluorescence from primer dimers. The specificity of all individual amplification reactions was confirmed by melting-curve analysis. The assays used β-actin as the endogenous internal housekeeping gene, which revealed less variability and better reproducibility in our experiments. Real-time expression values were calculated using the relative standard curve method. Standard curves were generated for each mRNA using 10-fold serial dilutions for both the target of interest and the endogenous control (β-actin) by measuring the cycle number at which exponential amplification occurred in a dilution series of samples. Values were normalized to the relative amounts of β-actin mRNA, which were obtained from a similar standard curve. In real-time PCR reactions, the same initial amounts of target molecules were used, and the cross point values of β-actin mRNA were constant in all samples.

Statistical Analysis

Data are reported as mean values ± standard deviations (SD). The two-tailed Student t test was used for two-group comparisons. Differences with P values <.05 were regarded as significant.

RESULTS

Survivin expression ratios were measured in cardiac myocyte samples in all groups (Fig 1). Intraperitoneal administration of EPO or MPS produced a significant increase in survivin expression ($P = .0006$ and $P = .0038$, respectively) compared with the trauma group, suggesting that EPO and MPS had antiapoptotic effects on cardiac myocytes after TBI. In contrast, when there was no important association we compared survivin expression ratios between EPO and MPS treatment groups ($P = .3027$). Treatment with vehicle did not produce a significant increase in survivin expression in cardiac myocytes after TBI ($P = .2827$).

Intraperitoneal administration of EPO or MPS produced significant increases in aven expression ($P = .0001$ and $P = .0033$, respectively) compared with other trauma groups,

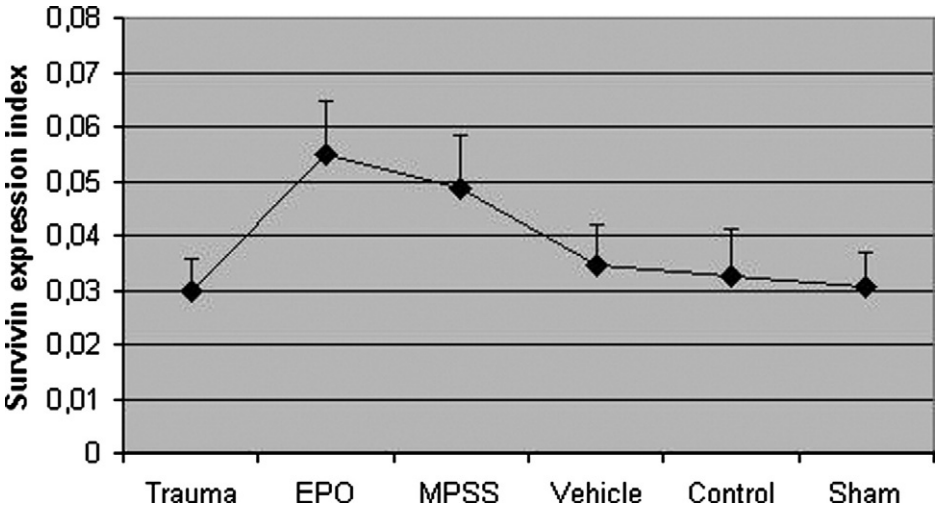

Fig 1. Shown are survivin expression ratios measured in the cardiac myocyte samples in all groups. Differences with *P* values <.05 were regarded as statistically significant.

suggesting that EPO and MPS showed antiapoptotic effects in cardiac myocytes after TBI (Fig 2). When we compared aven expression ratios between EPO and MPS treatment groups, there was no important association ($P = .2171$). Differences between trauma and vehicle-treated groups were not significant ($P = .4022$). Also, these results showed no significant differences in survivin and aven levels between the control and the sham-operated groups.

DISCUSSION

In addition to the bcl-2 family, which plays a significant role in aspects of cell death and survival, other proteins have also been observed to play a prominent role in the inhibition.[5–7,10] The most prominent member of this family is survivin, which has been characterized as a bifunctional protein that suppresses apoptosis and cell division. Despite the redundancy of cell-death pathways, current evidence suggests that overexpression of survivin becomes a requirement to preserve cell viability.[10] Recently, Blanc-Brude et al[11] showed that balloon-mediated arterial injury in rabbits results in expression of survivin in vascular cells. Expression of survivin in cultured smooth muscle cells was stimulated by serum or platelet-derived growth factor. It suppressed apoptosis and prevented caspase activation. These data identified survivin as a critical regulator of smooth muscle cell apoptosis after acute vascular injury.[11]

Aven is a conserved protein that has broad tissue distribution with prominent expression in heart, skeletal muscle, kidney, liver, pancreas, testis, and several established cell lines. Aven interferes with the ability of Apaf-1 to self-associate, thereby inhibiting Apaf-1–mediated activation of caspases.[9] Aven is less widely studied in cardiac myocytes, but its antiapoptotic features are known.

Limiting apoptosis throughout injury is related to enhanced survival and organ function.[12] It appears that induction of bcl-2 gene expression protects heart tissue after TBI. Recently, we have shown that EPO and MPS play important roles in the expression of bcl-2 and in decreasing

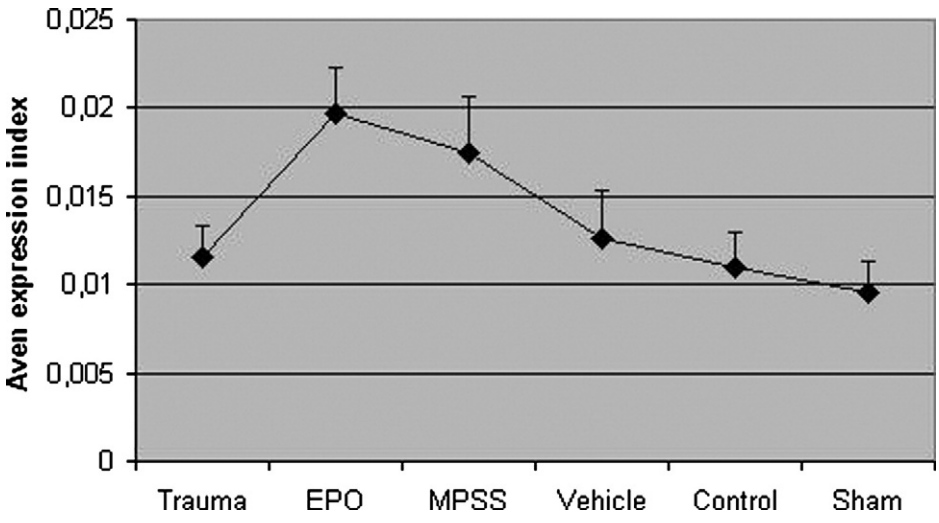

Fig 2. Shown are aven expression ratios measured in the cardiac myocyte samples in all groups. Differences with *P* values <.05 were regarded as statistically significant.

thiobarbituric acid-reactive substances, the end product of lipid peroxidation, in rat cardiac myocytes after TBI.[13,14] Increased survivin expression in response to cytokines raises the intriguing possibility that bcl-2 and survivin represent complementary survival pathways that are differentially regulated by cell-cycle status. In our study groups, survivin and aven levels were parallel, with a significant association. Although they act at different levels, it has been shown that two survival pathways, bcl-2 and survivin, may function in concert to prevent cell death,[15] a finding supported in our study.

Our data showed for the first time that survivin and aven are expressed in rat cardiac myocytes after TBI, suggesting that survivin and aven expression may represent mechanisms by which rat cardiac myocytes at risk of apoptosis retain their viability.

The data also documented that intraperitoneal administration of EPO and MPS produced significant increases in survivin and even expressions, suggesting that EPO and MPS protect cardiac myocytes after TBI. This observed phenomenon may be important for clinical purposes. Based on these results, we recommend comparative studies to evaluate the role of antiapoptotic signals survivin and aven in cardiac myocyte cell death, which may prove of value to sustain donor hearts after TBI.

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