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Original Contribution

Oxidative stress in head trauma in aging

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Abstract

Oxidative damage is proposed as a key mediator of exacerbated morphological responses and deficits in behavioral recovery in aged subjects with traumatic brain injury (TBI). In the present study, we show exacerbated loss of tissue in middle aged (12 months) and aged (22 months) Fisher-344 rats compared to young animals (3 months) subjected to moderate TBI. Analysis of 4-hydroxynonenal (4-HNE) and acrolein, neurotoxic by-products of lipid peroxidation, shows significant ($P < 0.05$) age-dependent increases in ipsilateral (IP) hippocampus 1 and 7 days post injury. In IP cortex, 4-HNE was significantly elevated 1 day post injury in all age groups, and both 4-HNE and acrolein were elevated in middle aged and aged animals 7 days post injury. Comparison of antioxidant enzyme activities shows significant ($P < 0.05$) age-dependent decreases of manganese superoxide dismutase in IP hippocampus and cortex 1 and 7 days post injury. Glutathione reductase activity also showed an age-dependent decrease. Overall, our data show increased levels of oxidative damage, diminished antioxidant capacities, and increased tissue loss in TBI in aging.

Keywords: Acrolein; Glutathione peroxidase; Glutathione reductase; Glutathione transferase; 4-Hydroxynonenal; Traumatic brain injury; Superoxide dismutase

Introduction

Traumatic brain injury (TBI) results in delayed dysfunction and death of neuron populations near and distant to the site of injury through secondary processes [1]. Current data show that neurons undergo a progressive degeneration involving increased excitatory amino acids, loss of ion equilibrium, decreased ATP production, aberrant proteolytic enzyme activity, reduction of reactive oxygen species (ROS) scavengers, and increased free radical formation [2–7]. Increased generation of ROS following TBI could lead to lipid peroxidation and formation of neurotoxic aldehydic products. Previous studies show increased lipid peroxidation, including significant elevations of thiobarbituric acid-reactive substances (TBARS) [8] and 8-isoprostaglandin $F_2\alpha$ [9] following TBI in young animals.

Studies of age-related changes following TBI indicate an exacerbated morphological response and a deficit in behavioral recovery, which is proposed to be mediated by secondary damage [10–12]. Although oxidative stress has been suggested to contribute to neuron loss following TBI, the study of oxidative stress in aging is still limited. Recently, 4-hydroxynonenal (4-HNE) and acrolein [13,14], two neurotoxic by-products of lipid peroxidation of arachidonic acid, have become of interest in a variety of neurological diseases involving oxidative stress [15–20]. To assess the possible involvement of oxidative damage in TBI in aging, we utilized a unilateral controlled cortical impact model of TBI in young (3 months),

Abbreviations: CON, contralateral; Cu/ZnSOD, copper/zinc superoxide dismutase; GPx, glutathione peroxidase; GRed, glutathione reductase; GST, glutathione transferase; 4-HNE, 4-hydroxynonenal; IP, ipsilateral; MnSOD, manganese superoxide dismutase; NBD-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; TBI, traumatic brain injury.

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middle aged (12 months), and aged (22 months) Fisher-344 rats and measured tissue sparing, and 4-HNE and acrolein levels 1 and 7 days post injury. Additional studies were performed to evaluate antioxidant enzyme activities in aging following TBI. These studies may provide insight regarding secondary injury in TBI in aging and may provide potential targets for clinical therapeutic intervention.

Materials and methods

Subjects and surgical procedures

The present studies were conducted in adult male Fisher-344 rats. All injury procedures were approved by the Institutional Animal Care and Use Committee of the University of Kentucky. Every effort was made to minimize both the possible suffering and the number of animals used. All animals were housed 2/cage on a 12 h light/dark cycle with free access to water and food. Twenty-one young (3 months), 24 middle aged (12 months), and 21 aged (22 months) rats (205–500 g/animal) were subjected to a unilateral cortical impact on the surface of the brain utilizing an electronic controlled pneumatic impact device (ECPI) as previously described [21]. Briefly, each animal was anesthetized with 2% isofluorane and placed in a Kopf stereotaxic frame with the incisor bar set at −5. Following a midline incision and retraction of the skin, a 6-mm-diameter craniotomy was made approximately midway between bregma and lamda with a Michele hand trephine (Miltex, NY). The skull disk was removed without disturbing the dura. The exposed brain was injured using the ECPI. The impact rod had a 5-mm-diameter beveled tip that was used to compress the cortex to a depth of 1.5 mm at 3.5 m/s. Following injury Surgicel (Johnson & Johnson, TX) was placed over the injury site, and the skull disk was replaced and sealed with a thin layer of dental acrylic. The incision was closed with surgical staples and the animals maintained at 35° to 37°C with a heating pad. Ten young, 10 middle aged, and 8 aged animals were allowed to survive for 1 day. Eleven young, 14 middle aged, and 13 aged animals were euthanized 7 days post injury.

Method for estimating tissue sparing

For studies of tissue sparing, 6 young, 6 middle aged, and 5 aged animals 7 days post injury were overdosed with sodium pentobarbital and transcardially perfused with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in PBS, and decapitated. Brains were removed, postfixed in 4% paraformaldehyde containing 10% sucrose at 4°C overnight, and subsequently cryoprotected in a 20% sucrose/4% paraformaldehyde solution for an additional 24 h. Coronal 50-μm sections were cut with a cryostat throughout the rostral-caudal extent of the injured cortex extending from the septal area to the posterior aspect of the hippocampus. Sections were stained with cresyl violet and subjected to image analysis (NIH Image v. 1.62). Quantitative assessment of spared tissue employed the Cavalieri method as described previously [22,23]. The amount of tissue sparing was expressed as percentage of the total cortical volume of the injured hemisphere compared to the uninjured hemisphere. In this way each animal is used as its own control. These methods obviate a need to adjust values due to possible differential shrinkage resulting from fixation and tissue processing. A total of 12 evenly spaced sections were used for each animal. All sections were assessed blind with respect to age group.

Tissue processing for lipid peroxidation and enzyme assay

For enzymatic and oxidative stress assays, animals were anesthetized using 5% isofluorane and subjected to cervical dislocation. Ipsilateral (IP) and contralateral (CON) hippocampi and cortices were dissected, immediately frozen in liquid nitrogen, and stored at −80°C until used for analysis. For analysis tissue samples were homogenized using a chilled Dounce homogenizer on ice in 1 ml Hepes buffer (pH 7.4) containing 137 mM NaCl, 4.6 mM KCl, 1.1 mM KH_2PO_4, 0.6 mM $MgSO_4$, pepstatin A, leupeptin, aprotinin, and PMSF. Samples were centrifuged at 100,000*g* for 1 h at 4°C and the supernatant was used for analyses. Total protein concentrations were determined using the Pierce BCA method (Sigma, St. Louis, MO).

4-Hydroxynonenal and acrolein assay

Dot-blot analyses of 4-HNE and acrolein-modified proteins were carried out using a Schleicher & Schuell dot-blot apparatus as described by Saiki et al. [24] with modification. Briefly, 20 μg of 100,000*g* supernatant protein was loaded in triplicate onto nitrocellulose in a 40–50 μl volume. Vacuum was applied until the solution was evacuated from the wells. After air drying, blots were incubated overnight in 5% dry milk in 0.05% Tween 20/Tris buffered saline (TTBS) to block nonspecific binding of the primary antibody. Blots were probed with a 1:2000 dilution of anti-4-HNE (Alpha Diagnostic, San Antonio, TX) or anti-acrolein (United States Biological, Swampscott, MA) polyclonal antibodies for 1 h at room temperature, washed in TTBS 3 × 10 min, and then incubated with a 1:3000 dilution of horseradish peroxidase-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch, West Grove, PA) for 1 h. Detection of bound antibodies was achieved by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) and exposure of the membrane to Hyperfilm (Amersham). Staining intensity was quantified using Scion Image (NIH). The average intensity of triplicate dots was calculated for 4-HNE or acrolein content in an individual sample. To validate linearity of response of the dot blots, aliquots of 100,000*g* protein from a representative rat were incubated for 4 h at 37°C with increasing concentrations of 4-HNE (10, 25, 50, 100 μM) or acrolein (1.0, 5.0, 10, 25 μM). The acrolein and 4-HNE-modified proteins were subjected to dot-blot analyses as described above.