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Regular Article

Protein-energy malnutrition impairs functional outcome in global ischemia

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Abstract

We investigated whether protein-energy malnutrition (PEM) exacerbates brain injury in global ischemia. It was hypothesized that PEM would increase secondary brain damage by worsening ischemia-induced depletion of glutathione (GSH) and increasing oxidative stress. Adult male gerbils were fed an adequate protein (12.5%; C) or low protein (2%; PEM) diet for 4 weeks and subjected to 5 min of bilateral carotid artery occlusion (Ischemia) or sham surgery (Sham). At 12 h post-ischemia, GSH and markers of oxidative stress were measured in hippocampus and neocortex. The remaining gerbils were tested in the open field on days 3, 7, and 10, with viable hippocampal CA1 neurons assessed on day 10. Although the habituation of C-Ischemia gerbils in the open field was normal by day 7, PEM-Ischemia gerbils failed to habituate even by day 10 and spent greater time in the outer zone ($P < 0.05$). Mean (±SEM) total number of viable CA1 neurons at 10 days post-ischemia were C-Sham = 713 (13), C-Ischemia = 264 (48), PEM-Sham = 716 (12), and PEM-Ischemia = 286 (66). Although PEM did not increase CA1 neuron loss caused by ischemia, a subset (4/12) of PEM-Ischemia gerbils showed dramatic reactive gliosis accompanied by extensive neuronal loss. Hippocampal protein thiols were decreased by PEM and ischemia. Although the mechanism is yet to be established, the finding that PEM worsens functional outcome following global ischemia is clinically relevant since 16% of elderly are nutritionally compromised at the time of admission for stroke.
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Introduction

Previous attempts to reduce stroke-induced brain injury with neuroprotective drugs targeting single early cellular events have been notably unsuccessful in clinical trials. Novel approaches and combination therapy are inevitable, given the complexity of the cell death cascade (Fisher and Brott, 2003). Modifiable factors adversely affecting outcome, such as compromised nutritional states, also need to be delineated.

The elderly are at high risk for compromised nutritional status (Gariballa et al., 1998b). Although most attention has been paid to malnutrition arising from stroke-related deficits such as dysphagia, protein-energy malnutrition (PEM) already exists in at least 16% of elderly at the time of admission for stroke (Axelsson et al., 1988; Dávalos et al., 1996; Gariballa et al., 1998a,b). Correlations have been drawn between PEM at stroke admission and increased risk of morbidity and mortality (Food Trial Collaboration, 2003; Dávalos et al., 1996; Gariballa et al., 1998a,b). No studies have been definitive, however, primarily because of small sample size. Even the large randomized multicenter FOOD Trial has a major weakness because, as noted by the authors, assessment of nutritional status was not standardized (Food Trial Collaboration, 2003).

The mechanisms by which PEM could alter the extent of brain injury in stroke have also not been explored, although there are specific pathways by which PEM could influence the complex cascade of secondary events responsible for brain damage. The latter includes ATP depletion, glutamate excitotoxicity, membrane depolarization, increased intracellular calcium, and production of reactive oxygen and nitrogen species that

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results in oxidative stress and inflammation (del Zoppo et al., 2000; Juurlink and Sweeney, 1997). Reduced glutathione (GSH) is critically important in controlling oxidative stress in neural cells (reviewed in Juurlink, 1999) and is rapidly depleted in brain ischemia–reperfusion (Candelario-Jalil et al., 2001), which enhances ischemic brain injury (Mizui et al., 1992). As cysteine is limiting for GSH synthesis, dietary sulfur amino acids can regulate GSH concentration (reviewed in Taylor et al., 1996). GSH in lung and liver is decreased by low-protein diets and PEM (Bauman et al., 1988; Taylor et al., 1992). The brain is not completely protected since acute, severe sulfur amino acid deficiency also modestly depresses brain GSH concentration and exacerbates injury in rat global hemispheric hypoxia–ischemia (Bobyn et al., 2002). We therefore hypothesized that the more clinically relevant condition of PEM would also increase neural damage following global ischemia by limiting supply of cysteine for GSH synthesis during the acute period when demand for GSH synthesis is high.

The objectives of this study were to investigate whether PEM exacerbates brain injury in a gerbil model of global ischemia by assessing both histological and functional outcomes. Neuronal survival was assessed by counting viable hippocampal CA1 neurons at 10 days post-ischemia. The ability to habituate to an open field was utilized as a measure of hippocampal function (Nurse and Corbett, 1994). Whether PEM altered outcome by depressing GSH and increasing oxidative stress in the acute period was examined by measuring GSH concentration, protein thiols, and glutathione reductase activity after 12 h of post-ischemic reperfusion.

Materials and methods

Dietary intervention

Adult male Mongolian gerbils (Charles River Canada, Saint-Constant, QC) were randomized to adequate protein (control diet, C) or low protein diet containing 12.5% and 2% protein, respectively (Dyets, Inc., Bethlehem, PA), and fed for 28 days. Diets were based on the AIN-93M diet (Reeves et al., 1993) with τ-butylhydroquinone omitted. Since gerbils fed 2% protein voluntarily reduce intake, the result is a mixed protein-energy malnutrition (PEM). Animals were housed at 22°C with a 12-h light/dark cycle, 3 per Plexiglas cage with food and water provided ad libitum. Biweekly body weights and daily food intakes were recorded. All animal procedures were in compliance with guidelines of the Canadian Council on Animal Care.

Ischemia model

On day 28, gerbils were subjected to bilateral carotid artery occlusion (Ischemia) or sham surgery (Sham) as previously described (Colbourne and Corbett, 1995). Under 1.5–2.0% isoflurane with 1 L/min O_2, the common carotid arteries were isolated. Core body temperature was maintained at 37 ± 0.5°C with a rectal probe and homeothermic blanket (Harvard Apparatus). Brain temperature was approximated with a tympanic membrane probe and maintained at 36.5 ± 0.2°C during occlusion with a water-heated blanket wrapped around the head. Micro-aneurysm clips (60 g pressure) were applied to the arteries for 5 min. For Sham animals, procedures were identical but without arterial occlusion. All gerbils were placed under a heat lamp after surgery. Groups were as follows: C-Sham (n = 21); C-Ischemia (n = 24); PEM-Sham (n = 21); PEM-Ischemia (n = 24).

Indices of PEM

At 12 h post-surgery, half the gerbils were anesthetized, perfused trans-cardially with heparinized saline, and livers and brains collected on ice. Liver GSH concentration, an index of PEM (Taylor et al., 1992), was analyzed using high performance liquid chromatography (Paterson et al., 2001). Liver lipid, increased in PEM (Rana et al., 1996), was analyzed as previously described (Miyazawa et al., 1994). Brain hippocampus and neocortex were dissected on ice and stored at −70°C until analysis for oxidative stress (see below).

Open field

The remaining gerbils continued pre-surgery diets for 10 days. On days 3, 7 and 10 post-surgery, animals were placed in an open field (75 × 75 × 75 cm) for 10 min as previously described (Colbourne and Corbett, 1995). Activity was videotaped and then analyzed (EthoVision Basic, Noldus Information Technology): (1) using the entire field and (2) by dividing the field into outer zone (approximately 8.5 cm width from the outer walls of the field or 1.5× mid-hip gerbil body width) and remaining inner zone.

Histology

On day 10, gerbils were anesthetized and perfused trans-cardially with heparinized saline followed by 10% phosphate-buffered formalin. Intact heads were refrigerated in formalin for 18–24 h and brains removed for paraffin embedding. Coronal brain sections were cut at 6 μm and stained with hematoxylin and eosin (H&E). With observer blinded to treatment, the number of viable-looking CA1 neurons, non-eosinophilic with defined cell membrane and nucleus, were counted bilaterally at 400× magnification in three sections (levels) representing the entire anterior–posterior axis of the hippocampus. Neurons were counted in medial, middle, and lateral sectors (sector length = 0.2 mm) of the hippocampal CA1 region at −1.7 and −2.2 mm to bregma and in a single sector (middle CA1) at −2.7 mm (Loskoto et al., 1975). The method is modified slightly from that of Colbourne and Corbett (1995). These sectors were consistently counted by placing a microscope grid one grid width medial from CA2 neurons which are easily distinguished from CA1 neurons based on their size (lateral sector), at the apex of CA1 (middle sector), and on the rising portion of CA1 in an area distinct from subiculum (medial sector). Neuron counts were summed

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