Brain cell injury following cerebral ischemia and reperfusion: positive effects of ICAM-1/ neuroprotective role of Taurine

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Abstract

Proinflammatory cytokines play an important role in the development of cerebral reperfusion injury. During reperfusion, activated neutrophil interact with endothelial cells and release cytokines which mediate inflammation. Some conventional drugs have been shown in animal experiments to provide effective neuroprotection. Although the actual mechanism is still unclear. Our data are consistent with the concept that functional outcome after stroke is dependent on the immune cell composition which develops following ischemic brain injury. Furthermore; the present study showed the mechanism of reperfusion injury and the potential therapeutic strategies of Taurine.

Keywords: Brain cell injury; Cerebral ischemia and reperfusion; ICAM-1; Taurine

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Introduction

On average, strokes in the USA strike once every 40 seconds and cause death every 4 minutes, with an estimated 41.6% death rate in 2007 [1]. With an aging population, the absolute numbers are likely to rise. Among survivors, work capacity is compromised in 70% of victims, and 30% need assistance with self-care. Hence, the disease burden is great. Some risk factors, like hypertension and diabetes, impair protective vascular mechanisms that keep CBF stable during reductions in blood pressure (cerebrovascular autoregulation), facilitating the occurrence of ischemia if intravascular pressure drops [2, 3]. These vascular alterations

increase the brain's vulnerability to ischemia after arterial occlusion because they compromise the development of collateral flow arising from adjacent non-ischemic vascular territories, which is vital to the survival of the ischemic per-infarct zone [4]. To accomplish cerebral ischemia, novel approaches are required that expand upon our evolving mechanistic understanding of the fundamentals of cell survival and death processes as well as tissue repair.

Pro-inflammatory cytokines and apoptosis have been suggested to be one of the main contributors to neuronal death in acute ischemic stroke [5]. Strong evidence for neuronal apoptosis is seen in numerous I/R animal models [6]. Moreover, apoptosis is evident in patients suffering from ischemic stroke [7, 8]. During stroke, a number of apoptosis regulatory gene products are activated [9, 10]. Over-expression of anti-apoptotic proteins such as Bcl-2 and Bcl-xL have been shown to promote cell survival after focal cerebral ischemia [11].

Taurine is a kind of endogenous free amino acid (2-amino ethane sulfonic acid) in tissue and a potent antioxidant agent its function may in part be to adjust calcium homeostasis in cells, anti-oxidative stress, anti-inflammatory and cell protector [12]. It has been reported that Taurine can protect against lung injury following I/R, but the mechanism is unclear [13]. Taurine plays multiple roles in the CNS including acting as a neuro-modulator, an osmoregulator, a regulator of cytoplasmic calcium levels, a trophic factor in development, and a neuroprotectant.

In neurons Taurine has been shown to prevent mitochondrial dysfunction and to protect against endoplasmic reticulum stress associated with neurological disorders [14]. Furthermore, it is shown that Taurine has beneficial effects on myocardial ischemia-reperfusion injury [15, 16] cardiomyopathy, congestive heart failure [17] and pulmonary edema [18]. The aim of this study was to investigate the neurooprotective role of Taurine administration following cerebral I/R.

Materials and Methods

Animal models

Experiments were performed on male Sprague-Dawley rats (250 to 300 g) purchased from (Jackson Laboratory). The rats received a standard diet with free access to tap water. All procedures were done according to guidelines from the American Physiological Society and were approved by local authorities at F. Edward Hébert School of Medicine, Bethesda, MD.

Animals were quarantined for a minimum of seven days before entering into the study. Following quarantine period, rats were put in cages for three days before the study.

Study design

Three groups, including randomly chosen 9-rats in each of them, were constituted. Sham group rats were fed with standard rats feed and underwent the same anesthetic and surgical procedures for an identical period of time, but without transient cerebral ischemia. Ischemia group rats were fed with standard feed and cerebral ischemia was established. Treatment group rats were fed with Taurine and cerebral ischemia was established. Taurine was given with dose 200 mg/kg/day via oral gavage method for 3-days in addition to standard feed to provide standardization.

Rat model of transient focal cerebral ischemia

The surgical protocol was performed similar to methods described previously [19, 20, 21]. In brief, transient cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) by insertion of a nylon filament (diameter 0.24–0.28 mm). After 2 hours of ischemia, the nylon filament was carefully pulled out to establish reperfusion. Rats that did not show neurological deficits immediately after reperfusion (neurological score <1) were excluded from the study. Rats that showed neurological deficits immediately after reperfusion (neurological score >0) but were found to be experiencing skull base or subarachnoid hemorrhage were also excluded from the study. Sham-operated animals underwent the same surgical procedures without occlusion of the MCAO.

Tissue preparation for TNF-α, IL-1β, IL-6, ICAM-1 measurement

Following decapitation, the brain was removed and washed in cooled 0.9% saline, kept on ice and then put on filter paper, then weighed and homogenized using a high intensity ultrasonic liquid processor and brain tissues were homogenized in ice-cold 1:10 (w/v) 0.1 M phosphate-buffered saline (PBS) (pH 7.4), containing protease inhibitor cocktail and 0.2% Triton X-100 for 30 seconds (26). The homogenates were centrifuged at 14,000×g for 20 min at 4◦C and the supernatant was collected for determination of TNF-α, IL-1β, IL-6according to the manufacturer's instructions and guidelines using enzyme-linked immunosorbent assay (ELISA) kits (Quantikine®/R&D Systems.USA and Uscn. Life Science Inc., USA).

Histological analysis

Cerebral damage was assessed by the histological examination of brain sections at the level of the coronal cortex from the sham group, I 2 h groups and each I/R group. The animals were deeply anesthetized with chloral hydrate, and subsequently transcardially perfused with 200 mL heparinized 0.9% saline followed by 500 mL 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (pH 7.4). The rats were decapitated, and the brains were immersed in 4% paraformaldehyde for 3 d, processed for paraffin embedding, and sectioned (5 μm thick) on a rotary microtome. Coronal sections consisting of the cortex were selected and processed for hematoxylin-and-eosin (HE) staining.

Measurement of infarct volume in the brain

Samples from all three groups (n = 9 for each group) were used for analysis. At 6 hours after reperfusion, rats were killed and their brains were quickly removed and frozen at −20°C for 15 minutes. Coronal brain sections (2 mm thick) were stained with 2% TTC at 37°C for 20 minutes followed by fixation with 4% paraformaldehyde. The staining images were recorded using a digital camera (Canon Oxus 950IS) and then quantified using an Image J (ver 1.37c, NIH). To compensate for the effects of brain edema, the corrected volume was calculated using the following equation: Percentage hemisphere lesion volume (%HLV) = {[total infarct volume − (left hemisphere volume − right hemisphere volume)]/right hemisphere volume} ×100%. Infarct volume measurements were carried out by an investigator blinded to the treatment groups as describe previously [22].

Western blot

Western blot analysis was performed to measure the expression of the ICAM-1 protein in all of the groups, as describe previously [23, 24].

Cytokine ELISA

Brain tissue homogenate was prepared using samples taken at all four points in time. Levels of TNF-α, IL-1β, IL-6were determined using commercially available ELISA kits according to the manufacturer's instructions.

Statistical analysis

The quantitative data were expressed as the means±standard deviation and analyzed using a one-way analysis of variance (ANOVA) and the Bonferroni *post hoc* test; $P<0.05$ was considered statistically significant.

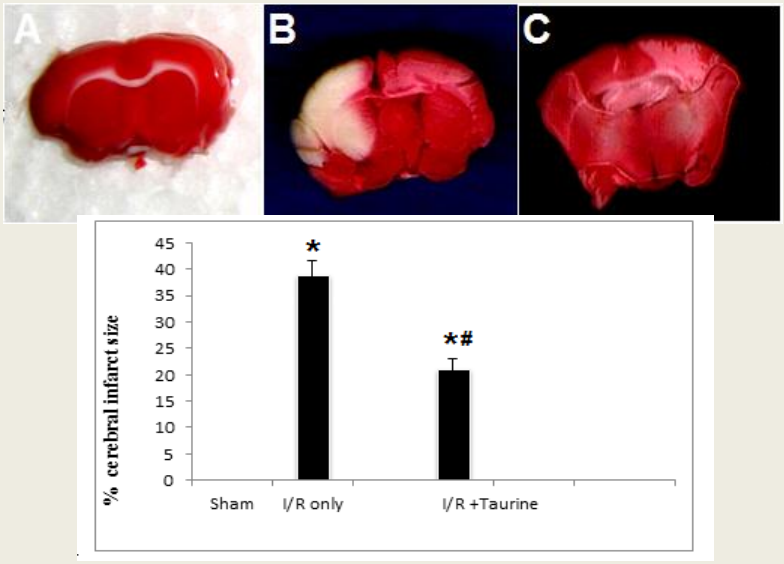

Figure 1.

Taurine reduced brain infarction after ischemia and reperfusion

TTC stain showing; (A) Normal brain without cerebral infarction, (B) Cerebral infarction area (41%) after I/R, (C) Treated with Taurine reduced cerebral infarction area (15%).

Results

Taurine protects brain from I/R injury

To evaluate the neuroprotective effects of Taurine after cerebral ischemia and reperfusion, the cerebral infarction area in the rat's brain was measured by 2,3,5-triphenyltetrazolium chloride (TTC) staining (Fig. 1) The cerebral infarct size of the Taurine treated group, was significantly lower than that of non-treated rat; $P<0.05$.These results strongly suggest that in murine models, Taurine can reverse the induction of ischemia-induced injury activity *in vivo* and reduce cerebral infarct size by more than 50%.

Histological changes after cerebral I/R

Using H&E staining, we found that there were no morphological injuries to the cortical neurons in the sham group, as these neurons displayed normal integrity of their cell structure (Fig. 2 A). After cerebral I/R, the neurons began to appear damaged with necrosis "infarct foci" (Fig. 2 B). The treated rats with Taurine show reduce the necrosis (Fig. 2 B).

Role of ICAM-1 following cerebral I/R

We investigated the involvement of ICAM-1 in the process of cerebral I/R inflammatory response. ICAM-1 expression demonstrate a dramatic decrease following cerebral I/R in mice treated with Taurine (Fig. 3).

Taurine reduce pro-inflammatory cytokines expression

In the brain, microglia produces TNF-α, IL-1β and IL-6 cytokines, which are major early response cytokines that trigger a cascade of inflammatory mediators. These cytokines are played an important role in the regulation of inflammation. C-reactive protein is an exquisitely sensitive systemic marker of inflammation and tissue damage. In the present study, the neuroprotective actions of Taurine administrated before cerebral ischemia could be due to its inhibitory effects on microglia and subsequent production of proinflammatory cytokines in the brain and periphery. Our data show that expressions of the proinflammatory cytokines TNF-α, IL-1β, IL-6 and C-R protein were significantly augmented after cerebral I/R compared with sham rats. Treatment with Taurine, expressions of TNF-α, IL-1β, IL-6 and inflammatory marker C-R protein were significantly reduced (Fig. 4).

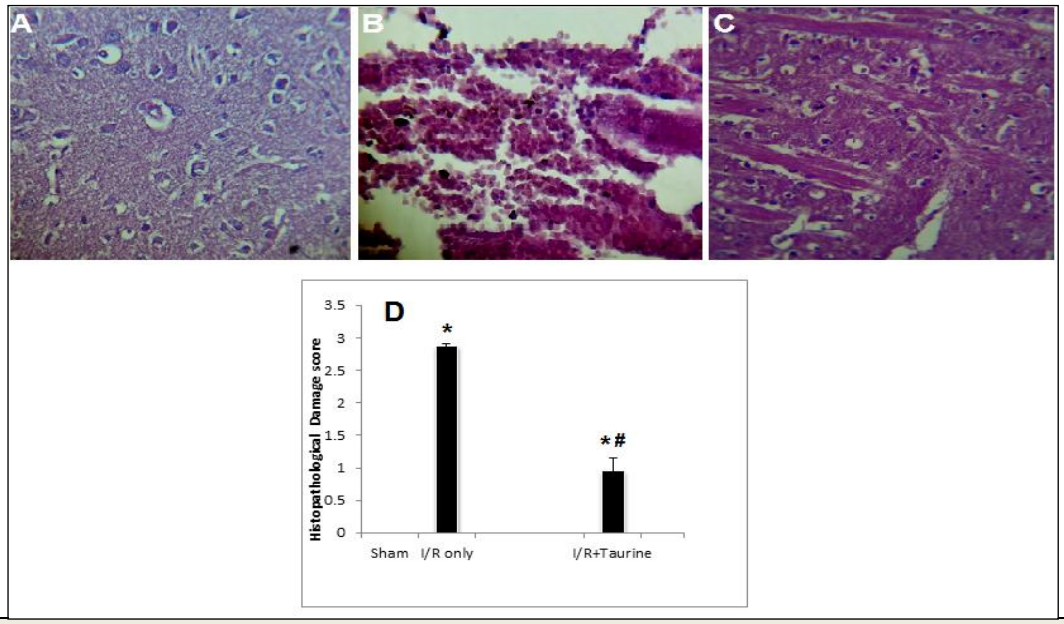

Figure 2.

Representative histological characteristics of brain sections were assessed by hematoxylin-and-eosin staining examination.

(A) Sham cerebral section revealed normal cortical tissue. (B) Damaged neurons were surrounded by activated glia cells. (D) Histological damage score. Values are mean ± SEM (n = 9 for each group); $^{*}P< 0.05$ *vs.* Sham, $^{\#}P< 0.05$ I/R+Taurine *vs.* I/R.

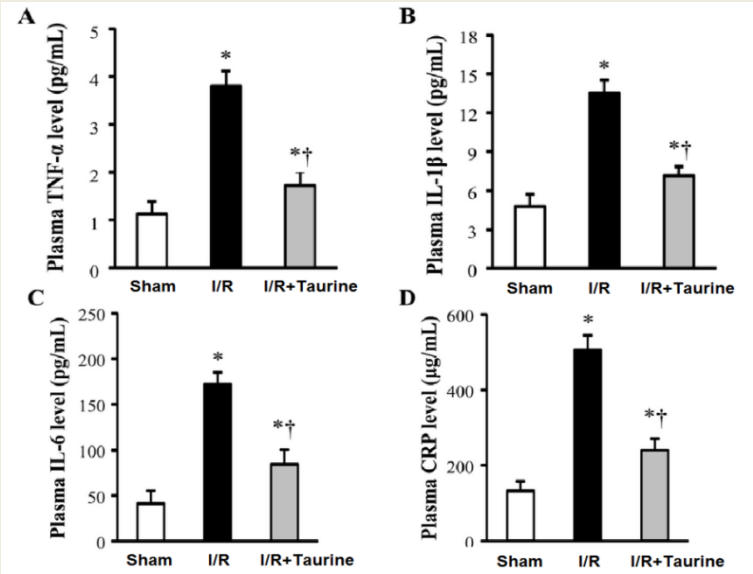

Figure 3.

Plasma levels of proinflammatory cytokines

(A) TNF-α, (B) IL-1β, (C) IL-6, (D) C-reactive protein after cerebral I/R. Values are mean ± SEM (n = 9 for each group); $^{*}P< 0.05$ *vs.* Sham; $^{\dagger}P< 0.05$ I/R+Taurine *vs.* I/R.

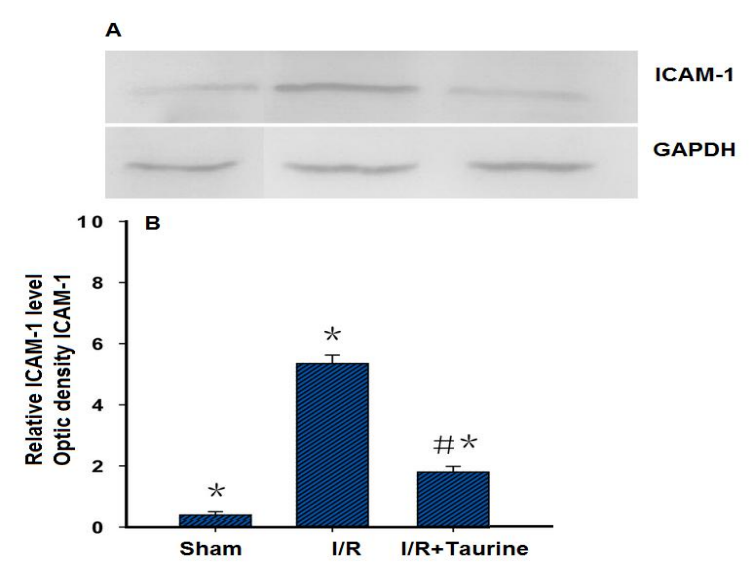

Figure 4.

Representative ICAM-1 protein expression

(A) Western blots showing representative ICAM-1 protein expression. ICAM-1 was blotted as a control and indicated no changes during the time course of the experiment. (B) Relative analysis of the ICAM-1 protein levels. Nine animals were included in each group. Each bar in B represents the means±SD; $^{*}P<0.05$ *vs.* Sham; $^{\#}P<0.05$ *vs.* I/R and sham.

Discussion

Ischemia and reperfusion (I/R) injury of the central nervous system (CNS) may contribute to the morbidity and mortality of stroke, head trauma, carotid endarterectomy, aortic aneurysm repair and deep hypothermic circulatory arrest. I/R injury of the CNS is characterized by disruption of the blood brain barrier, resulting in cerebral edema, increased intracranial pressure and leukocyte transmigration into the surrounding brain tissues [25]. Leucocytes may then release various proteases, lipid-derived mediators and ROS that may result in irreversible tissue damage, particularly within the ischemic penumbra [26]. Moreover, because of a loss of cerebral vasoreactivity, a reactive hyperemia may occur which may worsen the cerebral edema [27].

Thus I/R injury of the CNS may manifest clinically as worsened sensory, motor or cognitive functioning and death [28]. The present study demonstrated that, according to the selected biochemical and histological parameters of brain dysfunction, Taurine treatment modulated the course of ischemic cerebral in rats. This observation was supported by several key findings. Taurine treatment significantly ameliorated post-ischemic acute cerebral infarction and considerably limited the structural damage after ischemia. Histologically, Taurine treatment reduced the damage to brain. Untreated animals demonstrated typical changes: Moreover, Taurine treatment reduced the serum levels of cytokines which are indicators of impaired brain function. As mentioned above, despite intervention being after ischemic injury, cerebral I/R injury could be ameliorated with Taurine treatment.

The inflammatory responses in the brain to ischemic stroke are characterized by a rapid activation and proliferation of microglial cells, followed by the infiltration of circulating inflammatory cells, including neutrophils, T cells, monocyte/macrophages, and other cells in the ischemic brain region, as demonstrated in animal models and in stroke patients. The microglia are activated within minutes after onset of focal cerebral ischemia and may last for several weeks after initial injury [29]. Activated microglia produce a plethora of proinflammatory mediators in the brain, including TNF-α, IL-1β, IL-6 and ICAM-1 which contribute to the expansion of brain injury and the delayed loss of neurons [30].

In addition, expression of proinflammatory cytokines TNF-α, IL-1β and IL-6 in this brain area was also increased. These results are consistent with previous findings showing that cerebral ischemia injury substantially activates microglia and increases expression of proinflammatory cytokines and ICAM-1 in the blood, and increased immunoreactivity for proinflammatory cytokines is mostly co-localized with activated microglia [31], indicating

that activated microglial cells are the main source of proinflammatory cytokines in the brain after ischemic stroke. Furthermore, we found that early treatment with Taurine reduced the number of total microglia and the proportion of activated microglia in the peri-infarct cortical tissue, accompanied by decreased expressions of proinflammatory cytokines and ICAM-1. These findings provided evidence for suppressive effects of Taurine on microglial activation and release of proinflammatory cytokines *in vivo* in rats after ischemic stroke.

In conclusion, the present study demonstrates that administration of Taurine early before cerebral ischemia reduces infarct volume and improves neurological function by inhibition of microglia activation proinflammatory cytokine and ICAM-1 release in the brain. Taurine may be a promising therapeutic agent for the prevention and/or treatment of ischemic brain injury.

Competing interests

The authors have declared that no competing interests exist.

Author's Contributions

JF, AA, LY, CY performed the experiments. JF, CB analyzed the data. All authors approved the final paper.

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