Taurine exerts neuroprotective effects via anti-apoptosis in hypoxic-ischemic brain injury in neonatal rats

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= Abstract =

Purpose : Taurine (2-aminoethanesulfonic acid) is a simple sulfur-containing amino acid. It is abundantly present in tissues such as brain, retina, heart, and skeletal muscles. Current studies have demonstrated the neuroprotective effects of taurine, but limited data are available for such effects during neonatal period. The aim of this study was to determine whether taurine could reduce hypoxic-ischemic (HI) cerebral injury via anti-apoptosis mechanism.

Methods : Embryonic cortical neurons isolated from Sprague-Dawley (SD) rats at 18 days gestation were cultured in vitro. The cells were divided into hypoxia group, taurine-treated group before hypoxic insult, and taurine-treated group after HI insult. In the *in vivo* model, left carotid artery ligation was performed in 7-day-old SD rat pups. The pups were exposed to hypoxia, administered an injection of 30 mg/kg of taurine, and killed at 1 day, 3 days, 1 week, 2 weeks, and 4 weeks after the hypoxic insult. We compared the expressions of Bcl-2, Bax, and caspase-3 among the 3 groups by using real-time polymerase chain reaction (PCR) and western blotting.

Results : The cells in the taurine-treated group before hypoxic insult, although similar in appearance to those in the normoxia group, were lesser in number. In the taurine-treated group, Bcl-2 expression increased, whereas Bax and caspase-3 expressions reduced.

Conclusion : Taurine exerts neuroprotective effects onperinatal HI brain injury due to its anti-apoptotic effect. The neuroprotective effect was maximal at 1-2 weeks after the hypoxic injury. (Korean J Pediatr 2009;52:1337-1347)

Key Words : Taurine, Apoptosis, Hypoxic-ischemic brain injury, Neuroprotective effect

Introduction

Taurine (2–aminoethanesulfonic acid) is a simple sulfur– containing amino acid present in virtually all cells throughout the animal kingdom. In particular, it is enriched in electrically excitable tissues such as brain, retina, heart and skeletal muscles. Taurine has been implicated in major phenomena and one of the most abundant free amino acids in the central nervous system. In many mammals, its concentration even exceeds that of glutamate during ontogenic development[1]. Glutamate is the main excitatory transmitter in the brain stem. This sulfonic acid is thought to have a special role in the developing brain tissue. In other words taurine has been thought to be essential for the development and survival of neural cells. It is inhibitory and functions as a neuromodulator in the brain. Its function as a neurotransmitter implies the existence of specific taurine receptors and the neuromodulatory role, an interference with functions of other transmitter systems[2].

Taurine may also have potential for neuroprotection in cerebral ischemia[3]. It release is markedly enhanced under ischemic conditions. It protects neural cells from excitotoxicity induced by excitatory amino acids in the hippocampus[4] and cerebellum[5]. It has the ability to forestall harmful metabolic events evoked by ischemia and hypoxia[6] and to attenuate Ca^{2+} influx during ischemia[7]. Pretreatment with taurine (0.5, 1 or 2 mmol/L) could aid in the recovery

of synaptic function in rat hippocampus following a standardized hypoxic insult. Using *in vivo* rat brain ischemic models, several studies have documented the neuroprotective effect of taurine (200–300 mg/kg) under cell-damaging conditions[8].

The association of taurine with apoptosis, which is regarded as the major mechanism for the resolution of brain injury, has been examined in various cell types. Taurine inhibited arsenite-induced apoptosis of human PMN cells[9] and endothelial cell apoptosis[10]. Release of taurine from the cell through a CD 95 receptor and caspase dependent mechanism leads the triggering of apoptotic DNA fragmentation and cell shrinkage[11].

Numerous studies have shown that different experimental models of hypoxia and/or ischemia (e.g., anoxia, hypoxia, combined hypoxia, and ischemia) in neonatal animals can produce relatively predictable brain injuries[12-16] and that these brain injuries appear similar to those seen clinically in humans[17-21]. A commonly used neonatal rat model to investigate hypoxic-ischemic (HI) brain damage is the Vannucci-Rice model. This model involves a unilateral ligation of left common carotid artery following 8% O_2 for 1–2 hours. This procedure leads to cerebral damage restricted to the hemisphere ipsilateral to the occluded artery[16]. The neonatal rat HI model has been well characterized and extensively used to assess synthetic neuroprotective agents[22].

Neuroprotective drugs aim to salvage ischemic tissues, limit infarct size, prolong the window for reperfusion therapy or minimize post-ischemic reperfusion injury or inflammation. Current studies have demonstrated the neuroprotective effect of taurine in adult rat model, but limited data are available for those during the neonatal periods. Due to the dramatic differences in the physiology of perinatal and adult brains, the efficient treatment of HI encephalopathy in the perinatal period is likely different to those in adults.

The aim of this study was to determine whether taurine could reduce cerebral injury due to hypoxic-ischemic in the developing brain through anti-apoptosis using *in vitro* and *in vivo* models.

Materials and Methods

1. Embryonic cortical neuronal cell cultures

The rat embryonic cortical neuronal cell culture proce-

dure was based on the Brewer method[23]. Pregnant Sprague-Dawley (SD) rats at 18 days gestation (E18) were anesthetized with ether for 5 minutes at room temperature and the uterus were removed. The brains of the fetal pups were dissected and cortical tissues were digested for 5 minutes at 37℃ in Hanks' Balanced Salt Solution (HBSS) (GibcoBRL, NY, USA) containing 0.25% trypsin. They were rinsed five times with 5 mL of HBSS containing 1 mM sodium pyruvate and 10 mM HEPES (pH 7.4). The cells were transferred into 1 mL of Hanks' solution, pipetted 6–7 times with a small-bore Pasteur pipette, and dispersed. The cells were counted and inoculated into 10 cm dishes containing Neurobasal (GibcoBRL, NY, USA) plating media supplemented with B27 supplement (GibcoBRL, NY, USA) (100 mL Neurobasal, 2 mL B27 supplement, 0.25 mM 2-mercaptoethanol). Cell density was at about 2×10^6 cells/mm^2. They were cultured in 5% CO_2 incubator for forty-eight hours, after which cytosine arabinoside (3 μmol/mL) was added and left for another 48 hours to kill glial cells. The cultured cells were divided into two groups and were designated as the normoxia group and the hypoxia group. The normoxia group was cultured in 5% CO_2 incubators and the hypoxia group and the taurine-treated group before a hypoxic insult were placed in 1% O_2 incubators (94% N_2, 5% CO_2) for several hours. After verifying the desired amount of cellular injury in the hypoxia group, the taurine-treated group after a hypoxic insult was further divided. This produced four final groups; namely– normoxia, hypoxia, taurine-treated group before a hypoxic insult and taurine-treated group after a hypoxic insult.

2. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay

The MTT assay was used for estimation of cell viability and growth as originally described by Mosmann[24]. MTT was dissolved at a concentration of 5 mg/mL. Ten μL of the 5 mg/mL MTT stock solution was added to each well. After 4 hour of incubation at 37℃, media was removed and added 100 μL of the lysing buffer (Dimethyl sulfoxide (DMSO): 95% ethanol=1:1). Absorbance of the samples was read at 492 nm using a microtiter plate enzyme-linked immunosorbent assay (ELISA) reader. The amount of formazan produced is proportional to the number of live and metabolically active cells.

3. Animal protocols

This study was performed in accordance with the approved animal use guidelines of the Catholic University of Daegu. The protocol for the newborn animal model of HI brain injury was based on a modification of the Levine preparation described by Rice et al[16]. Left carotid artery ligation was done in 7-day-old SD rat pups under ether anesthesia. The neck was incised in the midline and the left common carotid artery was permanently ligated with 5-0 silk. Total time of surgery in each animal never exceeded 5 minutes. Following surgery, pups were returned to their mother for recovery and feeding for 1 hour. The pups were then exposed to a 2.5 hours period of hypoxia (8% oxygen, 92% nitrogen) by placing them in an airtight chamber partially submerged in a temperature controlled water bath to constantly maintain the ambient temperature inside the chamber at 37℃. The pups received an intraperitoneal injection of 30 mg/kg of taurine through the right lower quadrant of the abdomen 30 minutes before or after placement in the hypoxic chamber. Then, the pups were returned to their dam and sacrificed at 1 day, 3 days, 1 week, 2 weeks and 4 weeks after a hypoxic insult under ketamine. Whole brain tissues were obtained for analysis.

4. RNA extraction and real-time PCR

Total RNA was extracted with TRIzol reagent (Invitrogen corporation, Carlsbad, CA, USA). Briefly, total tissues (or cells) were homogenized in 1 mL of TRIzol reagent and total RNA was separated from DNA and proteins by extracting with chloroform and precipitating using isopropanol. The precipitate was washed twice in 75% ethanol, air-dried, and re-diluted in diethylpyrococarbonate (DEPC) treated distilled water. The amount and purity of extracted RNA was quantitated with a spectrophotometer (Beckman, Peapack, USA). The RNA was then stored at -70℃ pending further processing.

For reverse transcription, 1 μg total RNA was reverse transcribed for 1 hour at 37℃ in a reaction mixture containing 20 U RNase inhibitor (Promega, Madison, WI, USA), 1 mM dNTP, (TaKaRa, Shiga, Japan), 0.5 ng Oligo (dT) 15 primer (Promega, Madison, WI, USA), 1 X RT buffer and 200 U M-MLV reverse transcriptase (Promega, Madison, WI, USA). The reaction mixture was then heated at 95℃ for 5 minutes to stop the reaction. The cDNA was stored at -20℃ pending further processing.

Real-time PCR was performed in 48-well PCR plates (Mini OpticonTM Real-Time PCR System, Bio-rad, USA) using the FINNZYMES DyNAmo SYBR green qPCR kit (Finnzymes, Beverly, MA, USA). Amplification conditions are shown in Table 1. It was the same for all apoptotic and oxidant mRNA assayed: 95℃ for 15 minutes, followed by 40 cycles of 95℃ for 45 seconds, annealing temperature for 45 seconds, and 72℃ for 45 seconds. Real-time PCR data were analysed using the LightCycler software (BIORad Lab, Hercules, CA, USA).

5. Protein isolation and western-blotting

Samples of cortical neuronal cells or brain tissue were homogenized and total proteins were extracted using a protein lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid [EDTA], 0.5% Nonidet P-40, 100 mM phenylmethylsulfonly fluoride [PMSF], 1 mg/mL leupeptin, 1 mg/mL aprotinin, and 1 M 1,4-dithio-DL-threitol [DTT]). The lysates were centrifuged to remove debris. The protein concentration of the supernatant was measured using a Bio-Rad protein assay (BIORad Lab, Hercules, CA, USA), using bovine serum albumin as standards. After adjusting the protein concentrations, equal amounts of samples were added to appropriate amount of 2 X sodium dodecyl sulfate (SDS) sample buffer (100 mM Tris-HCl [pH 6.8], 200 mM DTT, 20% glycerol, 4% SDS, and 0.2% bromophenol blue). Samples were boiled for 5 minutes, and loaded (40 μg) into 12% SDS-polyacrylamide gel. Electrophoresis was done and proteins were then electro-transferred to a polyvinylidine difluoride (PVDF) membrane (Millipore, Bedford, MA, USA) at a constant voltage of 10 V for 30 minutes. The membranes were blocked in TBS-T buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, and 0.1% Tween 20) containing 5% nonfat milk for 1 hour at room temperature. Proteins were visualized following

Table 1. Primer Pairs and Annealing Temperatures for Real-time Polymerase Chain Reaction (PCR)

Name	Primer Sequence (5'-3')	Annealing
Bcl-2	F: TTGACGCTCTCCACACACATG	57℃
	R: GGTGGAGGAACTCTTCAGGGA	
Bax	F: TGCTGATGGCAACTTCAACT	55℃
	R: ATGATGGTTCTGATCAGCTCG	
caspase-3	F: AATTCAAGGGACGGGTCATG	56℃
	R: GCTTGTGCGCGTACAGTTTC	
beta-Actin	F: TTGCTGATCCACATCTGCTG	53℃
	R: GACAGGATGCAGAAGGAGAT	

overnight incubation with corresponding specific primary antibodies against Bcl—2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), Bax and caspase—3 (Cell signaling Technology, Beverly, MA, USA) at 1:1,000 dilutions in TBS—T at 4℃. After four washes in a TBS—T buffer, the membranes were incubated with horseradish peroxidase—conjugated second antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 hour at room temperature. Immunoreactivity was detected using enhanced chemiluminescence (ECL)—plus western Blotting Detection System (Amersham Biosciences, Piscataway, NJ, USA). The bands in the exposed films were analyzed by a densitometer (Multi gauze software, Fuji Photofilm).

6. Statistical analysis

Data were analyzed using the SPSS version 12 statistical analysis package. Examined data were assessed using the T—test, GLM (general lineal model) and ANOVA. In each test, the data are expressed as the mean SD and a *P* value =equal to 0.05 was accepted as statistically significant.

Results

1. Images of cortical neuronal cultured cells from rat embryos with taurine treatment

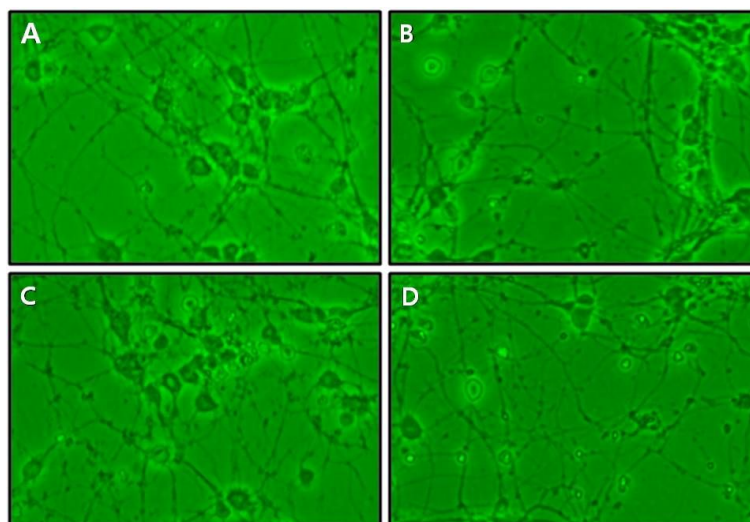The growth of embryonic cortical neuronal cells was observed with a phase contrast microscope (×400). Cells in the normoxia group (Fig. 1A) appeared normal, while cells in the hypoxia group (Fig. 1B) showed cellular swelling with indistinct nuclear shapes. Cells in the taurine—treated group before a hypoxic insult (Fig. 1C) had a similar appearance to those in the normoxia group, while the cells in the taurine—treated group after a hypoxic insult (Fig. 1D) were less numerous than in the taurine—treated group before a hypoxic insult.

2. Cell viability of cultured cortical neuronal cells, determined by the MTT assay

To determine the protective effects of taurine in the cultured cortical neuronal cells after a hypoxic insult, the relative cell viability of taurine was developed. In the hypoxia group, cells were reduced compared to the normoxia group. However, cell viability of the taurine—treated group before a hypoxic insult was similar to one in the normoxia group, while cell viability of the taurine—treated group after a hypoxic insult did not return to level of the normoxia group or the taurine—treated group before a hypoxic insult (Fig. 2).

3. The expression of Bcl—2, Bax and caspase—3 mRNA by real-time PCR in cultured cortical neuronal cells

The expression of Bcl—2 mRNA, which are indicators of the level of anti—apoptosis, was more reduced in the hy-

Fig. 1. High magnification (×400) photomicrographs of cultured embryonic cortical neuronal cells from 18—day-old pregnant Sprague—Dawley (SD) rats. Taurine was administered at 30 μg/mL. (A) Normoxia group; (B) Hypoxia group; (C) Taurine-treated group before hypoxic insult; (D) Taurine-treated group after hypoxic insult.

poxia group than in the normoxia group, and was increased in the taurine-treated group before a hypoxic insult compared to the hypoxia group. In contrast, the expression of
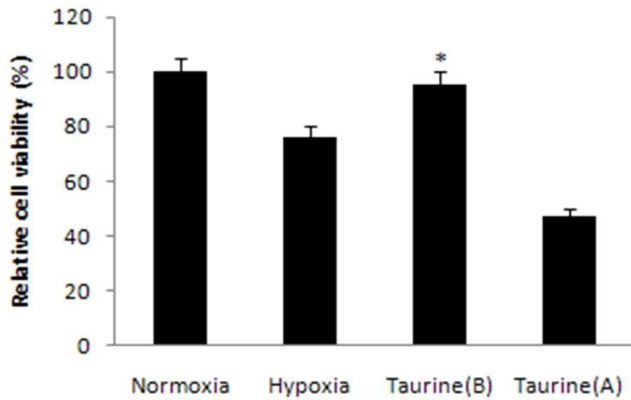

Fig. 2. Taurine attenuated hypoxic injury in rat brains. Cultured embryonic cortical neuronal cells were treated with taurine for 30 min before/after the hypoxic insult. Cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Taurine (B): Taurine-treated group before hypoxic insult; Taurine (A): Taurine-treated group after hypoxic insult. *P<0.05 compared with the normoxia group.

Bax & caspase-3 mRNA and the ratio of Bax/Bcl-2 expression, which are indicators of the level of pro-apoptosis, were more increased in the hypoxia group than in the normoxia group, and were decreased in the taurine-treated group before a hypoxic insult compared to the hypoxia group. The expressions of Bcl-2, Bax and caspase-3 mRNA in the taurine-treated group after a hypoxic insult was similar to those in the hypoxia group or did not return to level of the normoxia group or the taurine-treated group before a hypoxic insult (Fig. 3).

4. The expression of Bcl-2, Bax and caspase-3 by western blotting in cultured cortical neuronal cells

The expression of Bcl-2 was more reduced in the hypoxia group than in the normoxia group and was slightly increased in the taurine-treated group before and after a hypoxic insult compared to the hypoxia group. In contrast, the expression of Bax and the ratio of Bax/Bcl-2 expression were more increased in the hypoxia group than in the normoxia group and were decreased in the taurine-treated group before and after a hypoxic insult compared to the
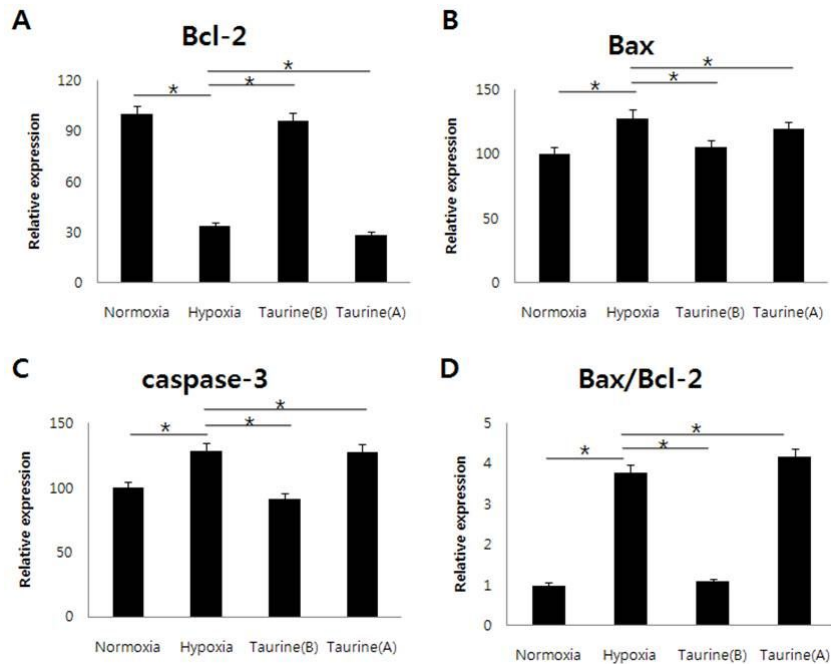
Fig. 3. Real-time polymerase chain reaction (PCR) for Bcl-2 (A), Bax (B), and caspase-3 (C) mRNA from cultured cortical neuronal cells from 18-day-old rat embryos (*in vitro*). The ratio of Bax/Bcl-2 (D) expression is also shown. Taurine was administered at 30 µg/mL. Data are presented as the ratios of band intensities for normoxia, hypoxia, taurine (B),and taurine (A) groups compared to those in the contralateral group. Taurine (B): Taurine-treated group before hypoxic insult; Taurine (A): Taurine-treated group after hypoxic insult. *P<0.05 compared with the normoxia group.

hypoxia group. However, the expression of caspase-3 were more increased in the hypoxia group than in the normoxia group and were decreased in the taurine-treated group before and after a hypoxic insult compared to the hypoxia group. The expressions of caspase-3 in the taurine-treated group after a hypoxic insult was similar to those in the hypoxia group (Fig. 4).

5. The expression of Bcl-2, Bax and caspase-3 mRNA at 1 day, 3 days, 1 week, 2 weeks, and 4 weeks after a hypoxic insult by real-time PCR in neonatal HI brain injury

At 1 day, 3 days, 1 week, 2 weeks, and 4 weeks after a hypoxic insult, the expression of Bcl-2 mRNA was more reduced in the hypoxia group than in the normoxia group, and was increased in the taurine-treated group before a hypoxic insult compared to the hypoxia group. In contrast, the expression of Bax and caspase-3 mRNA were more increased in the hypoxia group than in the normoxia group, and were decreased in the taurine-treated group before a

hypoxic insult compared to the hypoxia group. The expressions of Bcl-2 and Bax in the taurine-treated group after a hypoxic insult were similar to those in the hypoxia group and the expressions of caspase-3 in the taurine-treated group after a hypoxic insult was more decreased in proportion of the duration after a hypoxic insult (Fig. 5).

6. The expression of Bcl-2, Bax and caspase-3 at 1 day, 3 days, 1 week, 2 weeks, and 4 weeks after a hypoxic insult by western blotting in neonatal HI brain injury

At 1 week and 2 weeks after a hypoxic insult, the expression of Bcl-2 was more reduced in the hypoxia group than in the normoxia group, and was increased in the taurine-treated group before a hypoxic insult as compared to the hypoxia group. In contrast, the expression of Bax and caspase-3 were more increased in the hypoxia group than in the normoxia group, and decreased in the taurine-treated group before a hypoxic insult compared to the hypoxia group, However, at 1 day, 3 days and 4 weeks after a hy-
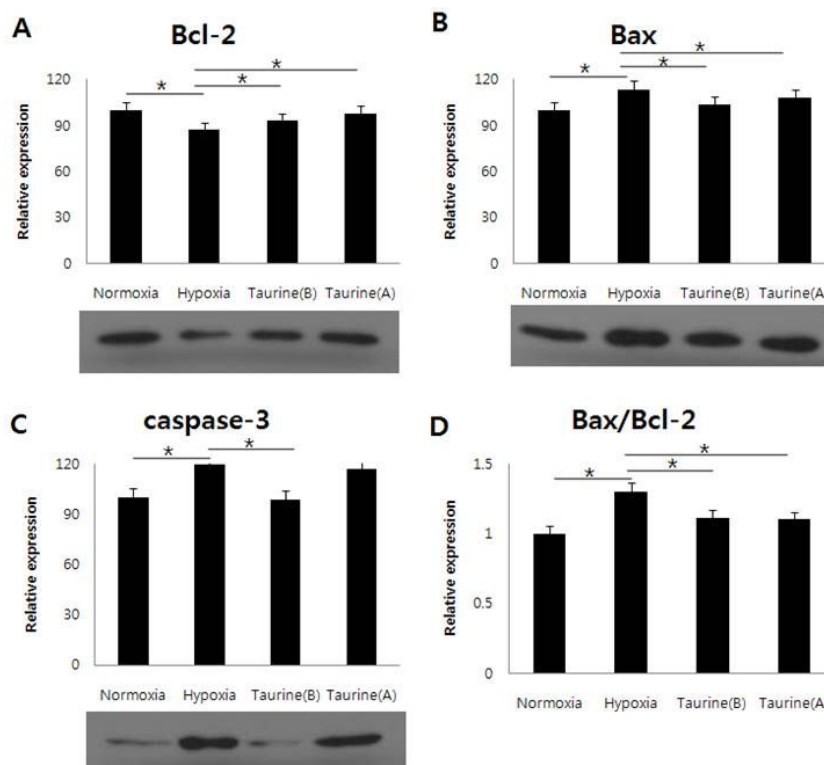
Fig. 4. Western blotting for Bcl-2 (A), Bax (B), and caspase-3 (C) from cultured cortical neuronal cells from 18-day-old rat embryos (*in vitro*). The ratio of Bax/Bcl-2 (D) expression isalso shown. Taurine was administered at 30 μg/mL. Data are presented as the ratios of band intensities for normoxia, hypoxia, taurine (B), and taurine (A) groups compared to those in the contralateral group. Taurine (B): Taurine-treated group before hypoxic insult; Taurine (A): Taurine-treated group after hypoxic insult. *P<0.05 compared with normoxia.
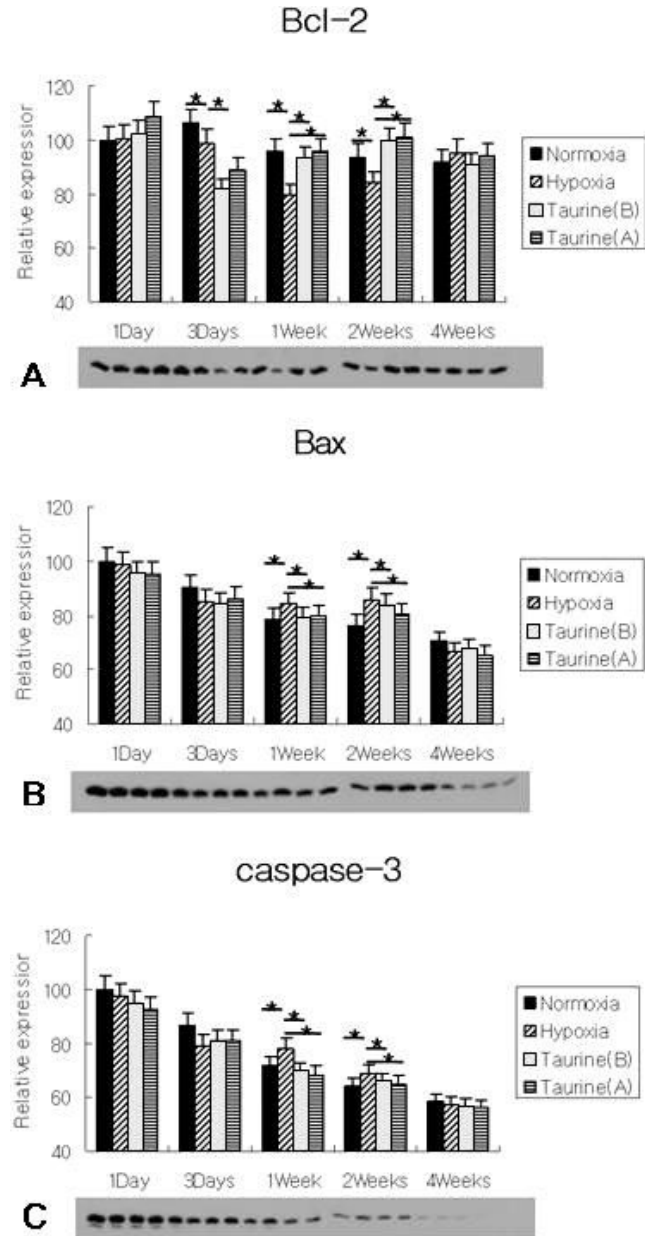
Bcl-2

A

Bax

B

caspase-3

C
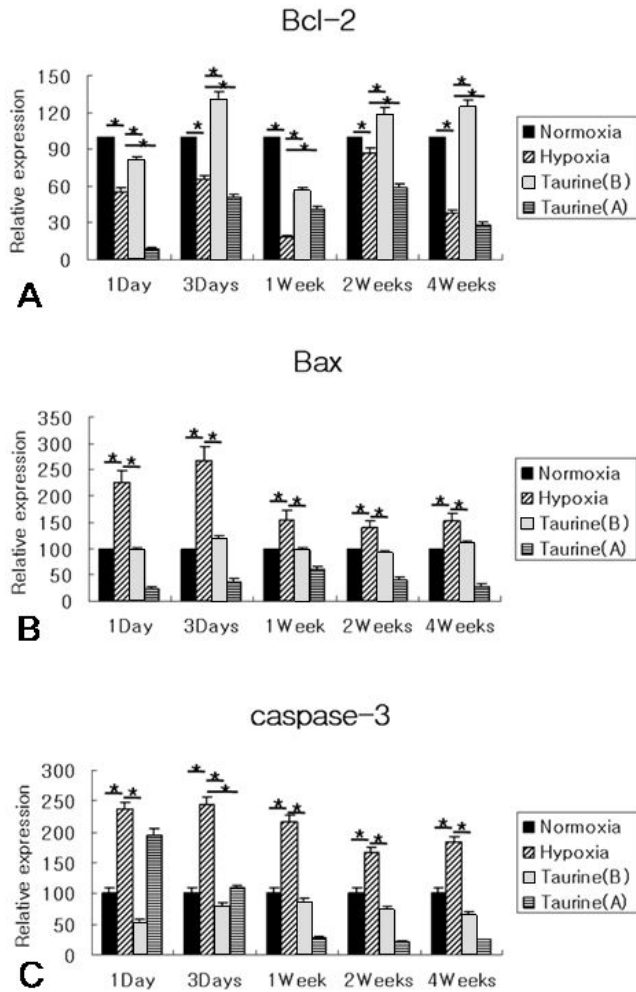
Fig. 5. Real-time polymerase chain reaction (PCR) for Bcl-2 (A), Bax (B),and caspase-3 (C) mRNA at 1 day, 3 days, 1 week, 2 weeks, and 4 weeks after hypoxic injury (*in vivo*). Taurine was administered at 30 mg/kg. Taurine (B): Taurine-treated group before hypoxic insult; Taurine(A): Taurine-treated group after hypoxic insult. $^*P < 0.05$ compared with the normoxia group.

poxic insult the expressions of Bcl-2, Bax and caspase-3 were different from the data of real-time PCR that we expected. Probably protein expression may be delayed and disappear early compare to mRNA expression (Fig. 6).

Discussion

Taurine has a number of physiological functions. It is a component of bile acids, which are used to help absorb fats and fat-soluble vitamins. It also helps regulate the heart beat, maintain cell membrane stability and prevent brain cell over-activity[25]. It is a major constituent of bile and can be found in lower amounts in the tissues of many animals including humans[26]. Taurine originated from the

Bcl-2

A

Bax

B

caspase-3

C

Fig. 6. Western blotting for Bcl-2 (A), Bax (B), and caspase-3 (C) at 1 day, 3 days, 1 week, 2 weeks, and 4 weeks after HI injury (*in vivo*). Taurine was administered at 30 mg/kg. Taurine (B): Taurine-treated group before HI insult; Taurine (A): Taurine-treated group after HI insult. $^*P < 0.05$ compared with the normoxia group.

Latin word taurus, which means bull or ox, as it was first isolated from ox bile in 1827 by Austrian scientists Friedrich Tiedemann and Leopold Gmelin[27]. It is often considered an amino acid in scientific literatures[27]. It is a vital nutrient for cats, and probably also for primates, since it is essential for the development and survival of neural cell[28].

HI encephalopathy triggers an immediate neuronal and

glial injury leading to necrosis secondary to cellular edema and lysis. Early congestion, fluid leak from increased capillary permeability and endothelial cell swelling may then lead to coagulation necrosis and cell death. The injury also triggers apoptosis resulting in delayed cellular death[29].

Taurine is known to protect neural cells from the excitotoxicity induced by excitatory amino acids[30]. In the central nervous system, it has been implicated in inhibitory neuromodulation or neurotransmission. Its function as a neurotransmitter implies the existence of specific taurine receptors and the neuromodulatory role, an interference with functions of other transmitter systems. It is known to exerts its protective function through inhibition of glutamate−induced calcium influx through L−, N− and P/Q−type voltage−gated calcium channels and N−methyl−D−aspartate receptor calcium channel and attenuation of glutamate−induced membrane depolarization and prevention of glutamate−induced apoptosis via preventing glutamate−mediated down−regulation of Bcl−2 and prevention of cleavage of Bcl−2 by calpain. It forestalls the harmful metabolic cascades evoked by ischemia and hypoxia and attenuates Ca^{2+} influx in ischemia. Taurine−containing neurons are fairly resistant to cerebral ischemia induced by the four−vessel occlusion model[31]. Taurine protects cerebellar granular cells exposed to kainate without affecting the production of reactive oxygen species in these cells[32].

The extracellular concentrations of taurine together with other amino acids have been measured by microdialysis in several *in vivo* animal models of ischemic injury. The levels were increased in the rat striatum[33] and rabbit cerebral cortex[34] after transient focal ischemia, in the rat cerebral cortex in the four−vessel occlusion model, in the rabbit spinal cord after aortic occlusion[35] and in the hippocampus after forebrain ischemia both in normal[36] and spontaneously hypertensive rats[37]. In this latter experimental paradigm, the ischemia−induced release of taurine was smaller in aged rats than in adults, indicating the age−related vulnerability of hippocampal neurons to ischemia[37]. In a global model of brain ischemia, taurine accumulated in the rat auditory cortex and cerebrospinal fluid[38].

In a hypoxic rat model, taurine prevented hypoxia−induced lactate accumulation and lipid peroxidation in the brain, liver, and heart tissues[39]. Besides its reported antioxidant effect, taurine has a GABA−A agonist activity. High concentrations of taurine is present in mammalian central nervous system and it is believed to be involved in osmore-

gulation and cell volume adjustments[28]. Growing evidences suggest that taurine may function as a potent inhibitory neurotransmitter or a modulator that regulates neuronal activity in many cerebral areas.

We studied the neuroprotective effect of taurine using an *in vitro* culture model of hypoxia and an *in vivo* animal model of neonatal HI brain injury. This study demonstrated that cultured cortical neuronal cells from 18−day−old rats survived better after hypoxia when taurine (30 μg/mL) was present. Cells in the normoxia group appeared normal, while cells in the hypoxia group showed cellular swelling with indistinct nuclear shapes. Increased number of surviving neuronal cells was prominent in the taurine−treated group before a hypoxic insult and the cells were morphologically similar to those in the normoxia group. Cells in the taurine−treated after a hypoxic insult were less numerous than those in the the taurine−treated before a hypoxic insult.

We studied the neuroprotective effects of taurine and its relation to the apoptosis cascade mechanism. Apoptosis markers, Bcl−2, Bax and caspase−3, from cytologic material were assayed by western blotting and real−time PCR.

HI injury induced apoptosis of neurons depends on the activation of caspases. Caspase−3, a widely studied caspase, plays an important role in neuronal cell death. It is well known that caspases are a family of intracellular proteins involved in the initiation and execution of apoptosis. The induction of apoptosis, whether through extrinsic or intrinsic mechanisms, results in the activation of initiator caspase, where caspase−3 is a common and key executor caspase. In addition, the Bcl−2 family of proteins are also involved in apoptosis. Pro−apoptotic proteins, such as Bax, Bid and Bak promote cell death, whereas anti−apoptotic proteins, such as Bcl−2 and Bcl−xL, may enhance cell survival. Programmed cell death, or apoptosis, is an active process that occurs naturally in the developing brain. Recently, apoptosis has been implicated in several disorders of the central nervous system as well as after a cerebral HI insult. HI−induced apoptosis as a common cause of injury in developing brain is currently a subject of intense investigation, and focal cerebral HI injury is a widely used model since one side of the brain can be used as an unequivocal control. Although apoptosis is considered to be more prominent as a mode of cell death in neonates compared to the adult brain, necrosis still predominates in immature brain affected by HI injury[40]. Immature cortical neurons are highly susceptible to apoptosis, and apoptosis

has a prolonged role in neurodegeneration after HI in the newborn rat. In addition, HI damage generally evolves more rapidly in the immature brain than its adult counterpart[16]. The prevention of apoptosis may potentially expand the therapeutic window for the treatment of perinatal HI brain injury.

Using the HI neonatal rat model, we compared the results of assays for some apoptotic markers from the control group, the hypoxia group, and the taurine-treated groups (pre-treatment, post-treatment). The results showed that in the taurine-treated group before a hypoxic insult, the Bcl-2, an anti-apoptotic protein, was relatively increased while Bax and caspase-3, both pro-apoptotic proteins, were relatively decreased. Bax/Bcl-2 ratio also decreased. These results suggest that the neuroprotective effect of taurine is associated with the apoptosis cascade.

The expression of Bcl-2 mRNA by real-time PCR in neonatal HI brain injury (in vivo) was more reduced in the hypoxia group than in the normoxia group, and was increased in the taurine-treated group before a hypoxic insult at all periods (1 day, 3 days, 1 week, 2 weeks, 4 weeks) compared to the hypoxia group. In contrast, the expression of Bax and caspase-3 mRNA were more increased in the hypoxia group than in the normoxia group, and were decreased in the taurine-treated group before a hypoxic insult at all periods compared to the hypoxia group. The expression of Bcl-2 by western blot in neonatal HI brain injury (in vivo) was more reduced in the hypoxia group than in the normoxia group, and was increased in the taurine-treated group before a hypoxic insult at 1 week and 2 weeks compared to the hypoxia group. In contrast, the expressions of Bax and caspase-3 were more increased in the hypoxia group than in the normoxia group, and were decreased in the taurine-treated group before a hypoxic insult at 1 and 2 weeks compared to the hypoxia group. However, at 1 day, 3 days and 4 weeks after a hypoxic insult the expressions of Bcl-2, Bax and caspase-3 were different from the data of real-time PCR that we expected. Probably protein expression may delay and disappear early compare to mRNA expression

In this study, we demonstrated the neuroprotective effect of taurine in hypoxic neonatal rat model through its effect on the apoptosis cascade. The above suggests that taurine has neuroprotective property over perinatal HI brain injury due to its anti-apoptotic effect, as evidenced by causing a decrease in Bcl-2 and increase in Bax and cas-

pase-3 expression. The neuroprotective effect of taurine administration after hypoxic brain injury was maximal at 1-2 weeks after HI injury.

In conclusion, taurine has neuroprotective effect when administered after hypoxia in the neonatal rat model. The apoptosis cascade are involved in this neuroprotective effect. Moreover, taurine is most effective when administered at 1-2 weeks after a hypoxic event.

한 글 요 약

신생 흰쥐의 저산소성 허혈성 뇌손상에서 항세포사멸사를 통한 taurine의 신경보호 효과

대구가톨릭대학교 의과대학 소아과학교실
동국대학교 의과대학 안과학교실*

정지은 · 김태열 · 박혜진 · 이계향 · 이경훈
최은진 · 김진경 · 정혜리 · 서억수* · 김우택

목 적: 타우린은 술폰 기를 산기로 하는 황 아미노산의 일종이며 뇌, 망막, 심장, 근육에 많이 분포되어 있다. 최근 국소적 뇌허혈에 대한 타우린의 신경보호효과에 관한 연구들이 발표되고 있으나 대부분 연구가 성인의 뇌졸중의 치료에 대한 연구이며 신생아시기의 저산소성 손상에 대한 효과를 구체적으로 연구한 바가 없다. 본 연구에서는 타우린이 저산소 상태로 유발된 뇌세포 배양과 신생 백서의 저산소성 허혈성 뇌손상에서 항세포사멸사를 통한 뇌보호 효과가 있는지를 알아보고자 실험하였다.

방 법: 재태기간 18일된 태아 흰쥐의 대뇌피질 세포를 배양하여 1% O₂ 배양기에서 저산소 상태로 뇌세포 손상을 유도하여 저산소군, 손상 전 후 타우린 투여군(30 μg/mL)으로 나누어 정상산소군과 비교하였다. 세포사멸사와 관련을 알아보기 위해 Bcl-2, Bax, caspase-3 primer와 항체로 실시간 중합효소연쇄반응과 western blotting을 하였다. 또한, 생후 7일된 백서의 좌측 총경동맥을 결찰한 후 저산소(8% O₂) 상태로 2시간 노출시켜서, 저산소성 허혈성 뇌 손상을 유발하였고, 뇌손상 전 후 30분에 타우린을 체중 kg당 30 mg을 투여하였다. 저산소성 허혈성 뇌손상 후 1일, 3일, 1주, 2주, 4주 째 뇌를 적출하여 Bcl-2, Bax, caspase-3 primer를 이용하여 실시간 중합효소연쇄반응을 하였고, 동일 항체로 western blotting하였다.

결 과: 저산소로 유발된 뇌세포 배양에서 정상군에 비해 저산소군에서 뇌세포 손상이 많았고 저산소 손상전 타우린 투여군에서 뇌세포 손상이 회복되었으며 저산소 손상 후 타우린 투여군에서는 저산소 손상 전 투여군보다 회복력이 떨어졌다. 실시간 중합효소연쇄반응과 western blotting을 이용한 저산소 상태의 태아 백서 뇌세포 배양 실험뿐만 아니라 저산소성 허혈성 뇌손상 동물 모델에서도 타우린을 투여한 경우 Bcl-2의 발현은 증가하고, Bax/Bcl-2의 비율, Bax와 caspase-3의 발현은 감소함을 보였다.

결 론：본 연구에서 타우린은 주산기 저산소성 허혈성 뇌손상에서 Bcl-2 발현 감소, Bax와 caspase-3 발현 증가를 유발시켜 항 세포사멸사 기전을 통한 신경보호 역할을 하는 것을 알 수 있었다. 그리고 이것은 저산소 손상 후 1주와 2주째에 가장 효과가 있었다.

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