EFFECTS OF TAURINE ON CEREBRAL BLOOD FLOW PERFUSION, CELL APOPTOSIS, AND INFARCT VOLUME IN ACUTE CEREBRAL ISCHEMIC RATS

Fei Zhou, Jingchun Guo, Ru Yang, Jing Gu, Hongbin Jin, Gencheng Wu, and Jieshi Cheng*

1. INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is one of the most abundant free amino acids in the mammals. This simple non-proteinaceous sulfur-containing amino acid has been thought to be a vital nutrient for development of the immature brain and survival of neural cells (Saransaari and Oja, 2000). Recently, taurine has been shown to be involved in many important physiological functions such as maintaining the structural integrity of the membranes, regulating calcium binding and transport, and functioning as an osmolality regulator, neuromodulator, neurotransmitter, and neuroprotective agent against L-glutamate-induced neurotoxicity (Foos and Wu, 2002). In addition, taurine has been shown to protect neural cells from excitotoxicity and prevent harmful metabolic events evoked by cell-damaging condition such as ischemia or hypoxia and epilepsy (Saransaari and Oja, 2002). Previous studies implied that taurine might be a potential and potent agent against cerebral ischemia. To corroborate its beneficial effects and explore their possible mechanisms, we investigated the effects of taurine on acute cerebral ischemia with respect of cerebral blood flow perfusion, cell apoptosis, neurological deficits and infarct volume.

2. MATERIALS AND METHODS

Adult male Sprague-Dawley rats (250 g±10 g) were randomly divided into 5 groups: ischemia (n=12), ischemia+taurine 10 mg/kg (n=8), ischemia+taurine 40 mg/kg (n=12),

* Department of Integrative Medicine, National Key Laboratory of Medical Neurobiology, Shanghai Medical College, Fudan University, China.

ischemia+taurine 80 mg/kg (n=8), and sham groups (n=6). Taurine was dissolved in saline and given (i.p.) at 5 minutes of ischemia and 5 minutes of reperfusion.

Transient cerebral ischemia was produced by a modification of the method of Longa *et al*. (1989). Under anesthesia, a segment of 4-0 monofilament nylon suture was inserted into the origin of the right middle cerebral artery to occlude the blood flow. After 1 h of occlusion, complete reperfusion of ischemic area was allowed by withdrawal of the suture. In the sham-operation group, the same surgical procedures were carried out except for the artery occlusion. Changes in the cerebral blood flow (CBF) of the ischemic cortex were monitored continuously from pre-ischemia to the early stage of reperfusion with a Laser-Doppler flowmeter (LDF, PeiFlux5000, Sweden) to ensure the success of artery obstruction and the observe the dynamic change of the CBF.

Neurological deficits were evaluated at 24 hours of reperfusion with 0~7 grade method. A rat exhibited the symmetrical behavior as a normal animal in 0 grade and could only lie on the left side because of the loss of supporting capability in 7 grade.

After 24 hours of reperfusion, the rats were quickly sacrificed. Eight brains in each group were removed and sliced into 2.0-mm thick sections, and then incubated in 20 g/l TTC for 30 min at 37^{o}C to observe the infarct area. The area/volume of infarcts was measured as in Swanson *et al*. (1990). Four brains in each group (ischemia, sham, and ischemia+taurine 40 mg/kg) were perfused with 40 g/l paraformaldehyde and cut into 30 μm thick coronal sections in a cryostat. To detect *in situ* fragmentation of double DNA damage histochemical staining with TUNEL was performed. The expression of active subtype of caspase-3 protein (caspase-3 p20) was observed by immunohistochemical methods. Immunostaining for caspase-3 was performed with the avidin-biotin peroxidase (ABC) method. The expression of caspase-3 mRNA was observed by *in situ* hybridization. The brain slices were incubated in hybridization buffer with the casapase-3 mRNA DIG-labeling oligonucleotide probe.

Changes in cerebral flow were evaluated as percentages, comparing to the base flow in pre-ischemia. Grades of neurological deficit were analyzed with the grading method. The cerebral infarct volume was estimated as percentage of the whole cerebrum. The number of TUNEL positive cells was counted and expressed as percentages in five (x200) random MCA areas. The level of expression of caspase-3 mRNA and protein was analyzed with the gray value. The statistical comparisons between two groups were performed with the unpaired *t* test. Significance between groups was assigned at $p<0.01$.

3. RESULTS

After 1 hour of ischemia and 24 hours of reperfusion, the degree of neurological deficits were severe (average 6 grade), and the infarct areas were larger than 30% of the whole brain in the model group. The infarct areas were distributed mainly in the ipsilateral striatum and cerebral cortex. Taurine 10 mg/kg had no significant effect on either neurological deficit score or cerebral infarct volume. However, the degree of neurological deficiency in the taurine 40 mg/kg and 80 mg/kg groups was slighter than that in the model group. The infarct volume in the taurine 40 mg/kg and taurine 80 mg/kg groups was likewise smaller than that in the model group (Table 1).

Continuous CBF monitoring in the local cortex showed that taurine increases the local cerebral blood flow. When the middle cerebral artery was successfully obstructed the local blood flow decreased immediately to less than 20% in pre-ischemia and persisted at the lower perfusion level during the whole ischemia stage. The lowered flow continued to the early stage of reperfusion, owing to long and severely low perfusion during the ischemic stage. When taurine (40 mg/kg) was given i.p. at the ischemic stage, the blood perfusion of the ischemic cortex started to increase by 50%~100% of the ischemic level from 10 min onwards and fluctuated about 30 min at a level higher than the ischemic level (Fig. 1).

Table 1. Neurological deficit score and cerebral infarct volume

Group		Neurological deficit score	Cerebral infarct volume (v/v%)
Sham		0	0*
Model		6(5~7)	31.2%±4.5%
Taurine	10 mg/kg	5(4~6)	29.3±4.6%
	40 mg/kg	3(2~4)	12.3%±1.3%*
	80 mg/kg	3(2~4)	12.0%±3.0%*

*Cerebral infarct volume is presented as mean±SD. Significant differences *vs*. model□*t*-test, p<0.01).

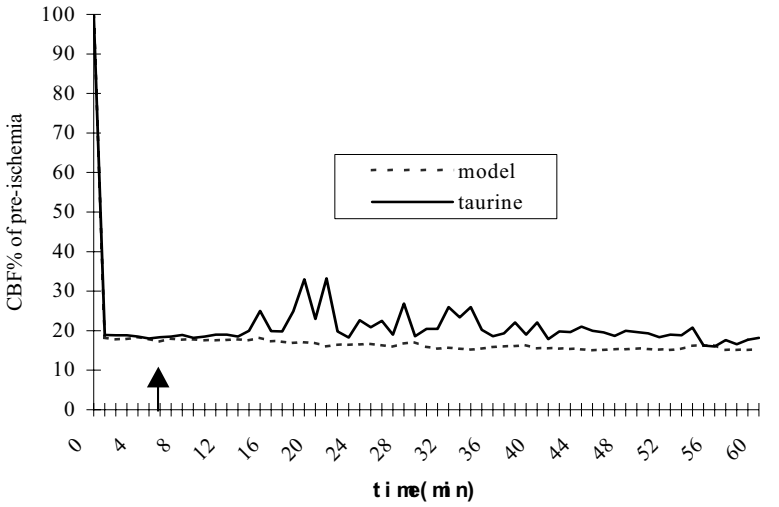

Figure 1. Effect of taurine on CBF in the local ischemic cortex. After taurine was given, the local blood flow increased from 10 min onward and fluctuated for 30 min (↑ dosing time).

We investigated effect of taurine on ischemic cell apoptosis. The results (Fig. 2) showed that there was a mild expression of caspase-3 mRNA and few immunoreactivities of caspase-3 p20 in the sham brain sections. When ischemia/reperfusion occurred, the expression of caspase-3 mRNA and immunoreactivities of caspase-3 p20 increased significantly. However in the ischemia+taurine group, the levels of expression of caspase-3 mRNA and caspase-3 p20 protein were lower. Similarly, TUNEL-positive cells were few in the sham group and increased in the model group, but were reduced in the ischemia+taurine group (Fig. 3). The decrease in the number of TUNNEL-positive cells and down-regulation of capase-3 mRNA and caspase-3 p20 protein in the taurine group imply that taurine is related to the anti-apoptotic pathways after ischemia and reperfusion.

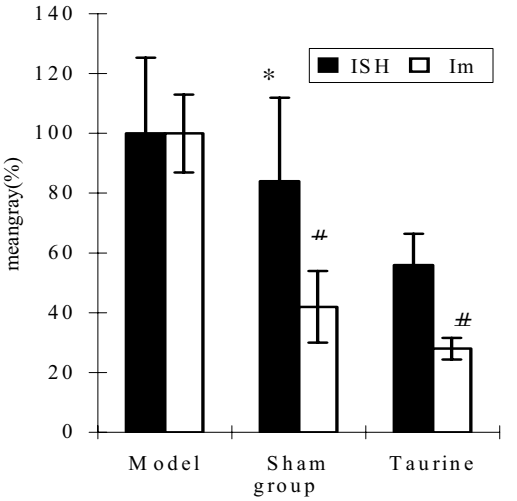

Figure 2. Effect of taurine on the expression of caspase-3 mRNA and caspase-3 p20 (mean gray% *vs*. model as 100%). *p<0.05, #p<0.01.

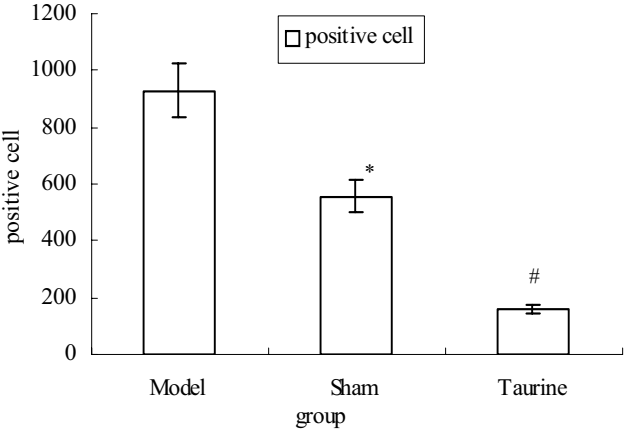

Figure 3. Amount of TUNEL-positive cells in the model and taurine groups (*vs*. model *p<0.05, #p<0.01).

4. DISCUSSION

In previous studies, elevated exteracellular levels of taurine were seen to enhance adenosine release in ischemia and contribute to the maintenance of homeostasis (Saransaari and Oja, 2003). Taurine could also be modified by NO (Saransaari and Oja, 2002) or electroacupuncture (Guo *et al.,* 2002). On the other hand, studies of Shuaib (2003) did not reveal neuronal protection of taurine. However, the results from our present research showed the protection of exogenous taurine by alleviating the neurological deficits and infarct volume.

Reduction of local cerebral blood flow is crucial to origin and development of ischemic cerebral injury. Our research demonstrates the CBF-increasing effect of taurine. We think that it is an important pathway of taurine to protect against cerebral ischemia by ameliorating microcirculation.

Apoptosis are likely to be involved in primary and secondary cell death in cerebral ischemia. Previous research reported that increased pro-caspase-3 immunoreactivity is seen in the penumbra following focal ischemia in the adult rats (Isidro and Planas, 2003). Recent studies provide direct evidences for distribution of caspase-3 activation following cerebral ischemia (Rami, 2003). Our present study showed that the expression of caspase-3 mRNA and caspase-3 (p20) protein and the number of TUENEL-positive cells increased in acute ischemic rats but decreased with taurine treatment. It suggests that taurine is involved in the cellular and molecular mechanism of anti-apoptosis process.

In summary, the results of our present studies showed that taurine could protect the cerebrum against acute cerebral ischemia by either increasing cerebral blood perfusion or suppression of cell apoptosis. It implies that taurine may be a potential and potent agent for cerebral ischemia therapy. However, further studies are needed to clarify how taurine affects cerebral blood flow and cell apoptosis.

5. ACKNOWLEDGMENT

This project was supported by grants the National Natural Science Foundation of China (Nos. 30030946 and 90209028) and the Foundation for the Author of National Excellent Doctoral Dissertation of P. R. China (Project No.10060101).

6. REFERENCES

Foos, T. M. and Wu, J.-Y., 2002, The role of taurine in the central nervous system and the modulation of intracellar calcium homeostasis, *Neurochem. Res.* **27**:21-26.

Guo J. C., Li, R., Zhao, P, and Cheng, J. C., 2002, Effect of taurine in combination with electroacupuncture on neuronal damage following transient focal cerebral ischemia in rats, *Acupunct. Electrother. Res.* **27**:129-136.

Isidro, F. and Planas, A. M., 2003, Signaling of cell death and cell survival following focal cerebral ischemia: life and death struggle in the penumbra, *J. Neuropathol. Exp. Neurol.* **62**:329-339.

Longa, E. Z., Weinstein, P. R., Carlson, S., and Cummins, R., 1989, Reversible middle cerebral artery occlusion without craniectomy in rats, *Stroke* **20**:84-91.

Rami, A., 2003, Ischemic neuronal death in the rat hippocampus: the calpain-calpastain-caspase hypothesis, *Neurobiol. Dis.* **13**:75-88.

Saransaari, P. and Oja, S. S., 2000, Taurine and neural cell damage, *Amino Acids* **19**:509-526.

Saransaari, P. and Oja, S. S., 2002, Taurine release in the development and adult mouse hippocampus: involvement of cyclic guanosine monophosphate, *Neurochem. Res.* **27**:15-20.

Saransaari, P. and Oja, S. S., 2002, Ischemia-induced taurine release is modified by nitric oxide-generating compounds in slices from the developing and adult mouse hippocampus, *Neurochem. Res*. **27**:395-402.

Saransaari, P. and Oja, S. S., 2003, Enhanced release of adenosine under cell-damaging conditions in the developing and adult mouse hippocampus, *Neurochem. Res*. **28**:1409-1417.

Shuaib, A., 2003, The role of taurine in cerebral ischemia: studies in transient forebrain ischemia and embolic focal ischemia in rodents, *Adv. Exp. Med. Biol*. **526**:421-431.

Swanson, R. A., Morton, M. T., and Tsao, W. G.., 1990, Semiautomated method for measuring brain infarct volume, *J. Cereb. Blood Flow Metab.* **10**:290-295.