Effects of *Gingko biloba* extract on glutamate-induced $[Ca^{2+}]_i$ changes in cultured cortical astrocytes after hypoxia/reoxygenation, H_2O_2 or *L*-glutamate injury

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Abstract: Aim To investigate glutamate-induced $[Ca^{2+}]_i$ changes in cultured rat neonatal cortical astrocytes after hypoxia/reoxygenation, H_2O_2 or high concentration of *L*-glutamate injury. In the meantime, the effects of *Gingko biloba* extract (*Gb*E) were examined. **Methods** $[Ca^{2+}]_i$ changes in astrocytes were monitored by laser scanning confocal microscopy with the Ca^{2+} sensitive fluorescent probe fluo-3. **Results** After astrocytes were impaired by hypoxia/reoxygenation, H_2O_2 (50 μmol·L^{-1}) or *L*-glutamate (0.25 mmol·L^{-1}), the exogenous glutamate (27 μmol·L^{-1}) could not induce increase of $[Ca^{2+}]_i$, but decrease by (3.3 ± 1.6)%, (81 ± 11)% and (81 ± 7)%, respectively. Pretreatment with *Gb*E (10 mg·L^{-1}) could not improve injured astrocytic glutamate response. But after pretreatment with *Gb*E (100 mg·L^{-1}), glutamate-induced $[Ca^{2+}]_i$ elevation of astrocytes after hypoxia/reoxygenation, H_2O_2 or high concentration of *L*-glutamate injury were (135 ± 98)%, (117 ± 93)% and (89 ± 36)%, respectively. Nimodipine (1.6 mg·L^{-1}) could also reverse the abnormal response of astrocytes after different injury. **Conclusion** Hypoxia/reoxygenation, H_2O_2 and high concentration of *L*-glutamate impaired astrocytes′ response to exogenous *L*-glutamate, and then bidirectional communication between astrocytes and neurons could not take place. *Gb*E could improve the abnormal responses and maintain the normal function of astroglical network. These effects support that *Gb*E has potential beneficial actions against brain injury.

Key words: *Gingko biloba* extract; astrocyte; $[Ca^{2+}]_i$; hypoxia/reoxygenation; glutamate; H_2O_2

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银杏叶提取物对低氧复氧、H_2O_2和谷氨酸损伤时谷氨酸引起的大鼠星形胶质细胞$[Ca^{2+}]_i$变化的影响

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摘要:目的 研究银杏叶提取物对低氧复氧、H_2O_2和 *L*谷氨酸损伤时谷氨酸升高大鼠星形胶质细胞$[Ca^{2+}]_i$的影响。方法 钙荧光探针 Fluo-3/AM标记胞浆内游离钙离子,激光扫描共聚焦显微镜测定$[Ca^{2+}]_i$的变化。结果 在低氧复氧、H_2O_2以及高浓度的 *L*谷氨酸损伤后,外源性谷氨酸(27 μmol·L^{-1})均不能引起培养乳大鼠星形胶质细胞正常的$[Ca^{2+}]_i$升高,反而使$[Ca^{2+}]_i$分别降低(3.3 ± 1.6)%,(81 ± 11)%和(81 ± 7)%;损伤前预先给予 *Gb*E(10 mg·L^{-1})不能明显改善星形胶质细胞的谷氨酸反应,但预先给予 *Gb*E(100 mg·L^{-1})后,27 μmol·L^{-1}谷氨酸可使损伤的星形胶质细胞$[Ca^{2+}]_i$分别升高(135 ± 98)%,(117 ± 93)%和(89 ± 36)%。结论 低氧复氧、H_2O_2以及高浓

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度的 *L*谷氨酸均能损伤星形胶质细胞的谷氨酸反应,影响神经细胞与胶质细胞的双向交流。*Gb*E能明显逆转不同损伤后谷氨酸诱导星形胶质细胞[Ca^{2+}]$_i$的异常变化,使星形胶质细胞在不同损伤时能维持正常功能,该作用可能与*Gb*E的脑保护作用有关。

关键词: 银杏叶提取物; 星形胶质细胞; [Ca^{2+}]$_i$; 低氧复氧; 谷氨酸; H_2O_2

There is growing evidence indicating that astrocytes play more active roles in the central nervous system in addition to the simple structural and trophic support for neurons. Astrocytes possess receptors for numerous transmitters such as glutamate, γ-aminobutyric acid (GABA), acetylcholine and so on, and may respond to neuronal activity by elevating [Ca^{2+}]$_i$ which can frequently propagate as waves within the cytoplasm of individual astrocyte and among adjacent astrocytes in confluent cultures[1]. Furthermore, astrocytes may also signal to neurons. Stimuli that increase the [Ca^{2+}]$_i$ in cultured astrocytes cause a Ca^{2+}-dependent release of glutamate from astrocytes that can affect the [Ca^{2+}]$_i$ in adjacent neurons[2,3]. The existence of signaling between astrocytes and the ability of astrocytes to signal to neurons suggest that astrocytes and neurons may function as an interdependent network in which bidirectional communication takes place and Ca^{2+} signaling is one of the major forms of communication among them[1-5].

Ischemia/reperfusion markedly enhances generation of intracellular oxygen free radicals and extracellular glutamate concentration, impairment of mitochondrial function and disruption of Ca^{2+} homeostasis. Ischemia/reperfusion induced high concentration of extracellular glutamate and oxygen free radicals, such as superoxide, hydrogen peroxide and peroxynitrite, have been implicated in the pathology of several neurological disorders[6]. *Ginkgo biloba* extract (*Gb*E) is a standardized mixture of active substances, including 24% flavonoid glycosides and 6% terpenoids, obtained from green leaves of the *G. biloba* tree. Some evidence obtained from animal models indicates that *Gb*E (75 and 100 mg·kg^{-1}·d^{-1} for 10 days) can improve the recovery of postischemic cardiac function in rats and *Gb*E can act as a free radical-scavenger, a potent inhibitor of NO production, and an inhibitor of lipid peroxidation[7,8]. In the present study, we tested whether hypoxia/reoxygenation, H_2O_2 or high concentration *L*-glutamate affect glutamate-induced [Ca^{2+}]$_i$ elevation in cultured cortical astrocytes, and the effects of *Gb*E were investigated in the meantime.

Materials and methods

Cell Cultures　Astrocytes from the cortex of newborn rats were prepared and maintained in culture as previously described[9]. In brief, rapidly dissected tissues were stripped from meninges and collected in Hanks buffered salt solution (HBSS). After trituration with a fire-polished Pasteur pipette, the pellet was digested with 0.125% trypsin for 10 min. The resulting cell suspension was resuspended in Dulbecco′s modified essential medium (DMEM, Gibco-BRL) supplemented with 10% fetal bovine serum (5% CO_2: 95% air, 37 ℃). After the bottom cell (astrocyte) layer became confluent (7 - 9 days), microglial and oligodendroglial were removed by shaking at 200 revolutions per minute for 18 hours. Astrocytes were detached with trypsin and transferred into 60 mm dishes. These first passage cultures became confluent and were used for experiments after 6 or 7 days.

Hypoxia/reoxygenation　The experiments of hypoxia/reoxygenation on astrocytes were performed according to the procedures of Goldberg *et al*[10]. Experimental cells in glucose-free Earle′s solution were placed in the chamber flushed continuously with mixed hypoxia gas (5% CO_2 + 95% N_2) to remove O_2. When P_{O_2} in the chamber was below 0.1% by observing the oxygen depletion indicators, this flushing was stopped. The hypoxia treatment consisted of incubating the cells in airtight O_2-deprived chamber at 37 ℃ for 2 h. Reoxygenation was performed by opening the chamber and subjecting it to normal atmospheric conditions. The cells were then incubated with fresh serum-free medium for 24 h. During hypoxia and reoxygenation, *Gb*E (10 and 100 mg·L^{-1}, Shanghai Xingling Pharmaceuticals Corporation, China) and nimodipine (1.6 mg·L^{-1}, Bayer Pharmaceuticals Corporation, Germany) were added to glucose-free Earle′s solution and fresh serum-free medium, respectively.

Calcium measurements　The ability of glutamate to evoke intracellular Ca^{2+} elevation in astrocytes was monitored by laser scanning confocal microscopy (LSCM, Bio-Tek Meridian Instruments, USA) with the Ca^{2+} sensitive fluorescent probe

fluo-3[11]. Cultures were washed twice with Mg^{2+}-free Earle′s solution and then incubated at 37 ℃ for 45 min with fluo-3/AM (3 mg· L^{-1}). After being washed with Mg^{2+}-free Earle′s solution containing glycin for 3 times, the indicator was allowed to deesterify for 45 min. The astrocytes loaded with fluo-3 were transferred to the stage of LSCM. The fluorescent intensity was detected with 488 nm blue laser for excitation and 530 nm for emission at room temperature. The fluorescent intensity was used to represent $[Ca^{2+}]_i$ changes in the astrocytes. After a 10 min stabilization period, the cells were photographed to obtain baseline images. Then exogenous glutamate (27 μmol· L^{-1}) was added and then serial images were taken in the same cells. The values of glutamate-induced $[Ca^{2+}]_i$ changes were represented by the peak value of the curve.

Statistics Data were expressed as $\bar{x} \pm s$. Statistical analyses were performed by *t*-test. *P* value of less than 0.05 was considered statistically significant.

Results

1 Modifications in astrocyte morphology by different injury

In normal cultured conditions, astrocytes appeared to be the typical star-shaped morphology and cells were most commonly found growing in clusters or small groups. After impaired by hypoxia/reoxygenation, H_2O_2 (50 μmol· L^{-1}) or *L*-glutamate (0.25 mmol· L^{-1}), astrocytes couldn′t connect with each other any more and many cells in the body had been shown to exhibit unusual morphologies. Pretreatment with *Gb*E or nimodipine, some of astrocytes recovered to normal morphologies and could extend towards each other.

2 Effects of *Gb*E on glutamate-induced $[Ca^{2+}]_i$ changes in cultured cortical astrocytes injured by H_2O_2

Experimental cells in serum-free medium were exposed to 50 μmol· L^{-1} H_2O_2 for 8 h[12]. *Gb*E (10 and 100 mg· L^{-1}) was added to serum-free medium at the same time. When a laser scanning confocal microscope (LSCM) was used, the exogenous glutamate-induced changes in $[Ca^{2+}]_i$ of cultured rat cortical astrocytes were observed.

In normal astrocytes, glutamate (27 μmol· L^{-1}) was shown to increase $[Ca^{2+}]_i$ from the resting level significantly ($P < 0.01$). After astrocytes were incubated with H_2O_2, the exogenous glutamate could not induce increase of $[Ca^{2+}]_i$, but a decrease of that. Pretreatment with *Gb*E (10 mg· L^{-1}), 27 μmol· L^{-1} glutamate-induced $[Ca^{2+}]_i$ was still decreasing, but pretreatment with *Gb*E (100 mg· L^{-1}) or nimodipine (1.6 mg· L^{-1}) could reverse the abnormal response of astrocytes injured by H_2O_2 significantly ($P < 0.01$) and there was a significant $[Ca^{2+}]_i$ elevation in injured astrocytes ($P < 0.05$, Figure 1 and Table 1).

3 Effects of *Gb*E on glutamate-induced $[Ca^{2+}]_i$ changes in cultured cortical astrocytes injured by *L*-glutamate

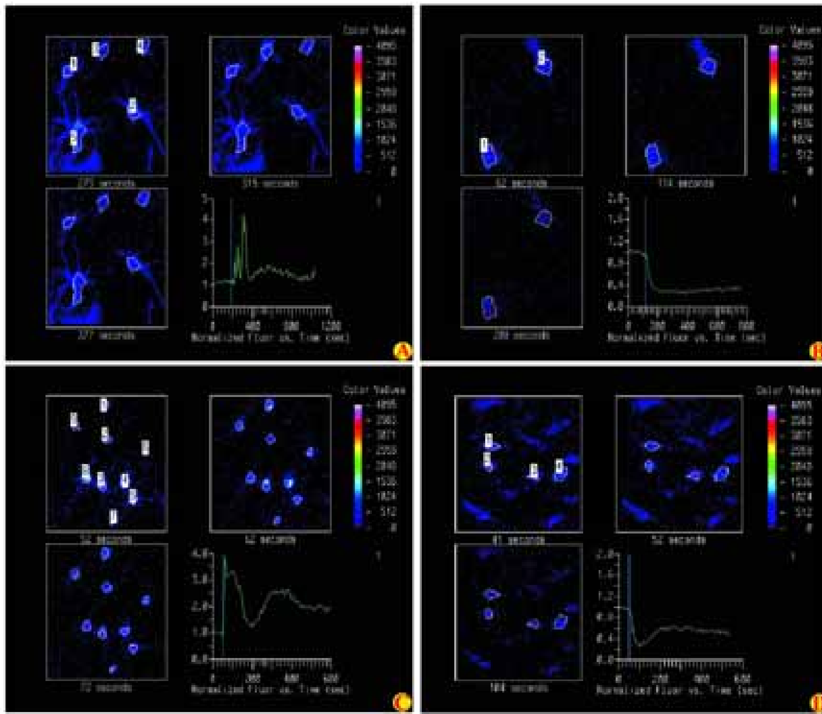
L-Glutamate (0.25 mmol· L^{-1}) was added to experimental cells in Mg^{2+}-free Earle′s solution containing glycin[13]. After 20 min, the cells were incubated with fresh serum-free medium for 24 h. During the courses, *Gb*E (10 and 100 mg· L^{-1}) was added to Mg^{2+}-free Earle′s solution and fresh serum-free medium, respectively.

The exogenous glutamate (27 μmol· L^{-1}) caused a significant $[Ca^{2+}]_i$ elevation in normal astrocytes ($P < 0.01$) and a $[Ca^{2+}]_i$ decrease in the astrocytes injured by 0.25 mmol· L^{-1} *L*-glutamate. Pretreatment with *Gb*E (10 mg· L^{-1}) could not improve the injured astrocytes′ response to exogenous glutamate, but there was a significant $[Ca^{2+}]_i$

Table 1 Effects of *Gb*E on 27 μmol· L^{-1} glutamate induced $[Ca^{2+}]_i$ changes in cultured cortical astrocytes injured by 50 μmol· L^{-1} H_2O_2

Parameter	Control	H_2O_2	Nimodipine	*Gb*E(100 mg· L^{-1})	*Gb*E(10 mg· L^{-1})
FI (Before)	1.02 ±0.04	1.00 ±0.08	1.01 ±0.05	1.07 ±0.05	0.99 ±0.05
FI (After)	4.1 ±1.0**	0.18 ±0.09**	2.4 ±1.0*	2.3 ±1.0*	0.4 ±0.4*
Increasing percentage /%	308 ±112	-81 ±11△△	133 ±103△△▲▲	117 ±93△△▲▲	-70 ±11△△$$

The fluorescent intensity (FI) was used to represent $[Ca^{2+}]_i$ changes. Before: The resting level of $[Ca^{2+}]_i$; After: $[Ca^{2+}]_i$ after adding 27 μmol· L^{-1} glutamate. $n = 5$ experiments, each experiment 4 - 6 cells. $\bar{x} \pm s$. Experimental cells in serum-free medium were exposed to 50 μmol· L^{-1} H_2O_2 for 8 h and *Gb*E (10 and 100 mg· L^{-1}) or nimodipine (1.6 mg· L^{-1}) was added, separately. The astrocytes were loaded with fluo-3/AM (3 mg· L^{-1}) for 45 min at 37 ℃ and 27 μmol· L^{-1} glutamate induced $[Ca^{2+}]_i$ changes were measured. * $P < 0.05$, ** $P < 0.01$ *vs* before; △△ $P < 0.01$ *vs* control (normal cultured condition), ▲▲ $P < 0.01$ *vs* H_2O_2; $$ $P < 0.01$ *vs* *Gb*E (100 mg· L^{-1})



A: Control; B: H_2O_2 (50 μmol· L^{-1}); C: *Gb*E (100 mg· L^{-1}); D: *Gb*E (10 mg· L^{-1})

Figure 1　Effects of *Gb*E on the [Ca^{2+}]$_i$ elevated by exogenous *L*-glutamate (27 μmol· L^{-1}) in cultured cortical astrocytes injured by 50 μmol· L^{-1} H_2O_2. Experimental cells in serum-free medium were exposed to 50 μmol· L^{-1} H_2O_2 for 8 h and *Gb*E (10 and 100 mg· L^{-1}) was added at the same time. After that the astrocytes were loaded with fluo-3/AM (3 mg· L^{-1}) for 45 min at 37 ℃. Glutamate (27 μmol· L^{-1}) was shown to increase [Ca^{2+}]$_i$ from the resting level significantly in normal astrocytes (control group, $P < 0.01$) and decrease [Ca^{2+}]$_i$ in H_2O_2 (50 μmol· L^{-1}) injured astrocytes. Glutamate-induced [Ca^{2+}]$_i$ was still decreasing in *Gb*E (10 mg· L^{-1}) group, but there was a significant glutamate-induced [Ca^{2+}]$_i$ elevation in *Gb*E (100 mg· L^{-1}) group ($P < 0.01$)

Table 2　Effects of *Gb*E on 27 μmol· L^{-1} glutamate induced [Ca^{2+}]$_i$ changes in cultured cortical astrocytes injured by 0.25 mmol· L^{-1} *L*-glutamate

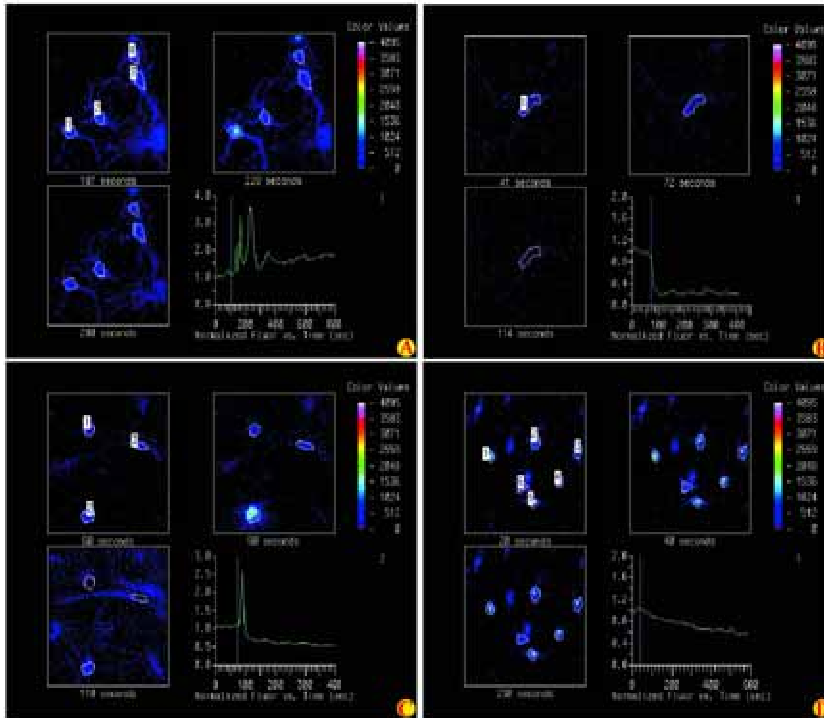
Parameter	Control	*L*-glutamate	Nimodipine	*Gb*E(100 mg· L^{-1})	*Gb*E(10 mg· L^{-1})
FI (Before)	1.24 ± 0.18	1.00 ± 0.04	1.02 ± 0.05	1.12 ± 0.10	1.04 ± 0.03
FI (After)	4.4 ± 1.6**	0.19 ± 0.08**	2.2 ± 0.4*	2.1 ± 0.4*	0.31 ± 0.15**
Increasing percentage /%	247 ± 95	- 81 ± 7△△	115 ± 39△△▲▲	89 ± 36△△▲▲	- 70 ± 16△△$$

The fluorescent intensity (FI) was used to represent [Ca^{2+}]$_i$ changes. Before: The resting level of [Ca^{2+}]$_i$; After: [Ca^{2+}]$_i$ after adding 27 μmol· L^{-1} glutamate. $n = 4$ experiments, each experiment 4 - 6 cells. $\bar{x} \pm s$. Experimental cells in Mg^{2+}-free Earle's solution containing glycin were exposed to *L*-glutamate (0.25 mmol· L^{-1}) for 20 min and *Gb*E (10 and 100 mg· L^{-1}) or nimodipine (1.6 mg· L^{-1}) was added, separately. * $P < 0.05$, ** $P < 0.01$ *vs* before; △△ $P < 0.01$ *vs* control (normal cultured condition); ▲▲ $P < 0.01$ *vs* *L*-glutamate; $$ $P < 0.01$ *vs* *Gb*E (100 mg· L^{-1})

elevation after pretreatment with *Gb*E (100 mg· L^{-1}) or nimodipine (1.6 mg· L^{-1}) ($P < 0.05$, Figure 2 and Table 2).

4　Effects of *Gb*E and hypoxia/reoxygenation on glutamate-induced [Ca^{2+}]$_i$ changes

When exogenous glutamate (27 μmol· L^{-1}) was added, there was a significant decrease of [Ca^{2+}]$_i$ in the astrocytes injured by hypoxia/reoxygenation ($P < 0.01$). Pretreatment with *Gb*E (10 mg· L^{-1}), there was no significant [Ca^{2+}]$_i$ changes induced by glutamate (27 μmol· L^{-1}) in injured astrocytes ($P > 0.05$). Pretreatment with *Gb*E (100 mg· L^{-1}) or nimodipine (1.6 mg· L^{-1}), astrocytic glutamate response was improved and there was a significant [Ca^{2+}]$_i$ elevation in injured astrocytes ($P < 0.01$, Figure 3 and Table 3).



A: Control; B: *L*-Glutamate (0.25 mmol· L^{-1}); C: *Gb*E (100 mg· L^{-1}); D: *Gb*E (10 mg· L^{-1})

Figure 2 Effects of *Gb*E on the [Ca^{2+}]$_i$ elevated by exogenous *L*-glutamate (27 μmol· L^{-1}) in cultured cortical astrocytes injured by 0.25 mmol· L^{-1} *L*-glutamate. Experimental cells in Mg^{2+}-free Earle′s solution containing glycin were exposed to *L*-glutamate (0.25 mmol· L^{-1}) for 20 min and *Gb*E (10 and 100 mg· L^{-1}) was added at the same time. After that the astrocytes were loaded with fluo-3/AM (3 mg· L^{-1}) for 45 min at 37 ℃. Glutamate (27 μmol· L^{-1}) was shown to increase [Ca^{2+}]$_i$ significantly in normal astrocytes (control group, $P<0.01$) and decrease [Ca^{2+}]$_i$ in *L*-glutamate (0.25 mmol· L^{-1}) injured astrocytes. Glutamate-induced [Ca^{2+}]$_i$ was still decreasing in *Gb*E (10 mg· L^{-1}) group, but there was a significant glutamate-induced [Ca^{2+}]$_i$ elevation in *Gb*E (100 mg· L^{-1}) group ($P<0.01$)

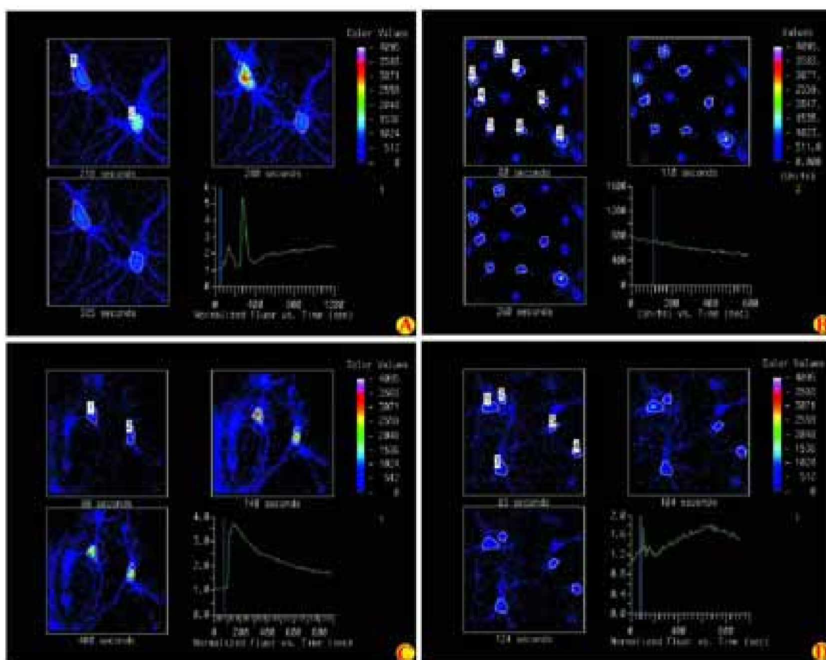
Table 3 Effects of *Gb*E on 27 μmol· L^{-1} glutamate induced [Ca^{2+}]$_i$ changes in cultured cortical astrocytes injured by hypoxia/reoxygenation (H-R)

Parameter	Control	H-R	Nimodipine	*Gb*E (100 mg· L^{-1})	*Gb*E (10 mg· L^{-1})
FI (Before)	1.2 ±0.4	1.02 ±0.03	1.03 ±0.03	1.00 ±0.04	1.03 ±0.05
FI (After)	4.1 ±0.8**	0.99 ±0.19	2.6 ±1.1*	2.4 ±1.0*	1.1 ±0.3
Increasing percentage /%	277 ±107	-3.3 ±1.6$^{\triangle\triangle}$	151 ±108$^{\triangle▲▲}$	135 ±98$^{\triangle▲▲}$	7 ±32$^{\triangle\triangle\$\$}$

The fluorescent intensity (FI) was used to represent [Ca^{2+}]$_i$ changes. Before: The resting level of [Ca^{2+}]$_i$; After: [Ca^{2+}]$_i$ after adding 27 μmol· L^{-1} glutamate. $n=5$ experiments, each experiment 4 - 6 cells. $\bar{x}\pm s$. Experimental cells in glucose-free Earle′s solution were incurred hypoxia for 2 h and then reoxygenation in fresh serum-free medium for 24 h. During hypoxia and reoxygenation, *Gb*E (10 and 100 mg· L^{-1}) or nimodipine (1.6 mg· L^{-1}) was added, separately. $^{*}P<0.05$, $^{**}P<0.01$ *vs* before; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$ *vs* control (normal cultured condition); $^{▲▲}P<0.01$ *vs* H-R; $^{\$\$}P<0.01$ *vs* *Gb*E (100 mg· L^{-1})

Discussion

The brain is particularly vulnerable to oxygen free radicals, which have long been thought to be important mediators of brain damage related to ischemia-reperfusion. The release of massive amounts of oxygen-free radicals during ischemia-reperfusion can also accelerate glutamate release[14]. In our experiments we found that hypoxia/reoxygenation, 50 μmol· L^{-1} H_2O_2 or high concentration of *L*-glutamate (0.25 mmol· L^{-1}) impaired astrocytes′ response, and extracellular physiological concentration of glutamate could not induce intracellular Ca^{2+} elevation in astrocytes. Thapsigargin incubation, microinjection of the Ca^{2+} chelator BAPTA, and photolysis of the Ca^{2+}



A: Control; B: Hypoxia/reoxygenation; C: *Gb*E (100 mg· L^{-1}); D: *Gb*E (10 mg· L^{-1})

Figure 3 Effects of *Gb*E on the $[Ca^{2+}]_i$ elevated by exogenous *L*-glutamate (27 μmol· L^{-1}) in cultured cortical astrocytes injured by hypoxia/reoxygenation. Experimental cells in glucose-free Earle′s solution were incurred hypoxia for 2 h and then reoxygenation in fresh serum-free medium for 24 h. During hypoxia and reoxygenation, *Gb*E (10 and 100 mg· L^{-1}) was added. After that the astrocytes were loaded with fluo-3/AM (3 mg· L^{-1}) for 45 min at 37 ℃. Glutamate (27 μmol· L^{-1}) was shown to increase $[Ca^{2+}]_i$ significantly in normal astrocytes (control group, $P < 0.01$) and decrease $[Ca^{2+}]_i$ in hypoxia/reoxygenation injured astrocytes. There was no significant $[Ca^{2+}]_i$ changes in *Gb*E (10 mg· L^{-1}) group, but there was a significant glutamate-induced $[Ca^{2+}]_i$ elevation in *Gb*E (100 mg· L^{-1}) group ($P < 0.01$)

cage NP-EGTA demonstrate that an intracellular Ca^{2+} elevation in astrocytes is necessary to evoke glutamate release and there is a dose-response relation between $[Ca^{2+}]_i$ elevations and glutamate release[3,15]. So Ca^{2+}-dependent glutamate release is reduced in astrocytes after injury, and then bidirectional communication between astrocytes and neurons can not take place. Deranged glia-to-neuron Ca^{2+} and glutamate signaling eventually leads to reduced viability of the surrounding neurons and may contribute to the secondary amplification of cell injury[16]. The possible mechanisms that glutamate cann′t induce intracellular Ca^{2+} elevation in astrocytes may be that hypoxia/reoxygenation or incubation with H_2O_2 and *L*-glutamate cause a variety of dysfunction of astrocytes such as inhibition of glutamate uptake, mitochondrial hyperpolarization, sarcoplasmic reticulum stores damage or cell apoptosis[17,18].

*Gb*E contains two major groups of active substances, flavonoids and terpenoids. It has been reported that *Gb*E can distinctly decrease the cerebral infarct size, improve the neurological deficits in rat focal cerebral ischemic models and protect against ischemia-induced neuron death and reductions in mitochondrial gene expression[19,20]. The antioxidant activities and/or the peroxynitrite/hydroxyl radical scavenging properties of *Gb*E may be involved in its neuroprotective actions and via their ability to inhibit nitric oxide (NO) synthesis and NO-stimulated PKC activity[21,22]. In this study, pretreatment with *Gb*E was shown to reverse the abnormal response to 27 μmol· L^{-1} glutamate of astrocytes injured by hypoxia/reoxygenation, H_2O_2 or high concentration of *L*-glutamate. Nimodipine which protects the brain from ischemic damage can also improve injured astrocytic glutamate response. In ischemic penumbra, neurons become highly vulnerable to spreading depression waves in absence of normal glia operation[23]. So the recovery of astrocytes to response to 27 μmol· L^{-1} glutamate might facilitate the maintaining of astroglical network and neurons functional recovery. These effects of *Gb*E support its potential beneficial actions against oxidative stress induced brain injury.

References

[1] Cornell-Bell AH, Finkbeiner SM, Cooper MS, *et al*. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling [J]. *Science*, 1990, **247** (4941): 470 - 473.

[2] Parpura V, Basarky TA, Liu F, *et al*. Glumate-mediated astrocyte-neuron signaling [J]. *Nature*, 1994, **369** (6483): 744 - 747.

[3] Parpura V, Haydon PG. Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons [J]. *Proc Natl Acad Sci USA*, 2000, **97** (15): 8629 - 8634.

[4] Bezzi P, Carmignoto G, Pasti L, *et al*. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes [J]. *Nature*, 1998, **391**(6664): 281 - 285.

[5] Duffy S, MacVicar BA. Adrenergic calcium signaling in astrocyte networks within the hippocampal slice [J]. *J Neurosci*, 1995, **15**(8): 5535 - 5550.

[6] Limbrick DD, Pal S, DeLorenzo RJ. Hippocampal neurons exhibit both persistent Ca^{2+} influx and impaiment of Ca^{2+} sequestration/extrusion mechanisms following excitotoxic glutamate exposure [J]. *Brain Res*, 2001, **89**(4): 56 - 67.

[7] Clostre F. *Ginkgo biloba* extract (EGb 761). State of knowledge in the dawn of the year 2000 [J]. *Ann Pharm Fr*, 1999, **57**(Suppl 1): 1S8 - 88.

[8] Varga E, Bodi A, Ferdinandy P, *et al*. The protective effect of EGb 761 in isolated ischemic/reperfused rat hearts: a link between cardiac function and nitric oxide production [J]. *J Cardiovasc Pharmacol*, 1999, **34**(5): 711 - 717.

[9] Naus CCG, Bechberger JF, Zhang YC, *et al*. Altered gap junctional communication, intercellular signaling and growth in cultured astrocytes deficient in connexin43 [J]. *J Neurosci Res*, 1997, **49**(5): 528 - 540.

[10] Goldberg WJ, Connor JR, Bernstein JJ. Glial fibrillary acidic protein immunohistochemistry of spinal cord astrocytes after induction of ischemia or anoxia in culture [J]. *J Neurosci Res*, 1987, **17**(2): 168 - 175.

[11] Segal M, Manor D. Confocal microscopic imaging of $[Ca^{2+}]_i$ in cultured rat hippocampal neurons following exposure to *N*-methyl-*D*-aspartate [J]. *J Physiol*, 1992, **448**: 655 - 676.

[12] Gotz ME, Ahlbom E, Zhivotovsky B, *et al*. Radical scavenging compound J811 inhibits hydrogen peroxide-induced death of cerebellar granule cells [J]. *J Neurosci Res*, 1999, **56**(4): 420 - 426.

[13] Atlante A, Calissano P, Bobba A, *et al*. Glutamate neurotoxiticity, oxidative stress and mitochondria [J]. *FEBS Lett*, 2001, **497**(1): 1 - 5.

[14] Martnez AD, Sáez JC. Regulation of astrocyte gap junctions by hypoxia-reoxygenation [J]. *Brain Res Rev*, 2000, **32**(1): 250 - 258.

[15] Araque A, Sanzgiri RP, Parpura V, *et al*. Calcium elevation in astrocytes cause an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons [J]. *J Neurosci*, 1998, **18**(17): 6822 - 6829.

[16] Bezzi P, Volterra A. A neuron-glia signalling network in the active brain [J]. *Curr Opin Neurobiol*, 2001, **11**(3): 387 - 394.

[17] Fauconneau B, Petegnief V, Sanfeliu C, *et al*. Induction of heat shock proteins by sodium arsenite in cultured astrocytes and reduction of hydrogen peroxide-induced cell death [J]. *J Neurochem*, 2002, **83**(6): 1338 - 1348.

[18] Smith IF, Boyle JP, Plant LD, *et al*. Hypoxic remodeling of Ca^{2+} stores in type I cortical astrocytes [J]. *J Biol Chem*, 2003, **278**(7): 4875 - 4881.

[19] Zhang Y, Gu D, Mao S, *et al*. Protective effects of *Ginkgo biloba* extract on focal cerebral ischemia and thrombogenesis of carotid artery in rats [J]. *Acta Pharm Sin* (药学学报), 1998, **33**(12): 901 - 905.

[20] Chandrasekaran K, Mehrabian Z, Spinnewyn B. Neuroprotective effects of bilobalide, a component of the *Ginkgo biloba* extract (EGb 761), in gerbil global brain ischemia [J]. *Brain Res*, 2001, **922**(2): 282 - 292.

[21] Bastianetto S, Zheng WH, Quirion R. The *Ginkgo biloba* extract (EGb 761) protects and rescues hippocampal cells against nitric oxide-induced toxicity: involvement of its flavonoid constituents and protein kinase C [J]. *J Neurochem*, 2000, **74**(6): 2268 - 2277.

[22] Calapai G, Crupi A, Firenzuoli F, *et al*. Neuroprotective effects of *Ginkgo biloba* extract in brain ischemia are mediated by inhibition of nitric oxide synthesis [J]. *Life Sci*, 2000, **67**(22): 2673 - 2683.

[23] Largo C, Cuevas P, Herreras O. Is glia disfunction the initial cause of neuronal death in ischemic penumbra? [J]. *Neurol Res*, 1996, **18**(5): 445 - 448.