JPP
Journal of
Pharmacy and Pharmacology

JPP 2005, 57: 1019–1025
© 2005 The Authors
Received January 25, 2005
Accepted April 18, 2005
DOI 10.1211/0022357056622
ISSN 0022-3573

Protective effects of baicalin on oxygen/glucose deprivation- and NMDA-induced injuries in rat hippocampal slices

Lu-Ying Liu, Er-Qing Wei, Yan-Min Zhao, Fang-Xue Chen, Meng-Ling Wang, Wei-Ping Zhang and Zhong Chen

Department of Pharmacology, School of Medicine, Zhejiang University, 353, Yan An Road, Hangzhou 310031, P.R. of China

Lu-Ying Liu, Er-Qing Wei, Yan-Min Zhao, Fang-Xue Chen, Meng-Ling Wang, Wei-Ping Zhang, Zhong Chen

Correspondence: E.-Q. Wei, Department of Pharmacology, School of Medicine, Zhejiang University, 353, Yan An Road, Hangzhou 310031, P.R. of China. E-mail: weieq2001@yahoo.com

Funding: This study was supported by the grant 30271498 (E.-Q. Wei) from the National Natural Science Foundation of China.

Abstract

Baicalin is a flavonoid derivative from *Scutellaria baicalensis* Georgi with various pharmacological effects. Recently, the neuroprotective effect of baicalin was reported. To confirm this effect and explore the possible mechanism, we have investigated the protective effect of baicalin on ischaemic-like or excitotoxic injury and the activation of protein kinase Cα (PKCα) in rat hippocampal slices. In-vitro ischaemic-like injury was induced by oxygen/glucose deprivation (OGD) and the excitotoxic injury by *N*-methyl-D-aspartate (NMDA). The viability and swelling of the slices were detected by triphenyltetrazolium chloride (TTC) staining and image analysis of light transmittance (LT), respectively. The translocation of PKCα was measured by immunoblotting. Baicalin was added during both injuries. Baicalin (0.1, 1, and 10 μmol L^{-1}) concentration-dependently inhibited OGD-induced viability reduction and acute neuron swelling, and inhibited the increased portion of PKCα present in the membrane fraction over the total PKCα. Baicalin ameliorated NMDA-induced viability reduction (not LT elevation) and inhibited the NMDA-increased membrane portion of PKCα at 1 μmol L^{-1}. We concluded that baicalin had a protective effect on ischaemic-like or excitotoxic injury in rat hippocampal slices, which might have been partly related to inhibition of PKCα translocation.

Introduction

Baicalin is one of the predominant flavonoid derivatives isolated from the dry roots of *Scutellaria baicalensis* Georgi (Huangqin). The effects of baicalin include free radical scavenging, antioxidant (Gao et al 1999; Shieh et al 2000), anti-inflammatory (Lin & Shieh 1996; Krakauer et al 2001; Chou et al 2003), antiviral (Li et al 1993; Wu et al 1995; Wu et al 2001) and vasodilatory effects (Duarte et al 1993) in peripheral organs or tissues. Recently, it has been reported that baicalin has a protective effect against cerebral ischaemia or other brain injuries. For example, baicalin inhibited the elevation of cerebral glutamate and aspartate induced by focal cerebral ischaemic injury in rats (Li et al 2003), and protected primarily cultured rat neurons against glutamate/*N*-methyl-D-aspartate (NMDA)- or glucose deprivation-induced injury (Lee et al 2003). However, the effect of baicalin against in-vitro ischaemic injury, such as oxygen/glucose deprivation (OGD), has not been studied.

OGD in rat hippocampal slices has been used widely as an in-vitro ischaemic model. During OGD, excitotoxicity leads to neuronal necrosis or apoptosis in ischaemic-vulnerable brain regions, mainly in the CA1 area of hippocampus (Hyrc et al 1997; Ientile et al 2001). In the early phase of the ischaemic injury, glutamate accumulation in the extracellular space and its receptor activation, especially the NMDA receptor, cause Na^{+}, Ca^{2+} and water influx to the cell, contributing to brain swelling (Mrsulja et al 1990). For assessing cell swelling, imaging light transmittance (LT) in brain slices has been developed (Mrsulja et al 1990).

In addition, the transduction pathways are activated in the ischaemic neuron injury. In these pathways, the protein kinase C (PKC) family is a typical class of key enzymes. It has been reported that in the hippocampal neurons only PKCγ and PKCα

responded to glutamate treatment out of the expressed isoforms. PKCγ was translocated to cytoplasmic, organelle-like structures, while PKCα redistributed to the plasma membrane and into the nucleus (Buchner et al 1999), which may play a more important role in acute and delayed cell death during ischaemia and excitoxicity. However, whether baicalin affects excitotoxicity and translocation of PKCα during in-vitro ischaemic injury remains unknown.

Therefore, to determine the protective effect of baicalin on ischaemic-like or excitotoxic injury and the possible mechanisms in rat hippocampal slices, we induced slice injury by OGD or NMDA, and observed the translocation of PKCα after injury. Ketamine, an NMDA receptor antagonist, was used as a positive control.

Materials and Methods

Reagents and chemicals

Baicalin (with purity > 95%) was kindly gifted by Professor Yong-Zhou Hu (Department of Medicinal Chemistry, School of Pharmaceutical Science, Zhejiang University, China). The compound was dissolved in dimethyl sulfoxide and diluted with normal artificial cerebrospinal fluid (nACSF) to 0.1, 1, or 10 μmol L^{-1} during the experiment. Ketamine was purchased from Shanghai Bio-Chem Co. (Shanghai, China) and diluted with nACSF to 10 μmol L^{-1}. NMDA was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA); rabbit polyclonal antibody against PKCα and goat anti-rabbit IgG linked to horseradish peroxidase (IgG-HRP) were from Zhongshan Biotech Co. (Beijing, China); and enchanced chemiluminescence (ECL) kit was from Renaissance, New England Nuclear-Dupont (Stevenage, UK).

Preparation of hippocampal slices

Male Sprague–Dawley rats (250–300 g, Certificate No 2001001, Experimental Animal Center of Zhejiang Academy of Medical Sciences) were used in this study. All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. After pentobarbital anaesthesia (40 mg kg^{-1}, i.p.), the rats were decapitated, and the brains were rapidly removed and hemisected. Each hippocampus was placed in cold nACSF (composed of (mmol L^{-1}): NaCl 124, KCl 4.9, $MgSO_4$ 1.3, $CaCl_2$ 2, KH_2PO_4 1.2, $NaHCO_3$ 25.6, glucose 10), and was serially cut along the sepotemporal axis to prepare 400 μm-thick slices using a tissue slicer. Slices were pre-incubated on a nylon net in the wells of an interface-style holding chamber thoroughly wetted with nACSF perfusion at 1 mL min^{-1} at 23–24°C, and gassed with warmed humidified 95% O_2 + 5% CO_2 (500 mL min^{-1}). After 60–90-min incubation, the slices were transferred to the image recording chamber.

OGD or NMDA injury

OGD was performed by changing nACSF to a glucose-free ACSF (NaCl 124, KCl 4.9, $MgSO_4$ 1.3, $CaCl_2$ 2, KH_2PO_4 1.2, $NaHCO_3$ 25.6, sucrose 10 (in mmol L^{-1}) at 35°C, pH 7.4) and gassed with a mixture of 95% N_2 + 5% CO_2 for 10 min, followed by 60-min reperfusion with oxygenated nACSF. NMDA injury was induced by 100 μmol L^{-1} NMDA in nACSF for 10 min, followed by 60-min reperfusion. Baicalin (0.1, 1 or 10 μmol L^{-1}) and ketamine (10 μmol L^{-1}) were added from 20 min before OGD or NMDA treatment to the end of the 60-min reperfusion.

Triphenyltetrazolium chloride (TTC) staining

To assess the slice viability, some slices were pre-incubated in 30 mL nACSF gassed with 95% O_2 + 5% CO_2 for 60 min, and then were treated with OGD or NMDA for 10 min followed by reperfusion for 60 min. At the end of the reperfusion, slices were stained with 0.2% TTC solution (w/v) for 30 min at 35°C. The slices were transferred to vials and weighed carefully. A TTC red product, formazan, in the slices was extracted by dimethyl sulfoxide at 4°C overnight. The absorbance at 490 nm of formazan was detected by Universal Microplate Recorder. The results were reported as absorbance per g tissue (A_{490} g^{-1}).

Light transmittance imaging

To real-timely assess the slice injury, LT changes were measured according to the reported method (Andrew et al 1996; Polischuk & Andrew 1996; Joshi & Andrew 2001). Individual slices were transferred to an image recording chamber and perfused with oxygenated nACSF at a rate of 1 mL min^{-1} at 35°C. Slices were transilluminated upward by a halogen lamp (100 V, 12 W) with a voltage-regulated power supply (Beijing Focus Instrument Co., China). The intrinsic optical signal of the slice was observed under a dissecting microscope (XTJ-3600, Shanghai Optical Instrument Import & Export Co., China) by a Panasonic (WV-CP240) charge-coupled device camera that connected to a Turbo TV (version 1.5, Integrated Micro Solutions). Typically, three frames were averaged for each image. The first acquired image served as a control (T_{con}), which was then subtracted from all subsequent images (T_{exp}) after acquisition with Adobe Photoshop (version 7.0). The data were presented as the percentage change of LT during and after OGD or NMDA injury. LT change (%) in the hippocampal CA1 stratum radiatum (RAD) region was calculated as the digital intensity of the subtracted image (T_{exp}–T_{con}) divided by the digital intensity of the control image (T_{con}) of that series:

$$LT = (T_{exp} - T_{con})/T_{con} \times 100\% = \Delta T/T\% \quad (1)$$

Histopathological assessment

After the experiment, hippocampal slices were fixed overnight in 10% buffered formaldehyde, then placed in 30% sucrose at 4°C for 24 h. The 10 μm-thick sections were cut

and stained with cresyl violet, and the morphology of neurons in the CA1 pyramidal layers was observed.

Protein preparation and immunoblotting

The treatment of slices was similar to that in TTC staining. At the end of reperfusion, the slices were homogenized in 100 vols (100 × wet weight of the slice) ice-cold 10 mmol L^{-1} Tris-HCl buffer containing sucrose 320 mmol L^{-1} at pH 7.4. The homogenate was centrifuged at 700 *g* for 10 min at 4°C. The supernatant was transferred to another tube, and centrifuged at 37 000 *g*, 4°C for 40 min. The second supernatant was used as the cytoplasm fraction, and the pellet was resuspended in 10 mmol L^{-1} Tris-HCl buffer at pH 7.4 as the membrane fraction. Protein concentration was determined by the Lowry method (Lowry et al 1951).

Protein samples (5 μg protein) of the subcellular fractions were separated by 7.5% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose filters in a Transblotter (BioRad, Richmond, CA). The filters were blocked for 1 h at room temperature with a solution of 10% (w/v) fat-free dry milk dissolved in Tris-buffered saline (TBS: 20 mmol L^{-1} Tris, pH 7.4, 140 mmol L^{-1} NaCl) containing 0.1% Tween-20 (TBST), then incubated with rabbit polyclonal antibody against PKCα (diluted 1:500 with TBST) overnight at 4°C. The filters were then washed (10 min × 3) with TBST and incubated with a goat anti-rabbit IgG-HRP (diluted 1:2000 with TBST) for 2 h at room temperature, followed by repeated washing for 120 min with TBST. Finally, the membranes were reacted with the ECL regents and exposed on an X-ray film. The protein bands on the X-film were quantitatively analysed with a laser densitometer (Ultro Scan XL, Pharmacia LKB Co, Sweden). To test the translocation of PKCα to the membrane fraction (activation), the results were represented as the portion of PKCα present in the membrane fraction over the total PKCα (membrane + cytoplasm fractions).

Statistical analysis

Results are expressed as mean ± s.d. The one-way analysis of variance was made using SPSS (version 11.0). When the analysis of variance manifested a statistical difference, Dunnett's test was applied. $P < 0.05$ was considered to be significant.

Results

Morphological changes of hippocampal CA1 neurons

After 10-min OGD or NMDA treatment and 60-min reperfusion, the neurons in the hippocampal CA1 region lost the distinct nuclear boundary, and had a vesiculated cytoplasm and were notably swollen. There were many holes and gaps in the cell layer, apparently due to the severe swelling seen in many of the neurons. In addition, the orientation of the neurons in the layer was much less uniform. When the hippocampal slices were treated with baicalin 10 μmol L^{-1} (OGD insult) or 1 μmol L^{-1} (NMDA insult), fewer gaps and holes, and better organized neurons were observed in the CA1 cell layer (Figure 1).

TTC staining in hippocampal slices

OGD and NMDA markedly decreased formazan production in the hippocampal slices, respectively ($P < 0.01$, Table 1). OGD insult (decreased by approximately 80%) was more severe than NMDA insult (decreased by approximately 40%). Baicalin (0.1, 1, or 10 μmol L^{-1}) concentration-dependently attenuated the decrease in formazan production induced by OGD ($P < 0.05$ or 0.01, Table 1); the most effective concentration was 10 μmol L^{-1}. Baicalin also attenuated the NMDA-induced injury but the significant effect was only found at 1 μmol L^{-1}. Ketamine (10 μmol L^{-1}) exhibited a protective effect on both injuries ($P < 0.01$, Table 1). Baicalin alone (10 μmol L^{-1}) had no direct effect (Table 1).

LT changes in hippocampal slices

Baicalin (10 μmol L^{-1}) and ketamine (10 μmol L^{-1}) alone did not change LT of hippocampal slices throughout the experiment over 70 min (Figure 2A). During 10-min OGD treatment, the LT in CA1 RAD increased by approximately 8% and peaked at 8–9 min, then decreased to a lower level below the baseline during the 60-min reperfusion (Figure 2B). Baicalin (1 and 10 μmol L^{-1}) inhibited the peak of $\Delta T/T\%$ ($P < 0.05$ or 0.01), but did not alter the peak time and the decrease of $\Delta T/T\%$ at 60 min after reperfusion ($P > 0.05$, Table 2). During 10-min NMDA (100 μmol L^{-1}) treatment, $\Delta T/T\%$ increased earlier and lasted longer compared with OGD treatment (Figure 2C). Baicalin (0.1 and 1 μmol L^{-1}) had a slight but not significant inhibiting effect on the peak of $\Delta T/T\%$ during NMDA treatment, and the high concentration (10 μmol L^{-1}) significantly delayed the peak time and increased $\Delta T/T\%$ after the 60-min reperfusion ($P < 0.01$, Table 2). Ketamine (10 μmol L^{-1}) effectively decreased the peak $\Delta T/T\%$ during both OGD and NMDA treatments and reversed the decreased $\Delta T/T\%$ after 60-min reperfusion, but only delayed the peak time after NMDA treatment ($P < 0.05$ or 0.01, Table 2).

Translocation of PKCα in hippocampal slices

After OGD or NMDA treatment, PKCα translocated to the membrane fraction (Figure 3), as the portion of PKCα present in the membrane fraction over the total PKCα increased by 25.8% or 44.2% (Table 3). Baicalin (0.1–10 μmol L^{-1}) inhibited OGD-induced PKCα translocation ($P < 0.01$). After NMDA-induced injury, baicalin 1 μmol L^{-1} had the same inhibiting effect, but

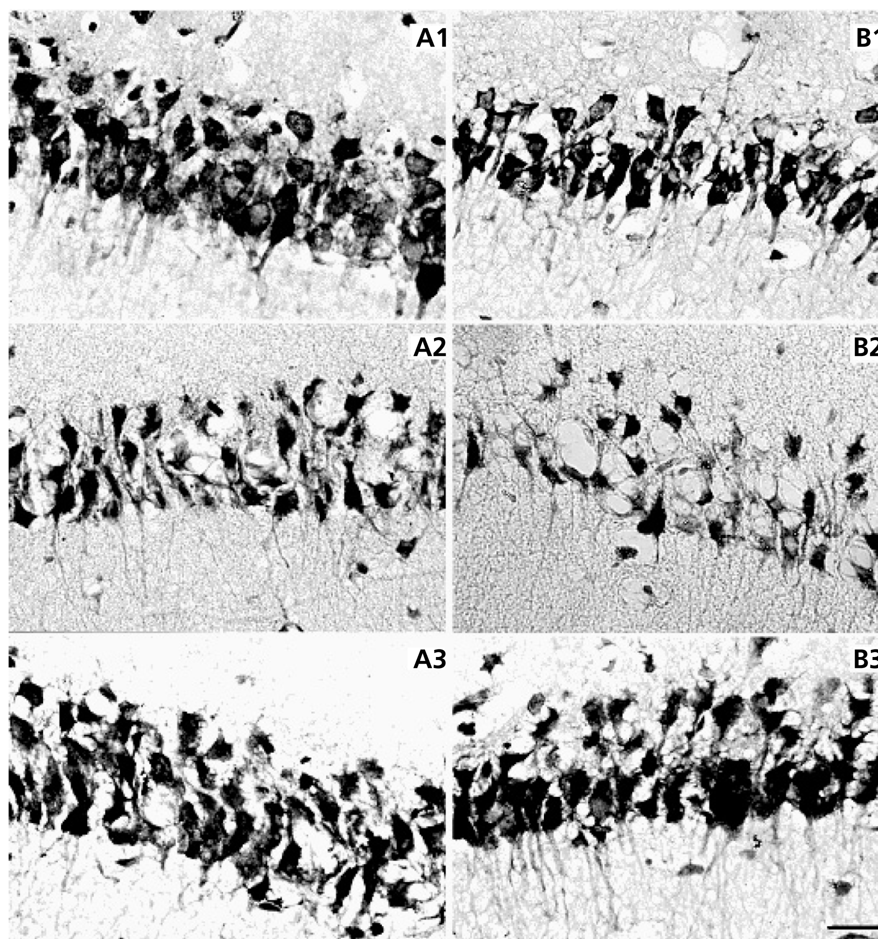

Figure 1 Histopathologic changes of the rat hippocampal CA1 region after oxygen/glucose deprivation (OGD) (A) or *N*-methyl-D-aspartate (NMDA; B) injury followed by 60-min reperfusion. 1: normal control; 2: OGD or NMDA treatment; 3: treated with baicalin (10 μmol L^{-1} for OGD and 1 μmol L^{-1} for NMDA injury). Sections were stained with cresyl violet, and bar = 25 μm.

Table 1 Effects of baicalin and ketamine on formazan production ($A_{490}\,g^{-1}$) after oxygen/glucose deprivation (OGD)- or *N*-methyl-D-aspartate (NMDA)-induced injury

Treatment (μmol L^{-1})	n	OGD	n	NMDA
Normal	16	47.1 ± 11.8	24	45.8 ± 8.7
Baicalin alone 10	4	53.4 ± 8.2	12	46.2 ± 9.9
Injury	9	9.93 ± 2.5**	24	28.4 ± 8.9**
Injury + baicalin 0.1	7	17.2 ± 1.0**##	12	34.5 ± 14.1**
1	6	19.7 ± 5.0**#	18	37.5 ± 11.9*##
10	7	28.7 ± 6.5**##	16	26.7 ± 8.8**
Injury + ketamine 10	4	42.7 ± 2.5##	7	41.5 ± 12.7##

*$P < 0.05$, **$P < 0.01$ compared with normal; #$P < 0.05$, ##$P < 0.01$ compared with OGD or NMDA treatment alone.

0.1 or 10 μmol L^{-1} had no significant effect. Baicalin 10 μmol L^{-1} alone had no significant effect on translocation of PKCα (Figure 3 and Table 3).

Discussion

Baicalin possessed a protective effect on ischaemic-like or excitotoxic injury in rat hippocampal slices, and this effect might have been partly mediated by inhibition of PKCα activation.

The supporting evidence for this protective effect on ischaemic-like injury is that baicalin inhibited the decreased slice viability in TTC staining (Table 1) and ameliorated morphological damage of CA1 pyramidal neurons (Figure 1) after OGD treatment. Furthermore, baicalin modulated LT changes during OGD and reperfusion injury (Figure 2 and Table 2). Imaging LT has been considered as a real-time method to monitor acute neuronal damage, and the elevated LT provides an indicator of cell swelling in the CA1 region in hippocampal slices (Andrew et al 1999). The higher the level of peak ΔT/T%, the more severe may be the cell swelling induced by OGD or NMDA insult. A delay in the peak ΔT/T% means that the acute cell damage happens more slowly. Thus, the inhibition of the peak (not peak time) of ΔT/T%

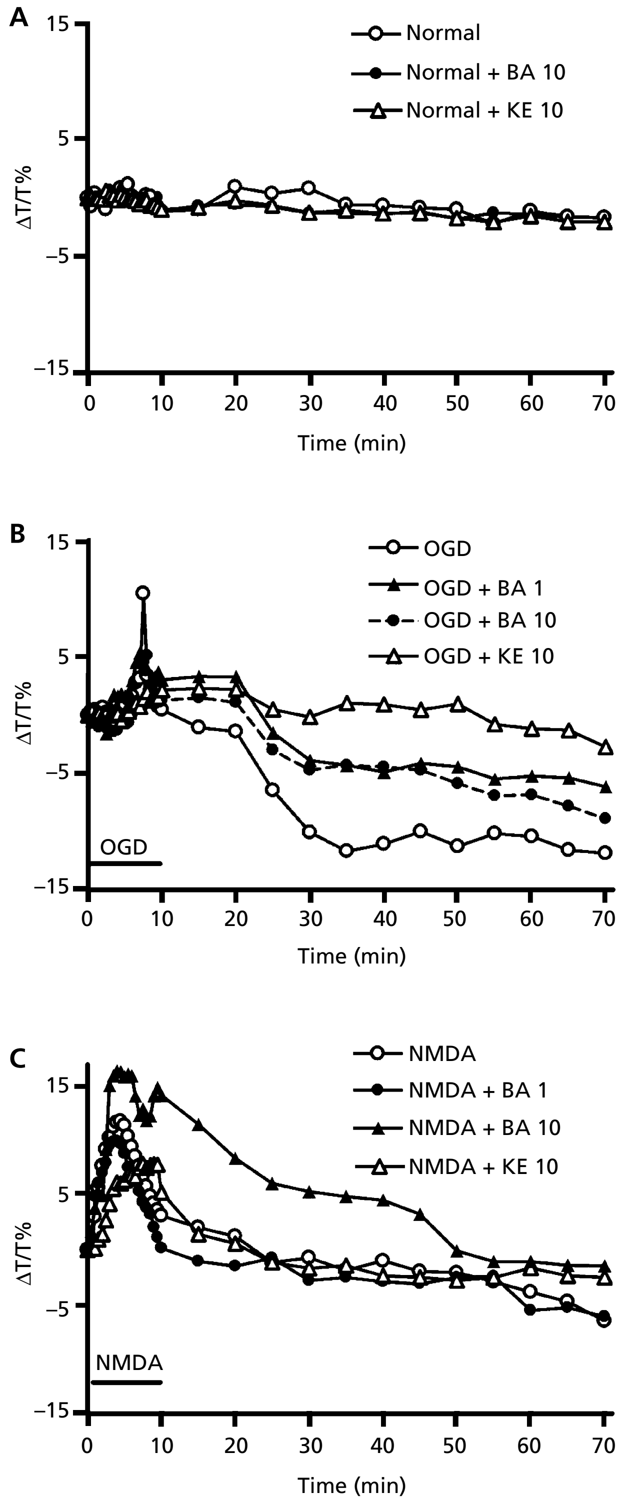

Figure 2 Typical time course of light transmittance in rat hippocampal slices after oxygen/glucose deprivation (OGD) or *N*-methyl-D-aspartate (NMDA) treatment and the effect of baicalin. A, Normal control (without injury); B, OGD treatment (10 min); C, NMDA treatment (100 μmol L^{-1}, 10 min); BA 1, treated with 1 μmol L^{-1} baicalin; BA 10, treated with 10 μmol L^{-1} baicalin; KE 10, treated with 10 μmol L^{-1} ketamine.

Table 2 Effects of baicalin and ketamine on light transmittance changes induced by oxygen/glucose deprivation (OGD) and *N*-methyl-D-aspartate (NMDA; 100 μmol L^{-1}) treatments in rat hippocampal slices

Treatment (μmol L^{-1})	n	Peak ΔT/T%	Peak time (min)	ΔT/T% after 60 min
OGD	21	7.6 ± 2.8	8.9 ± 1.8	−9.4 ± 4.9
OGD + baicalin 0.1	6	5.4 ± 2.6	9.4 ± 1.4	−8.9 ± 5.8
1	6	5.2 ± 2.1*	9.6 ± 1.7	−6.0 ± 2.4
10	6	4.0 ± 2.0**	8.0 ± 1.4	−10.8 ± 3.0
OGD + ketamine 10	6	4.5 ± 1.7**	9.8 ± 0.8	−3.9 ± 1.8##
NMDA	30	13.5 ± 5.5	4.8 ± 1.6	−8.0 ± 4.9
NMDA + baicalin 0.1	9	10.1 ± 3.4	3.8 ± 1.2	−6.7 ± 2.4
1	16	10.9 ± 4.6	3.9 ± 0.9	−6.1 ± 5.4
10	8	16.2 ± 2.8	6.5 ± 1.8##	−0.9 ± 3.3##
NMDA + ketamine 10	5	8.9 ± 1.3#	8.4 ± 2.2##	−2.4 ± 4.1#

*$P < 0.05$, **$P < 0.01$ compared with normal; #$P < 0.05$, ##$P < 0.01$ compared with OGD or NMDA treatment alone.

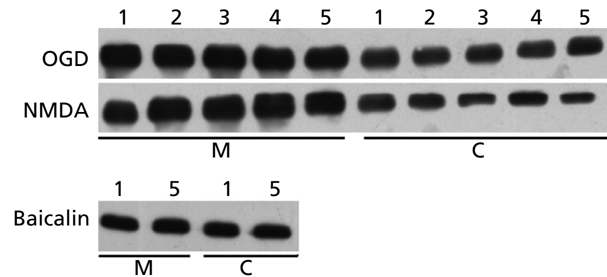

Figure 3 Translocation of PKCα after oxygen/glucose deprivation (OGD) or *N*-methyl-D-aspartate (NMDA) treatment in rat hippocampal slices. 1, Normal; 2, OGD or NMDA injury; 3, treated with 0.1 μmol L^{-1} baicalin; 4, 1 μmol L^{-1} baicalin; 5, 10 μmol L^{-1} baicalin. M, membrane; C, cytoplasm; baicalin, treated with baicalin alone.

Table 3 Effects of baicalin on the portion of PKCα present in the membrane fraction over the total PKCα in hippocampal slices after oxygen/glucose deprivation (OGD) or *N*-methyl-D-aspartate (NMDA) treatment

Treatment (μmol L^{-1})	n	Membrane/ (membrane + cytoplasm) (%)	
		OGD	NMDA
Normal	9	61.3 ± 4.1	44.3 ± 14.4
Baicalin alone 10	4	63.4 ± 1.6	58.4 ± 8.6
Injury	7	77.1 ± 4.0**	63.9 ± 15.0**
Injury + baicalin 0.1	7	68.1 ± 3.7##	52.4 ± 15.7
1	7	69.0 ± 3.8##	47.0 ± 13.3#
10	7	66.2 ± 5.7##	57.7 ± 12.3

**$P < 0.01$ compared with normal; #$P < 0.05$, ##$P < 0.01$ compared with OGD or NMDA treatment alone.

by baicalin implied that baicalin could decrease acute neuron swelling induced by OGD, but not shorten the swelling process. In addition, another acute damage called CA1 dendritic beading will occur and decrease the LT of the slices during reperfusion, which decreases the value of $\Delta T/T\%$ even below the baseline level (Andrew et al 1999). Baicalin, unlike ketamine, did not inhibit LT reduction during reperfusion, suggesting that its protective effect on the dendritic beading was limited.

Since excitotoxicity is one of the major pathological factors after ischaemic injury, we used NMDA to induce excitotoxicity in the experiments. NMDA-induced injury and the inhibition by baicalin in the slices were similar to those of OGD but there were some differences. As the results of the TTC staining assay showed, baicalin (0.1–10 μmol L^{-1}) inhibited OGD insult concentration-dependently, but inhibited NMDA insult only at 1 μmol L^{-1} (Table 1). As the results of the LT image analysis showed, baicalin (0.1–10 μmol L^{-1}) inhibited the LT elevation during OGD, but did not affect peak time and LT reduction after reperfusion. However, baicalin slightly inhibited LT elevation during NMDA treatment, but delayed the peak time and attenuated the LT reduction during reperfusion at a higher concentration (10 μmol L^{-1}) (Table 2 and Figure 2). This result suggested that baicalin mainly inhibited OGD-induced slice swelling (not dendritic beading), and partly inhibited NMDA-induced slice swelling (only delayed) and dendritic beading. Together with the potent effect of NMDA receptor antagonist ketamine, we propose that NMDA receptor activation partly mediated OGD insult, and that the protective effect of baicalin might have been partly related to NMDA receptor function. Baicalin might inhibit glutamate or aspartate release (Li et al 2003), intracellular calcium elevation after NMDA receptor activation (Lee et al 2003), or other unknown events.

In addition, we found that baicalin inhibited PKCα activation after OGD- or NMDA-induced injury as evidenced by the finding of membrane translocation (Table 3 and Figure 3). Although it has been reported that cytosolic PKC was not modulated by baicalin in leucocytes (Shen et al 2003), the translocation, an indicator of its activation, is more important to explain the effect of baicalin. One of the PKCα-dependent processes is the modulation of cell swelling by activation of calcium influx channel and anion (such as Cl^-) channels (Vieira et al 1997; Liu et al 2003), and so inhibition of PKCα translocation might explain the attenuating effect of baicalin on cell swelling in this study. Recent studies have demonstrated that activation of the classic PKC family, including PKCα, can phosphorylate NMDA receptor subunits and enhance NMDA receptor-mediated currents in the first minutes of ischaemia (Cheung et al 2001; Liao et al 2001; Wagey et al 2001; Zablocka et al 2001; Bickler et al 2004). Therefore, baicalin might modulate the function of NMDA receptor indirectly through inhibiting translocation of PKCα, so that the indirect inhibition might be related to the effect of baicalin on OGD insult. In addition to this mechanism, baicalin also regulates various intracellular processes, such as scavenging free radicals (Gao et al 1999; Shieh et al 2000), inhibiting intracellular calcium elevation and nuclear transcription factor (like NF-κB and AP-1) expression (Park et al 2004).

Conclusion

Baicalin had a protective effect on ischaemic-like injury; the effect might have been, at least in part, via inhibiting NMDA receptor function and PKCα activation. Our findings support further the possibility of a treatment for cerebral ischaemia and provide information for future studies of baicalin or other flavonoid derivatives.

References

Andrew, R. D., Adams, J. R., Polischuk, T. M. (1996) Imaging NMDA- and kainate-induced intrinsic optical signals from the hippocampal slice. *J. Neurophysiol.* **76**: 2707–2717

Andrew, R. D., Jarvis, C. R., Obeidat, A. S. (1999) Potential sources of intrinsic optical signals imaged in live brain slices. *Methods* **18**: 185–196

Bickler, P. E., Fahlman, C. S., Ferriero, D. M. (2004) Hypoxia increases calcium flux through cortical neuron glutamate receptors via protein kinase C. *J. Neurochem.* **88**: 878–884

Buchner, K., Adamec, E., Beermann, M. L., Nixon, R. A. (1999) Isoform-specific translocation of protein kinase C following glutamate administration in primary hippocampal neurons. *Brain Res. Mol. Brain Res.* **64**: 222–235

Cheung, H. H., Teves, L., Wallace, M. C., Gurd, J. W. (2001) Increased phosphorylation of the NR1 subunit of the NMDA receptor following cerebral ischemia. *J. Neurochem.* **78**: 1179–1182

Chou, T. C., Chang, L. P., Li, C. Y., Wong, C. S., Yang, S. P. (2003) The antiinflammatory and analgesic effects of baicalin in carrageenan-evoked thermal hyperalgesia. *Anesth. Analg.* **97**: 1724–1729

Duarte, J., Perez-Vizcaino, F., Zarzuelo, A., Jimenez, J., Tamargo, J. (1993) Vasodilator effects of quercetin in isolated rat vascular smooth muscle. *Eur. J. Pharmacol.* **239**: 1–7

Gao, Z., Huang, K., Yang, X., Xu, H. (1999) Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of Scutellaria baicalensis Georgi. *Biochim. Biophys. Acta* **1472**: 643–650

Hyrc, K., Handran, S. D., Rothman, S. M., Goldberg, M. P. (1997) Ionized intracellular calcium concentration predicts excitotoxic neuronal death: observations with low-affinity fluorescent calcium indicators. *J. Neurosci.* **17**: 6669–6677

Ientile, R., Macaione, V., Teletta, M., Pedale, S., Torre, V., Macaione, S. (2001) Apoptosis and necrosis occurring in excitotoxic cell death in isolated chick embryo retina. *J. Neurochem.* **79**: 71–78

Joshi, I., Andrew, R. D. (2001) Imaging anoxic depolarization during ischemia-like conditions in the mouse hemi-brain slice. *J. Neurophysiol.* **85**: 414–424

Krakauer, T., Li, B. Q., Young, H. A. (2001) The flavonoid baicalin inhibits superantigen-induced inflammatory cytokines and chemokines. *FEBS. Let.* **500**: 52–55

Lee, H. H., Yang, L. L., Wang, C. C., Hu, S. Y., Chang, S. F., Lee, Y. H. (2003) Differential effects of natural polyphenols on neuronal survival in primary cultured central neurons against glutamate- and glucose deprivation-induced neuronal death. *Brain Res.* **986**: 103–113

Li, B. Q., Fu, T., Yan, Y. D., Baylor, N. W., Ruscetti, F. W., Kung, H. F. (1993) Inhibition of HIV infection by baicalin – a flavonoid compound purified from Chinese herbal medicine. *Cell Mol. Biol. Res.* **39**: 119–124

Li, H., Wang, H., Chen, J. H., Wang, L. H., Zhang, H. S., Fan, Y. (2003) Determination of amino acid neurotransmitters in cerebral cortex of rats administered with baicalin prior to cerebral ischemia by capillary electrophoresis-laser-induced fluorescence detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **788**: 93–101

Liao, G. Y., Wagner, D. A., Hsu, M. H., Leonard, J. P. (2001) Evidence for direct protein kinase-C mediated modulation of N-methyl-D-aspartate receptor current. *Mol. Pharmacol.* **59**: 960–964

Lin, C. C., Shieh, D. E. (1996) The anti-inflammatory activity of Scutellaria rivularis extracts and its active components, baicalin, baicalein and wogonin. *Am. J. Chin. Med.* **24**: 31–36

Liu, X., Zhang, M. I., Peterson, L. B., O'Neil, R. G. (2003) Osmomechanical stress selectively regulates translocation of protein kinase C isoforms. *FEBS. Lett.* **538**: 101–106

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275

Mrsulja, B. B., Stanimirovic, D., Micic, D. V., Spatz, M. (1990) Excitatory amino acid receptors, oxido-reductive processes and brain oedema following transient ischaemia in gerbils. *Acta Neurochir. (Wien)* **51** (Suppl.): 180–182

Park, H. J., Lee, Y. W., Lee, S. K. (2004) Baicalin induces NAD(P)H:quinone reductase through the transactivation of AP-1 and NF-kappaB in Hepa 1c1c7 cells. *Eur. J. Cancer Prev.* **13**: 521–528

Polischuk, T. M., Andrew, R. D. (1996) Real-time imaging of intrinsic optical signals during early excitotoxicity evoked by domoic acid in the rat hippocampal slice. *Can. J. Physiol. Pharmacol.* **74**: 712–722

Shen, Y. C., Chiou, W. F., Chou, Y. C., Chen, C. F. (2003) Mechanisms in mediating the anti-inflammatory effects of baicalin and baicalein in human leukocytes. *Eur. J. Pharmacol.* **465**: 171–181

Shieh, D. E., Liu, L. T., Lin, C. C. (2000) Antioxidant and free radical scavenging effects of baicalein, baicalin and wogonin. *Anticancer Res.* **20**: 2861–2865

Vieira, L. L., Lafuente, E., Blum, J., Cabantchik, Z. I. (1997) Modulation of the swelling-activated amino acid channel of Leishmania major promastigotes by protein kinases. *Mol. Biochem. Parasitol.* **90**: 449–461

Wagey, R., Hu, J., Pelech, S. L., Raymond, L. A., Krieger, C. (2001) Modulation of NMDA-mediated excitotoxicity by protein kinase C. *J. Neurochem.* **78**: 715–726

Wu, J. A., Attele, A. S., Zhang, L., Yuan, C. S. (2001) Anti-HIV activity of medicinal herbs: usage and potential development. *Am. J. Chin. Med.* **29**: 69–81

Wu, X., Akatsu, H., Okada, H. (1995) Apoptosis of HIV-infected cells following treatment with Sho-Saiko-to and its components. *Jpn. J. Med. Sci. Biol.* **48**: 79–87

Zablocka, B., Gajkowska, B., Czechmanska, T., Domanska-Janik, K. (2001) Isoforms of protein kinase C in postsynaptic densities after cerebral ischemia. *Brain Res.* **889**: 105–111