Protective Effects of Berberine on Oxygen-Glucose Deprivation/Reperfusion on Oligodendrocyte Cell Line (OLN-93)

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Original Article

ABSTRACT

Background: Oligodendrocytes, the myelinating glial cells of central nervous system, are highly vulnerable to ischemic-induced excitotoxic insult, a phenomenon in which calcium overload triggers cell death. Berberine is an alkaloid extracted from medicinal herbs as Coptidis Rhizoma with several pharmacological effects like inhibition of neuronal apoptosis in cerebral ischemia.

Methods: We examined the effects of berberine (0.5-4 μM) and glutamate receptors antagonists (MK-801 [10 μM] and NBQX [30 μM]) on OLN-93 cell line (a permanent immature rat oligodendrocyte) during (30, 60, 240 min) oxygen-glucose deprivation (OGD)/24 h reperfusion. The cells were cultured in 12-well plates. The cells were exposed to glucose-free medium and hypoxia in a small anaerobic chamber. Cell viability was evaluated by MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay. The intracellular calcium levels also were evaluated by Ca^{2+}-sensitive indicator Fura-2/AM in presence or absence of berberine (2 μM) during 30 min chemical OGD by NaN3 (20 mM). Student's *t*-test and ANOVA were used for statistical analysis.

Results: Berberine, MK-801and NBQX significantly increased oligodendrocyte viability in all 3 time-scheduled oxygen-glucose deprivation/reperfusion. Berberine at 2 μM produced peak of protection, and increased cell viability to 83%, 77%, and 79% during 30, 60, 240 min ischemic experiments, respectively ($P < 0.001$). Berberine significantly attenuated intracellular Ca^{2+} rise induced by chemical ischemia, and this effect of berberine was significantly stronger than MK-801 and NBQX ($P < 0.001$).

Conclusions: We concluded that berberine protected OLN-93 oligodendrocyte against ischemic induced excitotoxic injury. Attenuation of intracellular Ca^{2+} overload by berberine may be the key mechanism that saved OLN-93 from excitotoxicity damage.

Keywords: Berberine, calcium, excitotoxicity, OLN-93 cell line, oxygen-glucose deprivation

INTRODUCTION

Oligodendrocytes have a vital function in formation and maintenance of axonal myelin sheet.[1,2] Oligodendrocyte

injury and myelin loss are principal pathological characteristics of demyelinating diseases such as multiple sclerosis (MS) which is a chronic degenerative central nervous system (CNS) disease.[3,4] Nowadays, the relevance of primary and/or secondary alterations in glutamate signaling leading to excitotoxic oligodendrocyte damage to MS is accepted.[5-7] In addition, excitotoxic injury to oligodendrocyte progenitors due to hypoxic ischemia is thought to contribute to pathogenesis of myelination disturbances in periventricular leukomalacia, which is the cerebral white matter damage in the premature infant.[8]

Excitotoxicity is a phenomenon, which is characterized by overactivation of glutamate receptors (GluRs); this event elicits cell death.[2,9] Excitotoxicity was explained in the late 1950s in retinal neurons for the 1st time.[2] Later, Olney *et al*. discovered that excitotoxic vulnerability existed in all central neurons that contained GluRs.[2] Since then, glutamate excitotoxicity has been described in both acute injury to the CNS and in chronic neurodegenerative disorders.[2]

Based on earlier findings, there are two types of ionotropic GluRs: N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxaz ole propionic acid (AMPA)/kainate (KA) receptors.[10,11] NMDA receptors have the main role in glutamate toxicity in neural cells; in addition, AMPA/kainate receptors contribute in neuronal excitotoxicity.[2] AMPA and kainate receptors are commonly present in astrocytes, oligodendrocytes and microglia.[2] While NMDA receptors are expressed in clusters on oligodendrocyte processes, AMPA and kainate receptors are diffusely located on oligodendrocyte somata.[12]

Ca^{2+} overload, during excitotoxicity process, can trigger cell death in both neuronal and glial cells.[2] In fact, Ca^{2+} overload mainly occurs through NMDA receptor, but the molecular properties of AMPA/kainate receptors in oligodendrocytes, (lack of GlutR2 subunit in AMPA receptors, and low spread of GlutR6 subunit in kainate receptors), result in more permeability to Ca^{2+} in these cells, so it makes them more susceptible to excitotoxicity insult.[2,12]

OLN-93 cell line derived from spontaneously transformed cells in primary rat brain glial cultures.[13] The OLN-93 cells in their morphological features and their antigenic properties resemble 5-10 days old (postnatal time) cultured rat brain oligodendrocytes.[13] According to the developmental stages of oligodendroglial cells, the OLN-93 cell line is identified as immature oligodendrocyte.[14,15]

In traditional, Chinese–Korean–Japanese medicine, some plants such as *Berberis* sp., *Coptidis* sp. rhizome, and *Phellodendri* sp. cortex; have been used to treat various inflammatory diseases.[16] Berberine is the major alkaloid extracted from these medicinal herbs.[16] Berberine has several pharmacological actions, including anti-inflammatory, antipyretic, anti-diarrheal, hypoglycemic, hypocholesterolemic, antidepressant, and Alzheimer's disease-amelioration effect.[16] Recently, it was reported that berbery extract reduced neuronal damage in the gerbil hippocampus after transient forebrain ischemia.[16]

In this study, we evaluated the effects of berberine on OLN-93 oligodendrocytes during oxygen-glucose deprivation/reperfusion (OGD/R), which is a reliable model of ischemia and excitotoxicity. Besides, intracellular calcium levels were measured as an important factor in excitotoxic phenomenon and its involvement in ischemic induced injury.

METHODS

Materials

Berberine was obtained from Sigma Chemical Co. (St. Louis, MO, USA). The stock solution was prepared at 1 mmol/l in deionized water and kept at –20°C. Dulbecco's Modified Eagle's medium (DMEM) with glutamine, glucose/glutamine-free DMEM, fetal bovine serum (FBS) were purchased from GIBCO. Penicillin-streptomycin, 3-[4,5-dime thylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), poly-L-lysine, dizocilpine (MK-801), NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f] quinoxaline-2,3-dione), and fura 2-AM were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals are of the purest grade available from regular commercial sources.

Cell culture

OLN-93 cell line was purchased from Pasteur Institute of Iran (Tehran, Iran). The cells were grown in DMEM medium supplemented with 10% FBS and 100 IU/ml penicillin and 100 μg/ml

streptomycin in a humidified 95% air and 5% CO_2 incubator at 37°C. The cells were subcultured twice a week by gentle scraping and cultured on poly-L-lysine-coated 12-well plates at a density of 5×10^4. Culture dishes were coated with poly-L-lysine 24 h before the experiment. Poly-L-lysine (150,000-300,000 MW) was dissolved in deionized water and the plate wells were filled with this solution (20 μg/ml). After 5 min standing at room temperature, the solution was aspirated and the plates were left to dry in a laminar flow hood overnight. Cells were used for experiments 24 h after seeding.

Oxygen-glucose deprivation and drug exposure

Procedures for Oxygen-glucose deprivation (OGD) were performed as described previously.[17] Briefly, the culture medium was replaced with glucose/glutamine-free DMEM, and cells were exposed to hypoxia for 30, 60, and 240 min in a small anaerobic chamber previously filled with 95% (v/v) N_2 and 5% (v/v) CO_2 at 37°C. To terminate the OGD, the chamber was opened and the medium was replaced with DMEM, and the cultures were then placed in an incubator with 5% CO_2 for 24 h. To examine the drug effects, cell cultures were treated 3 h before OGD with berberine, at concentrations of 0.5-4 μM. The preincubation time of 3 h was selected based on the previous studies.[18,19] These concentrations were chosen based on the results of preliminary experiments at nontoxic levels of berberine (data not shown). To investigate the effects of various inhibitors on OGD-induced cell death, MK-801, a noncompetitive antagonist of the NMDA receptor or NBQX, an AMPA/kainate receptor antagonist, was added to the medium 3 h before OGD.[19,20] All measurements were duplicated and each experiment was repeated at least 3 times. Every data point is therefore the mean of at least six measurements.

Analysis of cell viability

Oligodendroglial cell viability was measured using the colorimetric MTT assay, as previously described by Mosmann.[21] Briefly, cells were incubated with 0.5 mg/ml MTT in DMEM, at 37°C under 5% CO_2, for 3 h. The blue formazan reduction product, produced by the action of succinate dehydrogenase in living cells on the dye, was dissolved in 100 μl DMSO, and the optical density was read at 570 nm using a Dynex MMX microplate reader (Dynex, Richfield, MN, USA). Data were expressed as the percentage of viable cells in OGD-exposed plates compared with control normoxic plates determined by MTT reduction.

Measurement of intracellular free calcium

Measurement of intracellular free calcium concentration was performed using a Ca^{2+}-sensitive indicator Fura 2-AM with an Olympus IX-71 inverted microscope and CCD camera. Fluorescence emission images of intracellular Fura-2 at 510 nm, after excitation at two different 340 and 380 nm were acquired. Ratiometrically analysis of image pairs was carried out using ImageJ from the National Institute of Health (NIH), Bethesda, Maryland, USA.[22]

OLN-93 oligodendrocytes were preincubated with Fura-2 AM (5 μM) at room temperature. Incubation was continued for a further 15 min at 37°C.[22] Cells were placed in a flow-chamber and washed with isotonic buffer containing (in mM): 128 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES, pH 7.4 for 10 min.[23,24] The flow rate was 0.5 ml/min and the temperature was kept on 37°C through all the process. Chemical OGD was started by flow of sodium azide (NaN_3: 20 mM) in glucose-free isotonic buffer for 30 min.[25-27] The concentration of sodium azide (20 mM) was selected based on the study by Marino *et al*.[27] Our experiments showed that this concentration was producing the acceptable elevation of intracellular calcium in OLN-93 cells during 30 min-chemical OGD (data not shown). Images were taken for 4-min-durations along with 10 min-pauses at start, middle and the end of 30 min chemical OGD. Berberine and other pharmacologic agents were dissolved in NaN_3 (20 mM) to compare the alteration of intracellular calcium levels in presence and absence of drugs during 30 min chemical OGD.

Statistical analysis

All results are expressed as means ± standard deviation, the significance of differences was evaluated with Student's *t*-test by Office Excel 2007 from Microsoft Inc., 1 Microsoft Way, Redmond, WA, USA. For multiple comparisons, we used one-way ANOVA (Tukey's *post-hoc*) by

SPSS 16 from International Business Machines Corporation, 1 New Orchard Road, Armonk, New York 10504-1722, USA. $P < 0.05$ was considered as statistically significant.

RESULTS

The effects of berberine, on oxygen-glucose deprivation/reperfusion-induced cell injury in OLN-93 cells

We examined the effect of berberine (0.5-4 μM) on 30, 60, and 240 min OGD followed by 24 h reperfusion in OLN-93 cultures.

MTT assay demonstrated that cell viability decreased to 53%, 46%, and 35% during 30, 60, and 240 min OGD/R, respectively. Pretreatment of cultures with berberine (0.5-4 μM), significantly attenuated OGD-induced cell injury in OLN-93 cells in a concentration-dependent manner (ANOVA; $P < 0.05$). The peak of protective effect was at 2 μM concentration for berberine through all 3 time-scheduled experiments. At this concentration, berberine increased cell viability to 83%, 77%, and 79% during 30, 60, and 240 min OGD/R, respectively (Student's *t*-test, $P < 0.001$) [Figure 1a-c].

The effects of MK-801 and NBQX on oxygen-glucose deprivation-induced cell injury in OLN-93 cells

To evaluate the effects of inhibitors of ionotropic GluRs on OGD/R-induced cell death, MK-801 (10 μM) and NBQX (30 μM) were added to medium 3 h before OGD. Our results demonstrated that MK-801 and NBQX could significantly protect the OLN-93 cells from OGD-induced cell death during all 3 time-scheduled assessments in compare with sham (Student's *t*-test, $P < 0.001$). MK-801 increased cell viability to 78%, 69%, and 66% during 30, 60, and 240 min OGD/R, respectively. In addition, NBQX improved cell viability to 72%, 67%, and 57% during 30, 60, and 240 min OGD/R, respectively [Figure 1a-c].

The effects of berberine, MK-801 and NBQX on intracellular calcium levels during 30 min chemical oxygen-glucose deprivation in OLN-93 cells

To determine the intracellular calcium levels during ischemia, the OLN-93 cells were exposed to sodium azide (NaN_3: 20 mM), as a model of chemical OGD, for 30 min. Sodium azide was able to induce 141% increase in intracellular Ca^{2+} concentration, which was statistically significant compared to the Ca^{2+} levels prior to chemical OGD treatment of OLN-93 cells bathed in Ca^{2+}-containing medium. Furthermore, the ischemia-evoked increase in intracellular Ca^{2+} levels was decreased to 108% by berberine (2 μM), 120% by MK-801 (10 μM) and 124% in the presence of NBQX (30 μM) compared with pre OGD levels. Reduction in intracellular Ca^{2+} levels in presence of berberine and ionotropic GluRs was significant in compared with chemical OGD in absence of these pharmacologic agents [Figure 2].

DISCUSSION

Oligodendrocyte loss is one of the hallmarks of demyelinating diseases, including MS.[2,7,21,28] In this regard, the excitotoxicity phenomenon by its subsequent intracellular calcium overload has been described as a cause of oligodendroglial death.[7,21]

We examined the effect of berberine (0.5-4 μM) on OLN-93 oligodendrocytes during 30, 60, and 240 min OGD followed by 24 h reperfusion. Furthermore, the alteration of intracellular calcium levels during 30 min chemical OGD by NaN_3 (20 mM) in the absence and presence of berberine (2 μM) was evaluated by Fura-2 AM method. The results revealed that 30, 60, and 240 min OGD/R could induce cell death in OLN-93 oligodendrocytes.

The excitotoxicity pathway is initiated by ischemic glutamate release and following activation of ionotropic GluRs and intracellular calcium overload.[29,30] Remarkable distribution of ionotropic GluRs in oligodendrocyte has been revealed by the existence of AMPA/kainate receptors on oligodendroglial somata and NMDA receptors on the oligodendroglial processes.[12,31,32] Therefore, NBQX, the AMPA/kainate receptor antagonist, and MK-801, the selective NMDA receptor antagonist, are two important pharmacologic agents to protect the oligodendrocyte against excitotoxicity-induced cell injury.[30,32] Based on the previous studies; OGD-induced injury to oligodendrocyte processes and somata is Ca^{2+}-dependent, but the route of Ca^{2+} influx into the processes and somata are different.[30,33] Salter and Fern[30] demonstrated

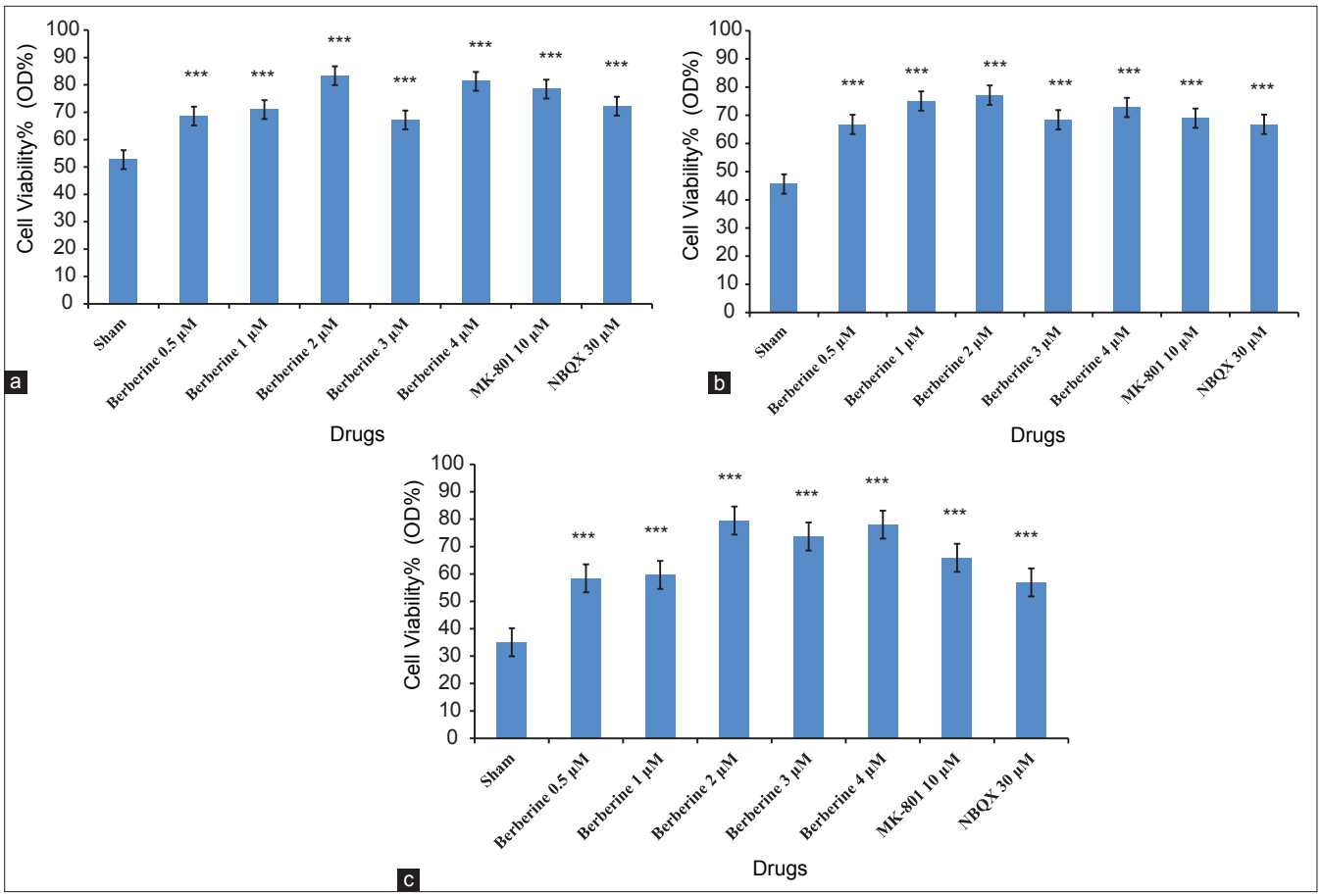

Figure 1: The effects of berberine (0.5-4 µM), and Dizocilpine (MK-801, 10 µM) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX, 30 µM) during (a) 30 min, (b) 60 min, (c) 240 min oxygen-glucose deprivation/24 h reperfusion induced cell injury in OLN-93 cell line (a permanent immature rat oligodendrocyte). The results are presented as mean ± standard deviation (***, $P < 0.001$)

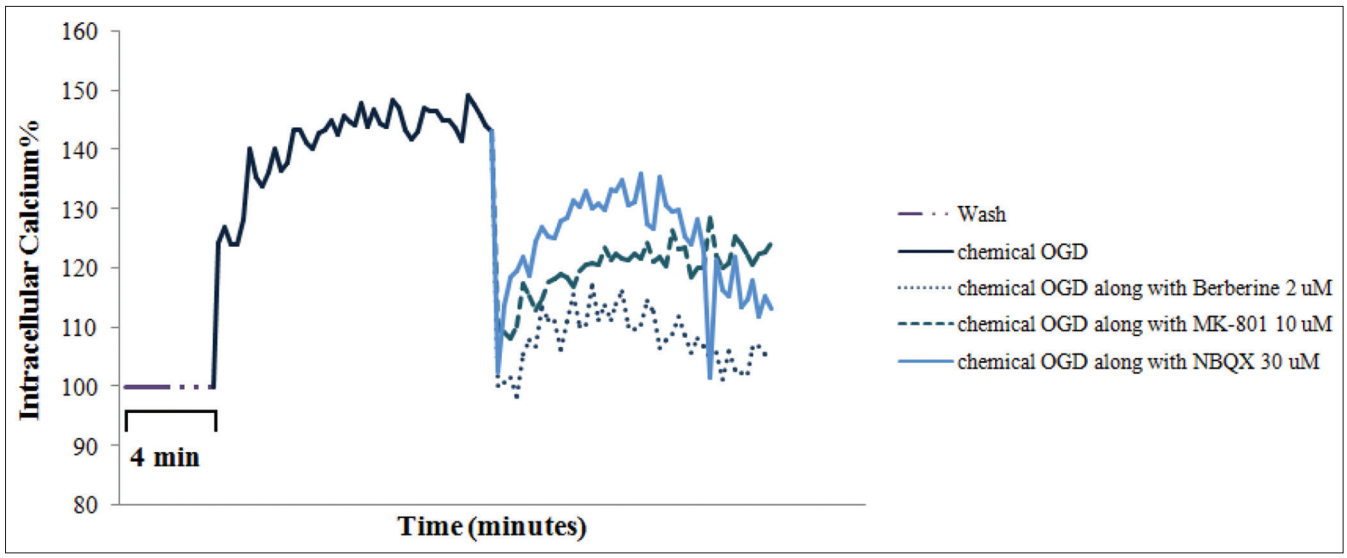

Figure 2: The results based on Fura 2-AM fluorescence ratio (F340 nm/F380 nm) to assess the intracellular Ca^{2+} levels during 30-min chemical oxygen-glucose deprivation by sodium azide (20 mM) in OLN-93 cell line (a permanent immature rat oligodendrocyte). Berberine (2 µM), Dizocilpine (MK-801, 10 µM) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f] quinoxaline-2,3-dione (NBQX, 30 µM) reduced intracellular Ca^{2+} levels significantly ($P < 0.001$)

that using NBQX (30 μM) to block Ca^{2+} influx through AMPA/kainate receptors during OGD had no effect on loss of processes but prevented the loss of oligodendrocyte somata. On the contrary, application of MK-801 (10 μM) could largely prevent OGD-induced injury to processes of oligodendroglial cells.[30] The protective effect of MK-801 occurred by blocking NMDA receptor, which is the route of Ca^{2+} influx into oligodendroglial processes.[30] Our findings revealed that blocking the AMPA/kainate receptors by NBQX (30 μM) as well blocking NMDA receptors by MK-801 (10 μM) could significantly protect the OLN-93 oligodendrocyte from excitotoxicity-induced cell injury during 30, 60, and 240 min-OGD/R trials. According to the developmental stages of oligodendroglial cells, the OLN-93 cell line is located between an oligodendroglial precursor cell and a late immature oligodendrocyte.[14,15,34,35] Thus, the position of ionotropic GluRs in OLN-93 follows other oligodendrocytes; consequently, our results are in accord with Salter and Fern's[30] findings.

The results demonstrated that berberine (0.5-4 μM) could significantly protect the OLN-93 cells during 30, 60, and 240 min OGD/R in a concentration-dependent manner. The peak action of berberine was at 2 μM concentration. This finding is consistent with the earlier experiments about the effects of berberine on neurons. By the way, Zhou *et al*.[36] found the neuroprotective effects of berberine on stroke models *in vitro* and *in vivo*; furthermore, Zhang *et al*.[37] suggested that berberine could regulate neuronal apoptosis in cerebral ischemia, which might be dependent on the degree of cell injury.

Excitotoxicity insult alters Ca^{2+} homeostasis as the cellular Ca^{2+} overload is a trigger for oligodendroglial death.[4,38] Pervious studies have revealed that excitotoxicity in oligodendroglial lineage could be prevented when Ca^{2+}-free medium was used.[30,38] Indeed, there are the evidences that Ca^{2+} entry through AMPA and kainate receptors alone is sufficient to trigger excitotoxicity in oligodendroglial cells, and the Ca^{2+} influx through NMDA receptors causes injury to oligodendrocyte processes.[30,38] Although, the oligodendrocyte processes are too small, NMDA receptors during excitotoxicity may raise intracellular Ca^{2+} to toxic levels within such a confined space.[30] This indicates that sufficient Ca^{2+} influx occurs through the NMDA receptors on processes to result in injury.[30] Because of the important role of Ca^{2+} in excitotoxic cell injury, we examined the intracellular calcium levels in absence and presence of berberine (2 μM) during 30 min-chemical OGD.

Sodium azide (NaN3), the well-established Complex IV inhibitor, was used to induce chemical hypoxia. Complex IV is a part of five complexes (Complex I-Complex V) which construct the mitochondrial respiratory chain; Complex IV defects in acute MS lesions affected oligodendrocytes, astrocytes, and axons.[25] *In vitro* model of brain ischemia by sodium azide increases intracellular Ca^{2+} in neurons.[27] The main source of calcium increase induced by NaN3 is extracellular, involving GluR activation in a first step and calcium channel opening in a second step.[27] Therefore, we used sodium azide in glucose-free isotonic buffer to investigate the intracellular Ca^{2+} overload during chemical OGD in OLN-93 oligodendrocytes.

The results revealed a significant rising in intracellular Ca^{2+} levels in OLN-93 oligodendrocyte due to 30 min-chemical OGD. Berberine (2 μM) could significantly reduce the intracellular Ca^{2+} levels. Furthermore, the antagonists of ionotropic GluRs: MK-801 (10 μM) and NBQX (30 μM) significantly attenuated the intracellular calcium levels during chemical OGD. This finding confirmed the results of MTT assay from 30 min-OGD/R trial. As mentioned above, the elevation of intracellular Ca^{2+} levels is a major cause of excitotoxicity-induced cell death. Therefore, attenuation of the intracellular Ca^{2+} overload by berbeine could be a major factor that protected the ONL-93 oligodendrocytes during OGD-induced excitotoxicity insult.

CONCLUSIONS

Our results demonstrated that berberine could protect OLN-93 oligodendrocyte against excitotoxicity-induced cell injury in a concentration-dependent manner during all 3 time-scheduled OGD/R ischemic models. The cell protection was partly due to prevention of intracellular calcium accumulation. Overall, these findings advocate the results of other studies about therapeutic potential of brebrine in the treatment of neuropathological conditions associated with

ischemic induced excitotoxicity. Furthermore, these observations support the hypothesis that Ca^{2+} current has a critical role during excitotoxicity insult.

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REFERENCES

1. Baumann N, Pham-Dinh D. Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev 2001;81:871-927.
2. Matute C, Alberdi E, Domercq M, Pérez-Cerdá F, Pérez-Samartín A, Sánchez-Gómez MV. The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 2001;24:224-30.
3. Matute C, Alberdi E, Domercq M, Sánchez-Gómez MV, Pérez-Samartín A, Rodríguez-Antigüedad A, *et al*. Excitotoxic damage to white matter. J Anat 2007;210:693-702.
4. Ibarretxe G, Sánchez-Gómez MV, Campos-Esparza MR, Alberdi E, Matute C. Differential oxidative stress in oligodendrocytes and neurons after excitotoxic insults and protection by natural polyphenols. Glia 2006;53:201-11.
5. Deng W, Yue Q, Rosenberg PA, Volpe JJ, Jensen FE. Oligodendrocyte excitotoxicity determined by local glutamate accumulation and mitochondrial function. J Neurochem 2006;98:213-22.
6. Giaume C, Kirchhoff F, Matute C, Reichenbach A, Verkhratsky A. Glia: The fulcrum of brain diseases. Cell Death Differ 2007;14:1324-35.
7. Matute C, Torre I, Pérez-Cerdá F, Pérez-Samartín A, Alberdi E, Etxebarria E, *et al*. P2X(7) receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. J Neurosci 2007;27:9525-33.
8. Deng W, Wang H, Rosenberg PA, Volpe JJ, Jensen FE. Role of metabotropic glutamate receptors in oligodendrocyte excitotoxicity and oxidative stress. Proc Natl Acad Sci U S A 2004;101:7751-6.
9. Rosin C, Bates TE, Skaper SD. Excitatory amino acid induced oligodendrocyte cell death *in vitro*: Receptor-dependent and-independent mechanisms. J Neurochem 2004;90:1173-85.
10. Kolodziejczyk K, Saab AS, Nave KA, Attwell D. Why do oligodendrocyte lineage cells express glutamate receptors? F1000 Biol Rep 2010;2:57.
11. Pitt D, Werner P, Raine CS. Glutamate excitotoxicity in a model of multiple sclerosis. Nat Med 2000;6:67-70.
12. Matute C. Oligodendrocyte NMDA receptors: A novel therapeutic target. Trends Mol Med 2006;12:289-92.
13. Richter-Landsberg C, Heinrich M. OLN-93: A new permanent oligodendroglia cell line derived from primary rat brain glial cultures. J Neurosci Res 1996;45:161-73.
14. Wilkins A, Majed H, Layfield R, Compston A, Chandran S. Oligodendrocytes promote neuronal survival and axonal length by distinct intracellular mechanisms: A novel role for oligodendrocyte-derived glial cell line-derived neurotrophic factor. J Neurosci 2003;23:4967-74.
15. Wejksza K, Rzeski W, Okuno E, Kandefer-Szerszen M, Albrecht J, Turski WA. Demonstration of kynurenine aminotransferases I and II and characterization of kynurenic acid synthesis in oligodendrocyte cell line (OLN-93). Neurochem Res 2005;30:963-8.
16. Cui HS, Matsumoto K, Murakami Y, Hori H, Zhao Q, Obi R. Berberine exerts neuroprotective actions against *in vitro* ischemia-induced neuronal cell damage in organotypic hippocampal slice cultures: Involvement of B-cell lymphoma 2 phosphorylation suppression. Biol Pharm Bull 2009;32:79-85.
17. Frantseva MV, Carlen PL, El-Beheiry H. A submersion method to induce hypoxic damage in organotypic hippocampal cultures. J Neurosci Methods 1999;89:25-31.
18. Rahbar-Roshandel N, Razavi L, Tavakoli-Far B, Mahmoudian M. Mebudipine and dibudipine protect PC12 cells against oxygen-glucose deprivation and glutamate-induced cell death. Pathophysiology 2008;15:227-31.
19. Tavakoli-Far B, Rahbar-Roshandel N, Rahimi-Moghaddam P, Mahmoudian M. Neuroprotective effects of mebudipine and dibudipine on cerebral oxygen-glucose deprivation/reperfusion injury. Eur J Pharmacol 2009;610:12-7.
20. Takatsuki K, Kawahara S, Takehara K, Kishimoto Y, Kirino Y. Effects of the noncompetitive NMDA receptor antagonist MK-801 on classical eyeblink conditioning in mice. Neuropharmacology 2001;41:618-28.
21. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
22. Ruiz A, Matute C, Alberdi E. Intracellular Ca^{2+} release through ryanodine receptors contributes to AMPA receptor-mediated mitochondrial dysfunction and ER stress in oligodendrocytes. Cell Death Dis 2010;1:e54.
23. Katnik C, Guerrero WR, Pennypacker KR, Herrera Y, Cuevas J. Sigma-1 receptor activation prevents intracellular calcium dysregulation in cortical neurons during *in vitro* ischemia. J Pharmacol Exp Ther 2006;319:1355-65.
24. Barreto-Chang OL, Dolmetsch RE. Calcium imaging of cortical neurons using Fura-2 AM. J Vis Exp 2009;23:e1067

25. Ziabreva I, Campbell G, Rist J, Zambonin J, Rorbach J, Wydro MM, *et al*. Injury and differentiation following inhibition of mitochondrial respiratory chain complex IV in rat oligodendrocytes. Glia 2010;58:1827-37.
26. Wang ZJ, Li GM, Tang WL, Yin M. Neuroprotective effects of stearic acid against toxicity of oxygen/glucose deprivation or glutamate on rat cortical or hippocampal slices. Acta Pharmacol Sin 2006;27:145-50.
27. Marino S, Marani L, Nazzaro C, Beani L, Siniscalchi A. Mechanisms of sodium azide-induced changes in intracellular calcium concentration in rat primary cortical neurons. Neurotoxicology 2007;28:622-9.
28. Follett PL, Deng W, Dai W, Talos DM, Massillon LJ, Rosenberg PA, *et al*. Glutamate receptor-mediated oligodendrocyte toxicity in periventricular leukomalacia: A protective role for topiramate. J Neurosci 2004;24:4412-20.
29. Deng W, Rosenberg PA, Volpe JJ, Jensen FE. Calcium-permeable AMPA/kainate receptors mediate toxicity and preconditioning by oxygen-glucose deprivation in oligodendrocyte precursors. Proc Natl Acad Sci U S A 2003;100:6801-6.
30. Salter MG, Fern R. NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 2005;438:1167-71.
31. Chen H, Kintner DB, Jones M, Matsuda T, Baba A, Kiedrowski L, *et al*. AMPA-mediated excitotoxicity in oligodendrocytes: Role for Na(+)-K(+)-Cl(-) co-transport and reversal of Na(+)/Ca(2+) exchanger. J Neurochem 2007;102:1783-95.
32. Kew JN, Kemp JA. Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology (Berl) 2005;179:4-29.
33. Yoshioka A, Yamaya Y, Saiki S, Kanemoto M, Hirose G, Beesley J, *et al*. Non-N-methyl-D-aspartate glutamate receptors mediate oxygen – Glucose deprivation-induced oligodendroglial injury. Brain Res 2000;854:207-15.
34. Back SA, Luo NL, Borenstein NS, Levine JM, Volpe JJ, Kinney HC. Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. J Neurosci 2001;21:1302-12.
35. Buckinx R, Smolders I, Sahebali S, Janssen D, Smets I, Ameloot M, *et al*. Morphological changes do not reflect biochemical and functional differentiation in OLN-93 oligodendroglial cells. J Neurosci Methods 2009;184:1-9.
36. Zhou XQ, Zeng XN, Kong H, Sun XL. Neuroprotective effects of berberine on stroke models in vitro and in vivo. Neurosci Lett 2008;447:31-6.
37. Zhang Q, Qian Z, Pan L, Li H, Zhu H. Hypoxia-inducible factor 1 mediates the anti-apoptosis of berberine in neurons during hypoxia/ischemia. Acta Physiol Hung 2012;99:311-23.
38. Alberdi E, Sánchez-Gómez MV, Marino A, Matute C. Ca (2+) influx through AMPA or kainate receptors alone is sufficient to initiate excitotoxicity in cultured oligodendrocytes. Neurobiol Dis 2002;9:234-43.

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