Original Research

The Determination of Brain Magnesium and Zinc Levels by a Dual-Probe Microdialysis and Graphite Furnace Atomic Absorption Spectrometry

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Key words: cerebral ischemia, magnesium, zinc, dual-probe, gerbil, microdialysis, GFAAS

Objective: The aim of this study was to develop a microdialysis-graphite furnace atomic absorption spectroscopy (MD-GFAAS) for monitoring dynamic changes of extracellular magnesium (Mg) and zinc (Zn) in the cortex of gerbils subjected to focal cerebral ischemia, that had been produced in anesthetized gerbils by occlusion of the right middle cerebral artery.

Methods: Two microdialysis probes were inserted into both sides of the cortex to simultaneously collect dialysates during cerebral ischemia. Dynamic changes in these analytes, on ipsilateral and contralateral sides of the brain, were assayed by MD-GFAAS. Optimal conditions and analytical precision of GFAAS were studied in the present assay.

Results: The present study demonstrated significant decreases in Mg (65% of baseline) and zinc (74% of baseline) maintained their levels within 3 h on the ipsilateral side of cortex during cerebral ischemia. Slight changes of Mg and Zn on the contralateral sides were also observed.

Conclusion: The derangement of extracellular Mg and Zn could be important in the progression of cell injury and may be associated with cerebral ischemia insult.

INTRODUCTION

Carotid occlusion in Mongolian gerbils has been employed extensively as a model of cerebral ischemia analogous to some forms of human stroke [1,2]. Isolated cerebral hemispheres and incomplete cerebral Willis' circle are unique anatomic features of gerbils [3,4]. Each brain hemisphere seems to have an independent blood supply, thus dual microdialysis probes can be used to monitor dynamic changes in neurochemicals, simultaneously, on ipsilateral and contralateral sides of the brain during cerebral ischemic events. Moreover, damage and metabolic changes on the ipsilateral side can be compared with those on the contralateral side, which being almost intact, can serve as a control [5,6].

Cerebral ischemia results in low oxygen and glucose supply and causes decreased ATP formation [7]. Various ATP-driven membrane-bound pumps or reuptake processes that usually work to keep the homeostasis of important metabolites or ions become retarded. Free magnesium would rise in cells in a poor energy state with less ATP. Previous studies of biochemical processes have mainly been based on whole brain homogenates from experimental animals, and findings obtained using such methods may not be directly applicable to various patterns of cerebral ischemia. There is growing optimism among researchers in the field of cerebral ischemia, as human stroke has at last become treatable and current research efforts delineate several new, potential therapies.

Mg and Zn are widely distributed in plants and animals, but in different concentrations. Mg plays an important role in a variety of fundamental cellular reactions, such as activating

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enzyme in chlorophyll, and modulators of intracellular metabolism [8] in tissues, proteins and membranes. Mg is a cofactor in hundreds of enzymatic reactions and is widely distributed in plants and animals [8]. Mg also modulates ATPases, which have central importance in energy metabolism [9,10]. An inverse association between dietary Mg intake and risk of stroke has been investigated, particularly in men with hypertension [11]. Neuroscientists have recently found that Mg might play an important role in cerebral ischemia [8–11].

The human body contains about 2–2.5 g of Zn; approximately 55% is located in muscle and 30% in bone, with 15% distributed among the other tissues [12,13]. Zn plays an important role in neurobiology. Zn deficiency is harmful to fetal brains. Zn is associated with the developing brain and central nervous system [14]. Zn may modulate nerve conduction in the brain and is also involved in the metabolism of the inhibitory neurotransmitter, gamma aminobutyric acid (GABA) [15].

Detailed mechanisms of the cellular or modulating functions of Mg and Zn require elucidation. Currently, clinical laboratories most frequently measure Mg and Zn levels, predominantly as total concentrations in biological samples, by photometry and atomic spectroscopy. Although determination of extracellular or free Mg concentration is difficult, it is necessary in order to understand the role of Mg in biological, physiological and pathological functions. Earlier technics require improvement with respect to sensitivity, selectivity and other interferences involved with using Mg-selected electrodes. Extracellular changes of metals caused by ischemic insult have not been fully investigated in neuronal tissues.

Microdialysis is widely used for sampling of region chemical substances from the extracellular fluid [16,17]. It is one of the most widely used technics for in vivo sampling of the chemical environment of the brain. The purpose of this present study was to attempt to use dual-probe microdialysis coupled with graphite furnace atomic absorption spectrometry (GFAAS) to investigate dynamic Mg and Zn levels of gerbil during cerebral ischemia.

MATERIALS AND METHODS

Six male gerbils (65–85 g) were allowed to acclimate to their environmentally controlled quarters (25°C and 12:12 h light-dark cycle) before the experiments. The gerbils were anesthetized with chloral hydrate (400 mg/kg body weight, i.p.) and the body temperature was maintained at 37°C with a heating pad (CMA/150). Following a midline incision, the skull was craniectomized to expose the right middle cerebral artery (MCA). An 8-0 suture was encircled the middle cerebral artery for later ligation. Two microdialysis probes (CMA/20-4, and a cut-off at 20 kDa) were stereotaxically implanted into the cortex (AP 0 mm, ML $\pm$ 5 mm, DV - 5.0 mm from Bregma). A focal ischemic lesion was induced by occlusion of the MCA. The dialysis probes were perfused with Ringer's solution at 2 μL/min using a CMA/100 infusion pump. Dialysis probes were perfused with Ringer's solution (147 mM Na^+; 2.2 mM Ca^{++}; 4 mM K^+; pH adjusted to 7.0) at 2 μL/min using a CMA/100 microinfusion pump. Dialysates were collected every 15 min in a CMA/140 fraction collector (Carnegie Medicin, Stockholm, Sweden).

Aliquots of dialysates (2 μL) diluted with water were injected and analysed by a GFAAS (Model Analyst 300, Perkin-Elmer, Uberlingen, Germany) via an auto sampler. All reagents used were of analytical grade and were purchased from E. Merck, all containers were soaked with 20% of nitric acid, rinsed with water and then dried in a clean room for later use. The concentrations of Mg and Zn in dialysates were calculated. The detection limits (signal-to-noise ratio = 5) for Mg and Zn in the present assay were 0.03 and 0.13 ng/mL, respectively.

To meet the challenge for analysing samples with low concentration and small sample size, it is necessary to choose an extremely sensitive technic. In the present study, microdialysis technic was used for collecting dialysates from the ischemic brain of gerbils.

RESULTS

The optimum operating conditions for the MD-GFAAS system were achieved in our previous study [18]. For the atomization of Mg and Zn, the temperature settings were controlled at 1700°C and 1300°C, respectively. In addition, Mg and Zn were sequentially detected at the wavelength of 285.2, and 213.9 nm, respectively.

The analytical performance of the MD-GFAAS system for the determination of extracellular Mg and Zn levels were evaluated in terms of calibration curves, detection limits, and recoveries as shown in Table 1.

The calibration curves of Mg concentrations ranged from 0.50 to 3.00 ng/mL and Zn concentrations ranged from 1.00 to 5.00 ng/mL (correlation coefficient value $>$ 0.995). The detection limits of Mg and Zn concentration were 0.03 ng/mL and 0.13 ng/mL, respectively. The C.V. values of precision of were tested using standard mixtures and pooled dialysate samples containing Mg and Zn ranged from 0.50 to 2.00 ng/mL were less than 3%.

Table 1. Analytical Performance of the On-Line Microdialysis (MD) Coupled with Graphite Furnace Atomic Absorption Spectrometry (GFAAS) System

Metals	Zn	Mg
Linear range (mg/mL)	1.00–5.00	0.50–3.00
Slope	0.1355	0.277
Correlation coefficient	0.999	0.998
Detection limit (mg/mL)	0.13	0.03
Recovery (%) (n = 6)	98	102

DISCUSSION

In general, stable basal levels of Mg and Zn in the cortex were obtained within 1–2 h after probe implantation. The mean concentrations of Mg and Zn in the basal dialysates were 1.71, and 1.80 ng/mL on the contralateral and 1.65, and 1.83 ng/mL on the ipsilateral sides, respectively. During cerebral ischemia, the mean Mg and Zn levels significantly decreased to 65 and 74% of baseline levels, respectively, and maintained their levels within 3 h in the ipsilateral sides whereas slight changes in the contralateral sides were detected shown in Figs. 1 and 2.

The purpose of this study was to employ a dual-probe microdialysis technic coupled with graphite furnace atomic absorption spectroscopy for monitoring dynamic changes of extracellular Mg and Zn in the cortex of gerbils subjected to focal cerebral ischemia. Two microdialysis probes were inserted into both sides of the cortex to simultaneously collect dialysates during cerebral ischemia. Dynamic changes in these analytes, on ipsilateral and contralateral sides of the brain, were easily assayed by MD-GFAAS.

Changes in biological trace metals on the ipsilateral (ischemic) side can be compared to the baseline level and to the simultaneous recording on the contralateral (nonischemic) side. Zn also has been reported to play an important role in neurobiology. Prenatally, the deficiency of Zn is harmful to the baby brain. Zn is associated with the developing brain and central nervous system [13]. Moreover, Zn may modulate nerve conduction in the brain and is also involved in the metabolism of the inhibitory neurotransmitter, gamma aminobutyric acid (GABA) [14].

The present study demonstrated significant decreases in Mg and Zn on the ipsilateral side of the cortex during cerebral ischemia. Slight changes of Mg and Zn on the contralateral sides were demonstrated.

CONCLUSION

The dual-probe microdialysis coupled with GFAAS technique provides advantages in the determinations of trace Mg and Zn levels in extremely small sample volumes and lower concentrations. In this study, the application demonstrates dynamic time profiles of extracellular Mg and Zn levels in the same animal during cerebral ischemia. Taken together, the derangement of extracellular Mg and Zn levels could be important in the progression of cell injury and may be associated with cerebral ischemic insult.

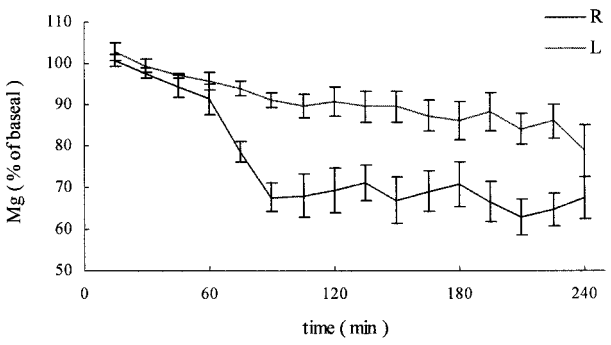

Fig. 1. Time profiles of the changes in Mg levels on the ipsilateral and contralateral gerbil cortex during MCA occlusion. Data are presented as mean ± SEM (n = 6).

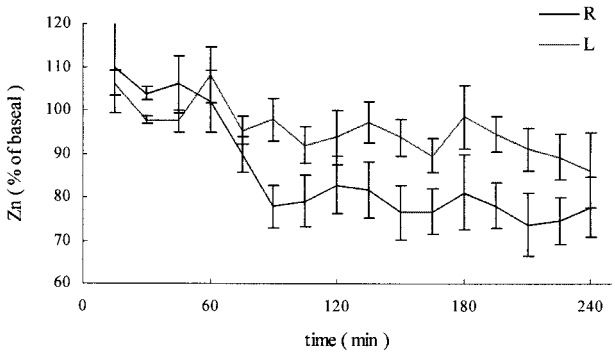

Fig. 2. Time profiles of the changes in Zn levels on the ipsilateral and contralateral gerbil cortex during MCA occlusion. Data are presented as mean ± SEM (n = 6).

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