Annals of Nuclear Medicine Vol. 20, No. 5, 377–380, 2006

Glial metabolic dysfunction caused neural damage by short-term ischemia in brain

Rie HOSOI,* Yuto KASHIWAGI,* Jun HATAZAWA,** Antony GEE*** and Osamu INOUE*

*Course of Allied Health Sciences, Graduate School of Medicine, Osaka University, Osaka, Japan
**Department of Nuclear Medicine and Tracer Kinetics, Osaka University Graduate School of Medicine, Osaka, Japan
***GlaxoSmithkline, Clinical Research Unit, ACCI, Cambridge, UK

Although several pieces of evidence have indicated that glial cells support neuronal cells in the ischemia-reperfusion brain, the direct contribution of glial cells to cell damage is not well known. The present study was designed to determine whether there are any changes in cell damage after a short-term middle cerebral artery occlusion (MCAO) when glial metabolism is suppressed. Injection of fluorocitrate (FC) or 10 minutes MCAO alone did not produce cell damage. However, 10 minutes MCAO in rats pretreated with FC caused significant cell damage. These data directly demonstrated that inhibition of glial metabolism might increase neuronal vulnerability to even a short-term transient ischemia.

Key words: glial cells, ischemia, fluorocitrate, cell damage

INTRODUCTION

BRAIN CONSISTS of heterogeneous cell populations of neurons and glial cells. Glial cells, mainly astrocytes, protect and maintain neuronal viability *in vitro* and *in vivo*. Glial cells are reported to provide structural, trophic, and metabolic support to neurons.[1] Furthermore, glial cells appear to play an important role in the maintenance of the extracellular space.[2] And it has been suggested that glial cells may be involved in brain damage induced by ischemia. A protective role of glial cells has also hypothesized based on the control of some potentially harmful extracellular agents such as excitotoxins and oxidants.

Fluorocitrate (FC) is an inhibitor of aconitase, an enzyme in the TCA cycle. The selectivity of the action of FC arises as it is selectively taken up by astrocytes. FC has been reported, at 1 nmol by local injection *in vivo*, to exert a maximal effect on changes in brain amino acid concentration 4 hours after injection.[3] In the same experimental condition, Hassel et al. reported that FC inhibited amino acid formation selectively in glial cells.[4]

The direct contribution of dysfunction of glial metabolism during ischemia to neuronal cell death is yet unknown. To test the effect of FC on the degree of neuronal cell damage induced by ischemia, in this study, rats were exposed to 10 minutes middle cerebral artery occlusion (MCAO) 4 hours after FC infusion.

MATERIALS AND METHODS

Male Wistar rats (8–9 weeks old) were purchased from Japan Clea (Tokyo, Japan). The rats were housed under a 12-hour, light-dark cycle and allowed free access to food and water. All experiments on the rats were performed with the permission of the Institutional Animal Care and Use Committee, School of Allied Health Sciences, Osaka University.

Surgery and stereotaxic procedures for fluorocitrate (Sigma-Aldrich Co., St. Louis, MO, USA) infusion in rat striatum were carried out as previously described.[5] Fluorocitrate (1 nmol/μl, 0.25 μl/min, 4 min) was infused through a cannula (33-gauge) into the left striatum of each rat while the rat was awake. At the same time, saline solution (1 μl) was infused through a cannula (33-gauge) into the right striatum. After 4 hours of FC infusion, rats

Received November 16, 2005, revision accepted March 27, 2006.

For reprint contact: Rie Hosoi, Ph.D., Course of Allied Health Sciences, Graduate School of Medicine, Osaka University, 1–7 Yamadaoka, Suita, Osaka 565–0871, JAPAN.

E-mail: hosoi@sahs.med.osaka-u.ac.jp

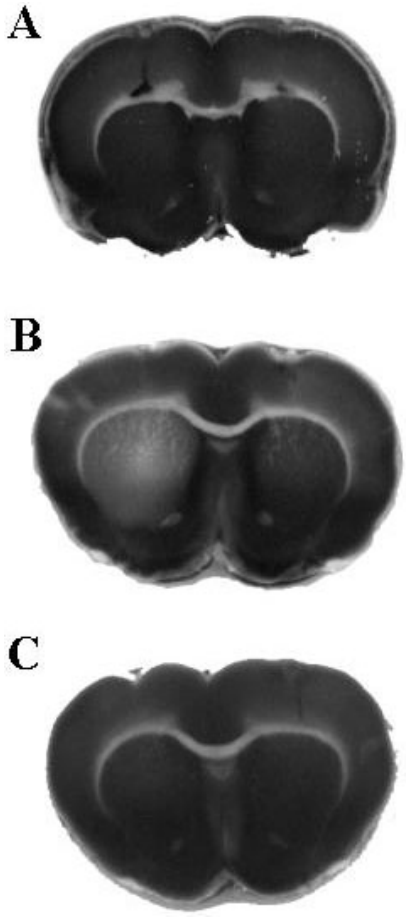

Fig. 1 Photographs of coronal sections in rats (A) treated with 10 minutes of MCAO 4 hours after saline injection; (B) pretreated with FC 4 hours before 10 minutes of MCAO; and (C) treated with FC infusion 4 hours before a sham operation. MCAO was induced in the left side of the images. Rat brains were dissected at 24 hours after MCAO or the sham operation, and then were stained with TTC.

Table 1 Effects of FC treatment and MCAO on the injured area

	number of injured animals/ experimental animals	injured area (mm^2) (mean ± SD)
saline-MCAO	0/10	—
FC-MCAO	13/15	12.4 ± 10.4*
FC-sham	1/10	6.9*

* mean of injured area in rat brains in which cell damage was detected

were prepared for MCAO. Surgery of transient focal cerebral ischemia has been described elsewhere.[6] The animals were initially inhaled with 4.5% halothane with anesthesia then maintained with 2.0% halothane during the operation on a heated mat. After 10 minutes of MCAO, the rats were reanesthetized and the intraluminal suture was removed for reperfusion. The rats were killed by decapitation under a light anesthesia at 24 hours after the reperfusion. The brains were quickly removed, and coronal slices (approximately 3 mm in thickness) were prepared. The slices were subsequently stained with 2,3,5-triphenyltetrazolium chloride (TTC, Wako Pure Chemical Industries, Osaka, Japan; 2% solution in PBS) at 37°C for 30 min. Photographic images of the coronal slices were used to detect brain injury. The cell injury, outlined in white, was measured by image analysis software (Scion Image for Windows).

RESULTS

The effect of FC on cell damage caused by MCAO of rat brain was examined. Rats were subjected to 10 minutes of MCAO 4 hours after FC (1 nmol) infusion. Figure 1 is a representative photograph of coronal brain slices from 24 hours after ischemia/reperfusion of MCAO rats. Animals that received 10 minutes of MCAO had no observable cell damage (Fig. 1-A, Table 1, n = 10). However, in animals pretreated with FC, 10 minutes of MCAO caused a significantly large degree of cell damage in the striatum as measured 24 hours post MCAO (Fig. 1-B, Table 1, n = 15). More than 80% animals showed cell damage, though their injured areas varied from 3.38 to 45.2 mm^2. Rats injected with FC without exposure to MCAO did not exhibit any observable cell damage except in one animal (Fig. 1-C, Table 1, n = 10).

DISCUSSION

The present results directly indicate that impairment of glial function makes neurons vulnerable to MCAO. Ten minutes MCAO itself caused no cell damage in the rat brain after 24 hours. One nmol FC infusion into the striatum resulted in a significant reduction in ^{14}C-acetate uptake throughout the striatum.[5] These results indicated that FC was diffused throughout the striatum. Under the lack of normal glial function caused by the FC injection, even such a short-term MCAO seriously compromised cell survival. As a result of nissle staining of other rat brains treated with FC and ten minutes MCAO treatment, neuronal loss was detected (data not shown). It is an unexpected result that FC injection with sham operation caused cell damage in one rat. In our previous study, FC (1 nmol) injection itself caused no cell damage.[5]

It is widely accepted that glial cells play a general supportive role in maintaining neurons. The importance of toxic extracellular levels of excitatory amino acids, as well as of high extracellular levels of inhibitory amino acids, are considered in relation to the pathophysiology of neuronal cell loss during cerebral ischemia. Such neurotransmitter amino acids, like glutamate, GABA, usually maintain a stable level through metabolic trafficking between neurons and glia. Normally, glucose is an efficient precursor of glutamate and GABA.[7] The carbons from glucose to the neurotransmitter glutamate and the

turnover time of the glutamate pool depend on both the rate of glycolysis and rate of entry glucose carbons into the TCA cycle. Under ischemic condition, the TCA cycle is suppressed because of oxygen decline, and the synthesis by transamination of glutamate from α-ketoglutarate, one of the TCA intermediates, is stopped.[8] Therefore, the glutamine-glutamate cycle between neuronal and glial cells is indispensable to maintain the glutamine-glutamate balance, especially in the ischemic brain. Glutamate uptake by glial cells normally prevents excitotoxic elevation of glutamate levels in the brain extracellular space, and this process appears to be a critical determinant of neuronal survival in the ischemic brain. In rabbit brain, it was reported that at the end of a 10-min ischemic period, glutamate concentration was markedly increased in the extracellular space and the levels returned to normal within 30 minuets of reperfusion.[9] So, lack of glutamate concentration maintenance by glial cells induced by FC injection is the critical factor that makes neurons vulnerable to MCAO.

In addition, glial cells have been postulated to play a role in maintaining extracellular ion concentrations. Previously, it has been shown that glial cells participate in the return of the extracellular potassium concentration ($[K^+]o$) to normal levels after seizures in the rat brain.[10] Exogenously added potassium in the rat brain caused a limited rise in $[K^+]o$ except for a transient peak, whereas after FC treatment, $[K^+]o$ increased and continue to rise following KCl perfusion.[11] Also it is reported that spreading depression (SD) provoked in the neocortex normally does not spread to the CA1, but during FC treatment it readily reached CA1 via the entorhinal cortex.[12] Neuronal injury in the penumbra of an ischemic focus has been attributed to recurrent SD.[13] So, it is easily understood that extension of neuron damage into the ischemic brain might occur due to failure of glial function. Besides potassium ion homeostasis, both intercellular and extracellular acidification, and reduction of the calcium concentration, which fluid is known to increase neuronal excitability,[14] were shown in FC treated rat brain.[11] These losses of sustaining ion homeostasis by glial dysfunction might cause the vulnerability of neurons to ischemia.

Traditionally, high lactate levels in the brain are associated with hypoxia/ischemia and considered deleterious, contributing to neuronal death by acidosis. However, more recently it has been proposed that lactate may serve as an alternative brain fuel to glucose under pathological conditions both *in vitro*[15] and *in vivo*.[16,17] Schurr et al. reported that blockade of lactate transport by alpha-cyano-4-hydroxycinnamate (4-CIN), a monocarboxylate transporter inhibitor, into neurons exacerbates neuronal ischemic damage.[18] A series of observations have led to postulating the existence of a glia-neuron lactate shuttle *in vivo* whereby lactate could be supplied by glial cells to neurons as a supplementary energy substrate. Furthermore, lactate has been shown to prolong the maintenance of electrical function in the glucose-free reperfusion/reoxygenation system.[19] Therefore, it is postulated that the lack of lactate supply is one of the factors that makes neurons vulnerable to ischemia.

The role of glial cells in the protection and maintenance of neurons is not a new concept, though the role of glial cells in neuroprotection is complex and poorly understood *in vivo*. Our study shows a direct link between glial metabolic dysfunction and neuronal death in the MCAO brain. Novel drugs targeted to glial cells may improve the functional performance of glial cells and thereby help minimize the death of neurons during the various phases of recovery from an ischemic attack. It would be interesting to examine this concept in other pathological conditions associated with activation or dysfunction of glial cells, including status epilepticus, Alzheimer's disease and Parkinson's disease.

REFERENCES

1. Chen Y, Swanson RA. Astrocytes and brain injury. *J Cereb Blood Flow Metab* 2003; 23: 137–149.
2. Walz W. Role of astrocytes in the clearance of excess extracellular potassium. *Neurochem Int* 2000; 36: 291–300.
3. Paulsen RE, Contestabile A, Villani L, Fonnum F. An *in vivo* model for studying function of brain tissue temporarily devoid of glial cell metabolism: the use of fluorocitrate. *J Neurochem* 1987; 48: 1377–1385.
4. Hassel B, Paulsen RE, Johnsen A, Fonnum F. Selective inhibition of glial cell metabolism *in vivo* by fluorocitrate. *Brain Res* 1992; 576: 120–124.
5. Hosoi R, Okada M, Hatazawa J, Gee A, Inoue O. Effect of astrocytic energy metabolism depressant on ^{14}C-acetate uptake in intact rat brain. *J Cereb Blood Flow Metab* 2004; 24: 188–190.
6. Abe K, Kashiwagi Y, Tokumura M, Hosoi R, Hatazawa J, Inoue O. Discrepancy between cell injury and benzodiazepine receptor binding after transient middle cerebral artery occlusion in rats. *Synapse* 2004; 53: 234–239.
7. Hassel B, Sonnewald U, Fonnum F. Glial-neuronal interactions as studied by cerebral metabolism of [2-^{13}C]acetate and [1-^{13}C]glucose: an *ex vivo* ^{13}C NMR spectroscopic study. *J Neurochem* 1995; 64: 2773–2782.
8. Haberg A, Qu H, Haraldseth O, Unsgard G, Sonnewald U. *In vivo* injection of [1-^{13}C]glucose and [1,2-^{13}C]acetate combined with *ex vivo* ^{13}C nuclear magnetic resonance spectroscopy: a novel approach to the study of middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab* 1998; 18: 1223–1232.
9. Hagberg H, Lehmann A, Sandberg M, Nystrom B, Jacobson I, Hamberger A. Ischemia-induced shift of inhibitory and excitatory amino acids from intra- to extracellular compartments. *J Cereb Blood Flow Metab* 1985; 5: 413–419.
10. Xiong ZQ, Stringer JL. Astrocytic regulation of the recovery of extracellular potassium after seizures *in vivo*. *Eur J Neurosci* 1999; 11: 1677–1684.
11. Largo C, Cuevas P, Somjen GG, Martin del Rio R, Herreras O. The effect of depressing glial function in rat brain *in situ* on ion homeostasis, synaptic transmission, and neuron

survival. *J Neurosci* 1996; 16: 1219–1229.
12. Largo C, Ibarz JM, Herreras O. Effects of the gliotoxin fluorocitrate on spreading depression and glial membrane potential in rat brain *in situ*. *J Neurophysiol* 1997; 78: 295–307.
13. Iijima T, Mies G, Hossmann KA. Repeated negative DC deflections in rat cortex following middle cerebral artery occlusion are abolished by MK-801: effect on volume of ischemic injury. *J Cereb Blood Flow Metab* 1992; 12: 727–733.
14. Davies G, Peterson DW. Normal extracellular calcium levels block kindled seizures. *Exp Neurol* 1989; 106: 99–101.
15. Schurr A, Payne RS, Miller JJ, Rigor BM. Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: further *in vitro* validation. *J Neurochem* 1997; 69: 423–426.
16. Maran A, Cranston I, Lomas J, Macdonald I, Amiel SA. Protection by lactate of cerebral function during hypoglycaemia. *Lancet* 1994; 343: 16–20.
17. Mendelowitsch A, Ritz MF, Ros J, Langemann H, Gratzl O. 17beta-Estradiol reduces cortical lesion size in the glutamate excitotoxicity model by enhancing extracellular lactate: a new neuroprotective pathway. *Brain Res* 2001; 901: 230–236.
18. Schurr A, Payne RS, Miller JJ, Tseng MT, Rigor BM. Blockade of lactate transport exacerbates delayed neuronal damage in a rat model of cerebral ischemia. *Brain Res* 2001; 895: 268–272.
19. Bock A, Tegtmeier F, Hansen AJ, Holler M. Lactate and postischemic recovery of energy metabolism and electrical activity in the isolated perfused rat brain. *J Neurosurg Anesthesiol* 1993; 5: 94–103.