Effects of Lifarizine and Nimodipine on Forebrain Lactate Levels in a Rat Model of Focal Cerebral Ischemia

Fokal Serebral İskeminin Rat Modelinde Ön Beyin Laktat Seviyeleri Üzerine Lifarizin ve Nimodipin'in Etkileri

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Abstract: ***Objective:*** The effects of lifarizine (RS-87476) and nimodipine on lactate concentrations in ischemic forebrain were evaluated in a rat model of bilateral carotid artery ligation.
Methods: Eighteen rats were randomly assigned to three groups (control, lifarizine treatment, and nimodipine treatment). The animals were anesthetized, the arteries were ligated, and assigned treatments were administered 15 min. later. After 72 hours of assigned treatment and reperfusion, the rats were decapitated and forebrain lactate levels were measured.
Results: The lactate levels in the lifarizine group were significantly lower than control levels ($p<0.05$). The levels in the nimodipine group were lower than control levels, but the difference was not significant ($p>0.05$).
Conclusion: These results suggest that lifarizine is more neuroprotective than nimodipine in the setting of focal cerebral ischemia in the rat.

Key words: focal cerebral ischemia, lifarizine, neuroprotective effect, nimodipine

Özet: ***Amaç:*** Lifarizin ve nimodipin'in ön-beyin iskemisi üzerine etkileri, ratlarda iki taraflı karotis arterlerin bağlanması ile oluşturulan deneysel modelde araştırıldı.
Yöntem: Rasgele seçilmiş 18 rat ile kontrol, lifarizin tedavi ve nimodipin tedavi grupları oluşturuldu. Anestezi edilip arterler balanıp 15 dakika sonra tedavilerine başlandı. Yetmişiki saatlik tedavi ve reperfüzyon sonrasında ratlar dekapite edildi ve ön-beyin laktat ölçümleri yapıldı.
Sonuç: Lifarizin tedavisi grubunda laktat miktarı kontrol grubundan önemli derecede düşük bulunmuştur. ($p<0.05$). Nimodipin grubunda seviyeler kontrol grubuna oranla düşük bulunmuş ancak bu fark anlamlı bulunmamıştır ($p>0.05$).
Yorum: Bu bulgular lifarizin'in nimodipine oranla daha etkili nöroprotektif bir ajan oldugunu düşündürmektedir.

Anahtar kelimeler: fokal serebral iskemi, lifarizin, nöroprotektif etki, nimodipin

INTRODUCTION

Stroke is defined as a sudden development of a focal neurologic deficit due to occlusion of a cerebral vessel, or spontaneous intracranial artery rupture with consequent hemorrhage in the brain parenchyma (4). Recently, a considerable amount of research has focused on pharmacological interventions that might help improve clinical outcome following stroke. Although various treatment modalities are used, there is still no clinically effective treatment for acute stroke (9).

Accumulation of sodium and calcium in the cell is a critical step in the process that leads to neuronal damage. Most pharmacological studies that have investigated treatment of neurological damage due to trauma or ischemia have focused on preventing influxes of calcium using calcium antagonists, such as calcium-channel blockers and N-methyl-D-aspartate (NMDA) receptor antagonists (5,14). In addition to calcium antagonists, some researchers have used sodium-channel blockers to treat cerebral ischemia. Reports have documented that lifarizine, a centrally active neuronal sodium-channel blocker, has neuroprotective efficacy in several in vivo models of focal and global cerebral ischemia, including four-vessel occlusion in the rat (1), and middle cerebral artery occlusion in the cat (6) and mouse (3).

The aim of this study was to compare the effects of this sodium/calcium-channel modulator (lifarizine, RS-87476) and a widely used calcium-channel antagonist (nimodipine) on forebrain lactate levels in the setting of focal ischemia in the rat.

MATERIAL AND METHODS

Eighteen adult Sprague-Dawley rats weighing 330-450 g (mean 385.5 g) were used. Each animal was anesthetized with an intraperitoneal (IP) injection of thiopental sodium (0.1 mg/kg). The femoral artery was cannulated to record mean arterial blood pressure (MABP), and 0.2 ml of heparinized saline (100 mU/ml) was administered. Blood pressure was measured with a pressure transducer (P1000 B, Narcobiosystem) and recorded with a four-channel physiograph (MKIII, Narcobiosystem). Body temperature was monitored with a rectal probe, and was kept between 36.5 °C and 37 °C with a heating pad.

Surgical Procedure

A vertical ventral midline incision was made in the skin of the anterior part of the neck. The left and right common carotid arteries were exposed in the paratracheal region, and were carefully dissected free of the accompanying vagosympathetic nerve trunks. A loose ligature was placed around each of the arteries. The rats were randomly assigned to one of three groups of six. All 18 animals underwent the carotid ligation procedure described above. The control group (Group 1) underwent ligation but received no drug treatment. The nimodipine treatment group (Group 2) received 0.5-mg/kg nimodipine IP twice daily for 3 days. The lifarizine treatment group (Group 3) received 0.5-mg/kg lifarizine IP three times daily for 3 days. After 72 hrs of treatment and reperfusion, each rat was re-anesthetized with thiopental sodium (0.1 mg/kg) IP and then decapitated. The heads were immediately placed in liquid nitrogen. Once they were frozen, each brain was removed and forebrain specimens were preserved in liquid nitrogen to halt enzymatic reactions prior to lactate level analysis. The forebrain specimens were homogenized, and the tissue concentration of lactate was measured by spectrophotometric methods (7,8).

Drug and Solutions

Lifarizine, an analogue of diphenylpiperazine in a 100 mg/ml formulation (RS-87476, which is 1-[Diphenylmethyl]-[4-methyl-(2-4-[methyphenyl] 4-methyl imidazol–5-yl)]; (Syntex®, USA) was prepared as a solution with 5% ethanol and sterile normal saline (0.9% NaCI). Nimodipidine (Nimotop-Bayer®, Germany) was used in 100 mg/ml form. Thiopental sodium USP (PENTOTHAL SODIUM 500 mg, Abbott) was used to anesthetize the rats, and was prepared by diluting with normal saline to a concentration of 0.1 mg/ml.

Statistical Analysis

The lactate and MABP data were statistically evaluated using the student's *t*-test and one-way ANOVA. All group data were expressed as mean±SD. A p value <0.05 was considered statistically significant.

RESULTS

In all three groups, MABP decreased immediately after the carotid arteries were ligated (111±4.52 mmHg before ligation and 81.67±8.16 mmHg after ligation; p>0.05). Table 1 shows the forebrain tissue lactate concentrations for each group. The lactate levels in both the lifarizine and nimodipine treatment groups were lower than the control level. The level in the lifarizine treatment group was significantly lower than that in the

Table 1: The forebrain lactate concentrations for the three study groups (µmol/g). Values are listed as mean±SD. F=5.157, P <0.05

Group	µmol lactate/wet wt
Control	43.03±1.68
Nimodipine	41.65±1.91
Lifarizine	35.20±1.44

controls ($p<0.05$), whereas the lactate level in the nimodipine treatment group was not ($p>0.05$).

DISCUSSION

Many pathophysiological processes in the brain are associated with a rise in tissue lactate content, and one of these is hypoxia. If cellular oxygen falls below the critical level needed for mithochondrial oxidative phosphorylation, the level of the reduced form of nicotinamide adenine dinucleotide-NADH rises. This leads to altered glucose degradation, with pyruvate being reduced to form lactate, instead of decarboxylated to form acetyl-CoA. Thus, lactate levels are considered good indicators of inadequate oxygen supply to tissue (10). Increased lactate production is usually considered to occur with activation of anaerobic glucose metabolism due to inadequate oxygen supply, and the eventual result of lactate build-up is tissue acidosis (10). In our study, the forebrains of the rats that were treated with lifarizine after ischemic injury contained significantly lower lactate levels than the tissues from the control and nimodipine-treated animals.

The tissue's ability to recover after cerebral ischemia may depend on the level of lactic acidosis that develops during an ischemic episode. Even very mild or short-term ischemia causes significant damage to pyramidal neurons in the CA1 and CA4 regions of the hippocampus, and other neuronal and glial cells.

Experimental studies on ischemia have shown that certain kinds of drugs may greatly reduce the tissue injury caused by cerebral infarction. L-type and pre-synaptic (N-type) voltage-regulator calcium-channel antagonists, and anticonvulsants such as barbiturates and phenytoin reduce brain damage when they are administered at the start of or just after an ischemic event. Specifically, the L-type calcium-channel blocker nimodipine prevents contraction of smooth muscle in artery walls; superoxide dismutase, a free radical scavenger, increases the half-life of nitric oxide; and MK-801, an NMDA receptor/channel antagonist, increases cerebral blood flow in ischemic zones (11).

As mentioned above, accumulation of sodium and calcium in the cell is an important step in the pathological process that leads to cell death after cerebral ischemia (5,12,13,14). Lifarizine helps minimize the recurrent depolarization induced by ischemia because it blocks neuronal sodium and calcium channels, thus preventing influxes of sodium and calcium ions into the cell (3). In vitro studies have shown that lifarizine causes allosteric activation of toxin zone 2 in sodium channels. This agent has also been shown to effectively block the sodium current in N1E-115 neuroblastoma cells (9).

Experiments on lifarizine treatment of ischemia caused by occlusion of the middle cerebral artery in cats revealed that this drug reduced the infarct area and cerebral edema, and stabilized energy reserves in the brain tissue (6). Other research on global ischemia caused by four-artery occlusion in rats showed that this agent prevented late neuronal death after 10 min of carotid ligation (1). In a study that involved magnetic resonance imaging, Brown et al. demonstrated that lifarizine treatment for cerebral ischemia reduced the amount of edema that developed, and also protected against striatal damage despite insufficient collateral blood supply to this brain area. The authors' conclusion was that lifarizine is effective at preventing neuronal death in the CA1 region after bilateral carotid occlusion in the brains of rats and other rodents (2). Blockage of sodium channels in neuronal tissues is a effective mechanism for reducing neuron damage after cerebral ischemia (3).

This is the first study that has investigated how lifarizine effects lactate levels in the ischemic rat brain. Our results indicate that lifarizine treatment and nimodipine treatment are both associated with reduced lactate production after forebrain ischemia in this animal model; however, nimodipine appears to be less effective than lifarizine. Lifarizine may somehow block the production of metabolites after initial ischemic injury. Our results suggest that lifarizine is a more effective neuroprotective agent than nimodipine in the setting of rat forebrain ischemia induced by bilateral carotid artery ligation.

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Neuroprotective agents in acute ischemic stroke.

Sareen D.

Ten classes of neuroprotective agents have reached phase III efficacy trials. They included calcium channel antagonists, NMDA receptor antagonists, lubeluzole, CDP-choline, the free radical scavenger tirilazad and ebselen, enlimomab, GABA agonist clomethiazole, the sodium channel antagonist fosphenytoin, magnesium, glycine site antagonist GV150526 and piracetam.