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Identification of proteins regulated by curcumin in cerebral ischemia



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ABSTRACT

Background: Curcumin is known to have a neuroprotective effect against cerebral ischemia. The objective of this study was to identify various proteins that are differentially expressed by curcumin treatment in focal cerebral ischemia using a proteomic approach.

Methods: Adult male rats were treated with vehicle or curcumin 1 h after middle cerebral artery occlusion. Brain tissues were collected 24 h after the onset of middle cerebral artery occlusion, and cerebral cortices proteins were identified by two-dimensional gel electrophoresis and mass spectrometry.

Results: We detected several proteins with altered expression levels between vehicle- and curcumin-treated animals. Among these proteins, ubiquitin carboxy-terminal hydrolase L1, isocitrate dehydrogenase, adenosylhomocysteinase, and eukaryotic initiation factor 4A were decreased in the vehicle-treated animal, and curcumin treatment attenuated the injury-induced decreases of these proteins. Conversely, pyridoxal phosphate phosphatase was increased in the vehicle-treated animal, and curcumin treatment prevented decreases in this protein. The identified altered proteins are associated with cellular metabolism and differentiation.

Conclusions: The results of this study suggest that curcumin exerts a neuroprotective effect by regulating the expression of various proteins in focal cerebral ischemia.

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1. Introduction

Stroke leads to severe neurologic impairment and causes high morbidity and mortality. One subtype of stroke, cerebral ischemia, involves several biochemical events including the release of calcium, activation of excitatory neurotransmitters, and the generation of excessive nitric oxide and free radicals [1,2]. The accumulation of these reagents triggers neuronal degeneration and neuronal death [2,3].

Curcumin is a major component of *Curcuma longa* Linn. (Zingiberaceae) and is commonly present in turmeric [4]. It exhibits several pharmacologic properties such as anti-inflammatory and antioxidant agent [5,6]. Curcumin exerts neuroprotective effects in a variety of neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, and cerebral ischemia [7,8]. Moreover, curcumin protects neuronal cells against middle cerebral artery occlusion (MCAO) induced focal cerebral ischemia and counteracts oxidative

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stress resulting from traumatic brain injury [5,9]. Curcumin also diminishes infarct volume and improves neurologic scores in focal cerebral ischemia [10,11]. Although there is extensive evidence to support the neuroprotective effects of curcumin, little is known about how curcumin provides these benefits during focal cerebral ischemia. We postulate that the neuroprotective actions of curcumin occur through the modulation of various proteins. In this study, we used two-dimensional gel electrophoresis and mass spectrometry to identify the differential expression of proteins induced and suppressed by curcumin treatment for focal cerebral ischemia.

2. Materials and methods

2.1. Experimental animals and treatment

Sprague–Dawley rats (male, 225–250 g, $n = 56$) were purchased from Samtako (Animal Breeding Center, Osan, Korea) and were randomly divided into four treatment groups; vehicle + sham, curcumin + sham, vehicle + MCAO, and curcumin + MCAO ($n = 14$ per group). The animals were housed at 18°C–22°C with a 12 h light/12 h dark cycle and had free access to a pellet diet and tap water. All procedures minimized animal suffering and followed a protocol approved by the ethics committee concerning animal research at Gyeongsang National University (GNU-LA-25). A single dose of vehicle or curcumin (50 mg/kg) was given via intraperitoneal injection 1 h after the onset of MCAO as described previously [10,11]. Curcumin (Sigma, St. Louis, MO) was dissolved in a physiological saline solution including 1% dimethyl sulfoxide. Vehicle-treated animals were injected with the same volume of physiological saline.

2.2. Middle cerebral artery occlusion

Rats were anesthetized with Zoletil (Virbac, Carros, France, 50 mg/kg), and MCAO was performed according to a previously described method [12]. Briefly, the right common carotid artery, external carotid artery, and internal carotid artery were exposed through a midline cut. The tip of a 4/0 nylon filament was rounded with heat and inserted from the external carotid artery, through the internal carotid artery, and advanced until the tip occluded the origin of the middle cerebral artery. Sham animals were received an operation including the same surgical procedure without filament insertion. At 24 h after the onset of permanent occlusion, animals were decapitated, and the right cerebral cortex was isolated.

2.3. Neurologic behavioral test

The neurologic deficit score was determined based on 5-point score ($n = 16$), according to Longa *et al.* [12]. The neurologic behavior was evaluated after 24 h of ischemia. Accordingly, 0 score indicates no observable visible sign of injury (no ischemia), mild neurologic symptoms were depicted by score of 1 in which the rats did not fully extend the forepaw contralateral to MCAO, a score of 2 indicates occasional rotating movements by the rats, 3 indicates spontaneous circling by the rats most of the time, rats with severe

neurologic deficit were given a score of 4. These severely affected rats showed very limited movements with depressed consciousness.

2.4. Two-dimensional gel electrophoresis

For the extraction of proteins, samples were homogenized in buffer solution (8 M urea, 4% CHAPS, ampholytes, and 40 mM Tris-HCl), and the suspensions were centrifuged at 16,000 g . Protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA) according to the manufacturer's manual. Immobilized pH gradient (IPG, range pH 4–7 and pH 6–9, 17 cm; Bio-Rad) gel strips were incubated in rehydration buffer (8 M urea, 2% CHAPS, 20 mM DTT, 0.5% IPG buffer, and bromophenol blue) for 13 h. Proteins were loaded on IPG strips via sample cup, and first-dimension isoelectric focusing was performed using Ettan IPGphor 3 (GE Healthcare, Uppsala, Sweden) according to the following protocol: 200 V (1 h), 500 V (1 h), 1000 V–8000 V (30 min), and 8000 V (5 h). After first-dimension separation, the strips were incubated in equilibration buffer (6 M urea, 30% glycerol, 2% sodium dodecyl sulfate, 50-mM Tris-HCl, bromophenol blue) containing DTT and iodoacetamide. The strips were loaded onto gradient gels (7.5%–17.5%), and second-dimension electrophoresis was performed on Protein-II XI electrophoresis equipment (Bio-Rad) at 5 mA per gel for 2 h, followed by 10 mA per gel at 10°C until the bromophenol blue dye migrated off the bottom of the gel.

2.5. Silver staining, image analysis, and protein identification

The gels were fixed in a solution containing 12% acetic acid and 50% methanol for 2 h, washed with 50% ethanol, and then treated with 0.2% sodium thiosulfate. The gels were then washed with deionized water, stained by impregnation in a silver solution (0.2% silver nitrate), and developed in 0.2% sodium carbonate solution. The gel images were collected using Agfa ARCUS 1200™ (Agfa-Gevaert, Mortsel, Belgium). The PDQuest 2-D analysis software (Bio-Rad) was used to analyze differentially expressed protein spots among experimental groups. The detected protein spots were excised and destained, and the gel particles were digested in trypsin-containing buffer. The extracted peptides were analyzed by a Voyager-DE STR Biospectrometry Workstation (Applied Biosystems, Foster City, CA) for peptide mass fingerprinting. Database searches were carried out using MS-Fit and Pro-Found programs. SWISS-PROT and NCBI were used as the protein sequence databases.

2.6. Western blot analysis

Western blot analysis was performed as previously described [13]. The samples were dissolved in lysis buffer, and phenylmethanesulfonyl fluoride was added as a protein inhibitor. After sonication and centrifugation, protein concentration was determined by a bicinchoninic acid kit (Pierce, Rockford, IL) according to the manufacturer's manual. Total protein (30 μ g) was electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and transferred to

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