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Ferulic acid attenuates the cerebral ischemic injury-induced decrease in peroxiredoxin-2 and thioredoxin expression

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HIGHLIGHTS

- Ferulic acid protects brain tissues against cerebral ischemic injury.
- Ferulic acid prevents brain injury-induced decrease of peroxiredoxin-2.
- Ferulic acid prevents brain injury-induced decrease of thioredoxin.
- Ferulic acid prevents brain injury-induced decrease in the interaction between thioredoxin and ASK1.

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ABSTRACT

Ferulic acid, a phenolic phytochemical compound found in various plants, has a neuroprotective effect through its anti-oxidant and anti-inflammation functions. Peroxiredoxin-2 and thioredoxin play a potent neuroprotective function against oxidative stress. We investigated whether ferulic acid regulates peroxiredoxin-2 and thioredoxin levels in cerebral ischemia. Sprague-Dawley rats (male, 210–230 g) were treated with vehicle or ferulic acid (100 mg/kg) after middle cerebral artery occlusion (MCAO), and cerebral cortex tissues were collected 24 h after MCAO. Decreases in peroxiredoxin-2 and thioredoxin levels were elucidated in MCAO-operated animals using a proteomics approach. We found that ferulic acid treatment prevented the MCAO-induced decrease in the expression of peroxiredoxin-2 and thioredoxin. RT-PCR and Western blot analyses confirmed that ferulic acid treatment attenuated the MCAO-induced decrease in peroxiredoxin-2 and thioredoxin levels. Moreover, immunoprecipitation analysis showed that the interaction between thioredoxin and apoptosis signal-regulating kinase 1 (ASK1) decreased during MCAO, whereas ferulic acid prevented the MCAO-induced decrease in this interaction. Our findings suggest that ferulic acid plays a neuroprotective role by attenuating injury-induced decreases in peroxiredoxin-2 and thioredoxin levels in neuronal cell injury.

Ferulic acid, 4-hydroxy-3-methoxy cinnamic acid, is a phenolic phytochemical constituent of many grains, fruits, and vegetables. Ferulic acid exhibits an anti-oxidant effect through scavenging reactive oxygen species, and also inhibits inflammation [1,3]. Ferulic acid, because of its anti-oxidative and anti-inflammatory activities, has been shown to have therapeutic effects in various diseases such as cancer, diabetes, and neurodegenerative diseases [9,19,24]. Moreover, ferulic acid decreases infarct volume and apoptotic cell death in focal cerebral ischemic injury [4].

Peroxiredoxins and thioredoxin are ubiquitously expressed antioxidant proteins. These proteins prevent oxidative stress and regulate biological process such as cell proliferation and apoptosis [8]. Peroxiredoxins comprise a family of six anti-oxidative enzymes. Among these enzymes, peroxiredoxin-2 is a neuron-specific protein that is abundantly expressed in the brain [7,23]. Peroxiredoxin-2 has cytoprotective effects against oxidative stress in neuronal cell culture and has been shown to reduce brain injury after transient brain ischemia [2,5]. Moreover, over-expression of peroxiredoxin-2 improves neurological recovery after brain ischemic insults [5]. These studies demonstrate the fact that peroxiredoxin-2 plays an important role in response to oxidative stress in neurons [14]. A previous study demonstrated that over-consumption of peroxiredoxin-2 under oxidative stress reduces thioredoxin activation, leads to the activation of apoptosis signaling kinase 1 (ASK1) [21]. The production of reactive oxygen species due to the oxidation of thioredoxin leads to apoptosis by

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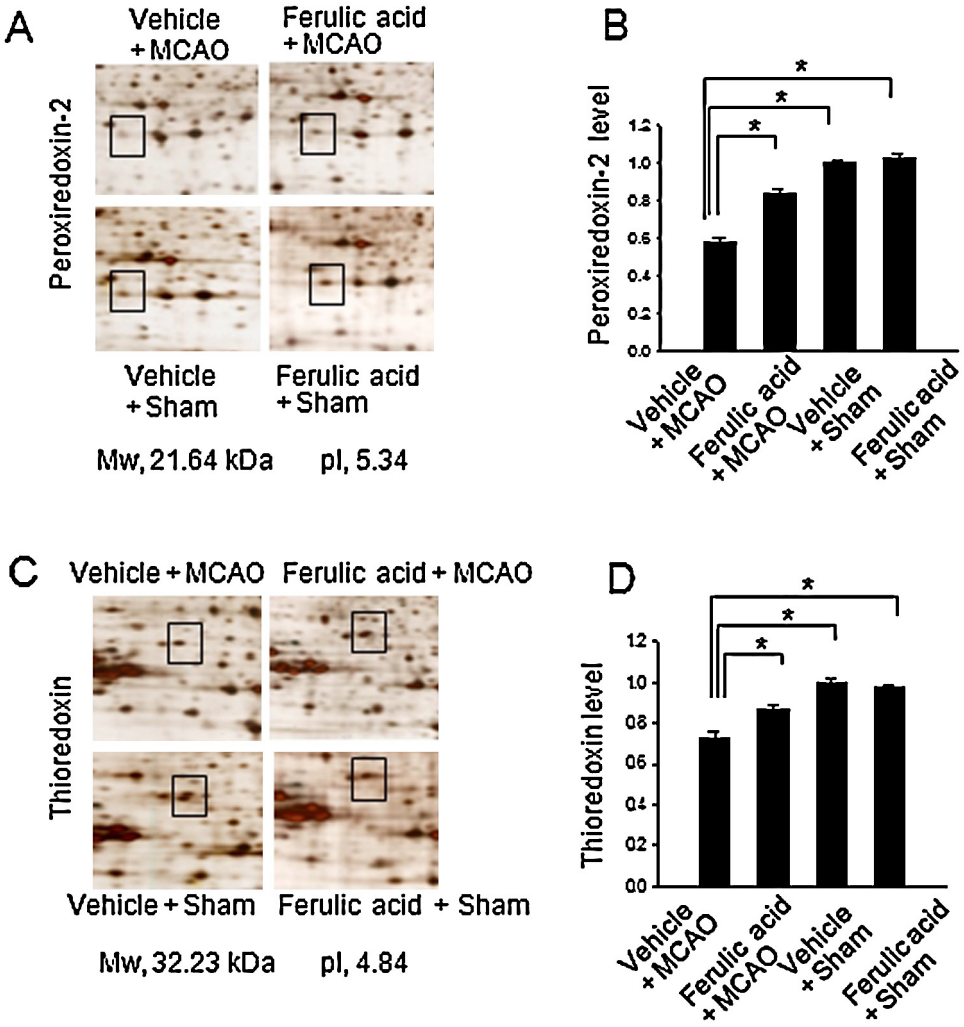

Fig. 1. Peoxiredoxin-2 (A and B) and thioredoxin (C and D) protein spots identified by MALDI-TOF in the cerebral cortices from vehicle + middle cerebral artery occlusion (MCAO), ferulic acid + MCAO, vehicle + sham, ferulic acid + sham animals. Squares indicate the protein spots. The ratio of intensity is described as spots intensity of these animals to spots intensity of vehicle + sham animals. Data are means ± S.E.M. $^*p < 0.05$. Mw and *IP* indicate molecular weight and isoelectric point, respectively.

activation of ASK1. Activated ASK1 phosphorylates and activates JNK, which ultimately induces the apoptotic cascade. Thus, it is accepted that thioredoxin binds to ASK1, thereby inhibiting stress-induced apoptosis [13,21]. Moreover, thioredoxin has neuroprotective functions against oxidative stress in brain ischemic injury and also exerts cytoprotective effects through mediation of neuronal cell differentiation and regeneration [18]. Thioredoxin reduces infarct volume and neuronal apoptosis after brain ischemic damage by attenuating oxidative stress and preventing caspase-3 expression [17,26]. Overexpression of thioredoxin in transgenic mice and administration of thioredoxin have been shown to decrease brain damage following transient focal cerebral ischemia [6,25]. In this proteomic study, we investigated that peroxiredoxin-2 and thioredoxin expression decreases in a focal cerebral ischemia animal model. Furthermore, we studied whether ferulic acid attenuates the decrease in peroxiredoxin and thioredoxin following brain ischemic injury.

Male Sprague-Dawley rats (210–230 g, $n = 60$) were used (Samtako Co., Animal Breeding Center, Osan, Korea). All animal experiments followed a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the Gyeongsang National University. Animals were maintained under controlled temperature and lighting (12 h/12 h light/dark cycle) and were randomly divided four groups, vehicle + sham group, ferulic acid + sham group, vehicle + middle cerebral artery occlusion (MCAO) group, and ferulic acid + MCAO group. Ferulic acid (Sigma, St. Louis, MO, USA) was dissolved in normal saline for the vehicle. A single dose of ferulic acid (100 mg/kg) or vehicle was injected intravenously immediately after MCAO [6].

The MCAO operation was induced as previously referenced [16]. Animals were anesthetized with sodium pentobarbital (30 mg/kg). Briefly, the right common carotid artery was exposed and the external carotid artery was cut. A 4/0 nylon monofilament with its tip rounded by heat was inserted into the external carotid artery and advanced through the internal carotid artery until it reached the origin of the middle cerebral artery. The sham-operated animals underwent the same surgical process, except for arterial blockade. The body temperature was maintained at 37 ± 0.5 °C throughout the surgical procedure with a temperature-controlled heating pad and overhead lamp. The brain tissues were collected at 24 h after the onset of MCAO.

A proteomic analysis was performed as previously described [10,12]. For the proteomic analysis, the right cerebral cortices were homogenized in lysis buffer (8 M urea, 4% CHAPS, ampholytes, and 40 mM Tris–HCl) and were centrifuged at 16,000 rpm for 20 min at 4 °C. The supernatant was removed and the pellets were dissolved in lysis buffer. Bradford method (Bio-Rad, Hercules, CA, USA) was used to determine total protein concentration. For the