available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Ferulic acid provides neuroprotection against oxidative stress-related apoptosis after cerebral ischemia/reperfusion injury by inhibiting ICAM-1 mRNA expression in rats

Chin-Yi Cheng[a], *Shan-Yu Su*[a,b], *Nou-Ying Tang*[c], *Tin-Yun Ho*[a], *Su-Yin Chiang*[a], *Ching-Liang Hsieh*[b,d,*]

[a]*Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan*
[b]*Department of Chinese Medicine, China Medical University Hospital, Taichung, Taiwan*
[c]*School of Chinese Medicine, China Medical University, Taichung, Taiwan*
[d]*Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan*

ARTICLE INFO

Article history:
Accepted 24 February 2008
Available online 18 March 2008

Keywords:
Ferulic acid
Intercellular adhesion molecule-1 mRNA
Macrophage-1 antigen mRNA
8-Hydroxy-2′-deoxyguanosine
4-Hydroxy-2-nonenal
Apoptosis

ABSTRACT

Our previous studies have shown that ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA) inhibits intercellular adhesion molecule-1 (ICAM-1) expression in the ischemic striatum after 2 h of reperfusion in a transient middle cerebral artery occlusion model in rats. The purpose of this study is to further investigate the neuroprotective effects of FA during reperfusion after cerebral ischemia. Rats were subjected to 90 min of ischemia; they were then sacrificed after 2, 10, 24 and 36 h of reperfusion. ICAM-1 and macrophage-1 antigen (Mac-1) mRNA were detected using semi-quantitative RT-PCR at 2 h of reperfusion. Mac-1, 4-hydroxy-2-nonenal (4-HNE), 8-hydroxy-2′-deoxyguanosine (8-OHdG), active caspase 3, neuronal nuclei (NeuN) and TUNEL positive cells were measured at 2, 10, 24 and 36 h of reperfusion. FA (100 mg/kg, i.v.) administered immediately after MCAo inhibited ICAM-1 and Mac-1 mRNA expression in the striatum at 2 h of reperfusion, and reduced the number of Mac-1, 4-HNE and 8-OHdG positive cells in the ischemic rim and core at 10, 24 and 36 h of reperfusion. FA decreased TUNEL positive cells in the penumbra at 10 h, and in the ischemic boundary and core at 24 and 36 h of reperfusion. FA curtailed active caspase 3 expression in the penumbra at 10 h and restored NeuN-labeled neurons in the penumbra and ischemic core at 36 h of reperfusion. FA decreased the level of ICAM-1 mRNA and the number of microglia/macrophages, and subsequently down-regulated inflammation-induced oxidative stress and oxidative stress-related apoptosis, suggesting that FA provides neuroprotection against oxidative stress-related apoptosis by inhibiting ICAM-1 mRNA expression after cerebral ischemia/reperfusion injury in rats.

* *Corresponding author*. Department of Chinese Medicine, China Medical University Hospital, No 2, Yuh-Der Road, Taichung, Taiwan. Fax: +886 4 22362871.
E-mail address: clhsieh@mail.cmuh.org.tw (C.-L. Hsieh).

Abbreviations: FA, ferulic acid; ICAM-1, intercellular adhesion molecule-1; Mac-1, macrophage-1 antigen; 4-HNE, 4-hydroxy-2-nonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; I/R, ischemia/reperfusion

0006-8993/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.brainres.2008.02.090

1. Introduction

Inflammatory response and oxidative stress are known to exacerbate the damage caused by acute cerebral ischemia/reperfusion (I/R) injury (Shin et al., 2006). Cytokines elicit the synthesis of P-selectin, E-selectin and intercellular adhesion molecule-1 (ICAM-1), adhesion molecules that facilitate the adhesion of activated leukocytes to endothelial cells (ECs). Activated leukocytes then infiltrate the ECs, cross the vascular wall and migrate into the brain parenchyma (Soriano et al., 1999; Ding et al., 2003; Khan et al., 2007). Macrophage-1 antigen (Mac-1, CD11b/CD18), a heterodimeric protein consisting of α (CD11b) and β (CD18) subunits expressed on the surface of activated leukocytes, facilitates cell–endothelium interactions (Hickstein et al., 1993; Caimi et al., 2001; Arumugam et al., 2004). Mac-1 acts as an activation marker for microglia/macrophages (Berliner et al., 2000; Nakase et al., 2004; Ueno et al., 2006), which release a host of neurotoxic compounds, including reactive oxygen species (ROS), nitric oxide (NO), cytokines and lipid peroxidation products, that worsen secondary ischemic neuronal insults (Miyahara et al., 2003; Kao et al., 2006; Kapadia et al., 2006). Evidence suggests that necrosis and apoptosis are the main characteristics of neuronal death following acute cerebral I/R injury (Kao et al., 2006). ROS, which are robustly produced by activated microglia/macrophages, attack neuronal components including lipid, protein and DNA, causing nuclear DNA oxidation and lipid peroxidation (Won et al., 2001; Imai et al., 2003). Superoxide anions, primary oxygen free radicals produced by mitochondria, are rapidly converted in the cell to hydrogen peroxide, which is subsequently converted to the highly reactive hydroxyl radical (Klein and Ackerman, 2003). This radical then hydroxylates the C-8 position of guanine residues in G–C rich regions of DNA, leading to a G:C to T:A transversion mutation (Won et al., 2001; Sakurai et al., 2003). The well-recognized oxidative stress markers in DNA and lipids are 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 4-hydroxy-2-nonenal (4-HNE), respectively. Eight-OHdG is mainly produced in neurons after transient cerebral ischemia, and the accumulation of unrepaired oxidative DNA lesions in the nucleus can lead to either tumorigenesis or apoptosis (Hayashi et al., 1999; Nagayama et al., 2000; Sakurai et al., 2003). Four-HNE, a product toxic to neuronal perikarya, is released from polyunsaturated fatty side chains and contributes to the dysfunction of cell membrane transporters via lipid peroxidation, which then leads to apoptosis (McCracken et al., 2000; Gordon et al., 2005; Lee et al., 2005). Previous studies demonstrated that drugs designed to inhibit recruited leukocytes/microglia markedly curtailed inflammation and oxidative stress-related apoptosis, and consequently provided neuroprotection in cerebral I/R injury (Storini et al., 2005; Kao et al., 2006).

Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA), a component of *Anglica sinensis (olivi) Didl. and Ligusticum chuoanxiong Hort.*, was shown to be a free radical scavenger and to have anti-inflammatory and antioxidation effects in a transient middle cerebral artery occlusion (MCAo) model (Wang et al., 2005). In our previous study, we found that FA (100 mg/kg) effectively inhibited ICAM-1 expression in the ischemic striatum and decreased the levels of superoxide anions in the ischemic brain parenchyma at 2 h of reperfusion after MCAo (submitted). The purpose of this study is to further investigate the neuroprotective effects of FA, and the possible mechanisms involved in the effects of FA against activated microglia/macrophages, oxidative stress and oxidative stress-related apoptosis in the penumbra and ischemic core areas during the cerebral I/R period.

2. Results

2.1. Physiological parameters

Blood gas parameters (including pH, pO_2 and pCO_2) and blood sugar levels were measured at 10 min before and 90 min after

Table 1 – Physiological parameters

	BS, mg/dl	pH	pO_2, mmHg	pCO_2, mmHg
10 min before ischemia				
2 h (n=6)				
Sham	119.0±29.2	7.30±0.03	97.5±10.9	28.7±7.0
Control	118.3±26.3	7.32±0.04	96.0±13.3	26.8±3.9
FA	124.7±24.6	7.28±0.03	96.3±5.8	26.2±6.7
10 h (n=5)				
Sham	111.8±24.1	7.30±0.04	104.0±12.9	28.4±5.0
Control	94.4±2.5	7.27±0.03	108.6±8.2	30.5±4.2
FA	94.0±25.2	7.25±0.03	107.6±8.0	33.1±4.4
24 h (n=6)				
Sham	120.0±29.0	7.30±0.05	90.8±6.9	29.9±3.2
Control	116.2±23.3	7.29±0.03	103.2±13.6	30.7±6.3
FA	118.8±16.0	7.31±0.03	95.0±7.3	29.7±5.3
36 h (n=3)				
Sham	119.3±48.5	7.32±0.02	92.7±8.5	31.5±3.4
Control	115.0±20.0	7.29±0.02	110.3±8.6	26.7±5.7
FA	98.3±28.6	7.27±0.03	105.7±9.9	25.2±2.1
90 min after ischemia				
2 h (n=6)				
Sham	110.7±26.1	7.31±0.02	102.8±10.3	22.4±5.8
Control	104.7±18.5	7.33±0.04	98.3±9.3	23.5±4.8
FA	126.2±3.3	7.35±0.03	91.3±17.4	21.7±5.9
10 h (n=5)				
Sham	144.6±43.3	7.33±0.04	87.6±6.3	22.8±3.0
Control	128.2±11.8	7.34±0.03	93.0±11.8	22.6±2.1
FA	109.4±28.8	7.35±0.03	89.8±5.1	26.6±4.1
24 h (n=6)				
Sham	138.7±44.4	7.31±0.02	93.2±8.7	28.3±4.0
Control	135.0±28.7	7.34±0.02	93.2±11.2	23.4±4.3
FA	118.5±28.1	7.35±0.05	92.5±6.2	23.3±3.3
36 h (n=3)				
Sham	153.3±30.6	7.34±0.02	81.3±8.3	23.3±5.2
Control	112.0±25.9	7.37±0.01	88.3±12.9	22.4±3.6
FA	120.7±34.2	7.37±0.03	93.7±7.0	20.7±1.1

Mean±SD. BS: blood sugar; sham: sham group; control: control group; FA: ferulic acid-treated group; 10 min before ischemia: 10 min before cerebral ischemia; 90 min after ischemia: 90 min after cerebral ischemia; 2 h: sacrificed at 2 h of reperfusion; 10 h: sacrificed at 10 h of reperfusion; 24 h: sacrificed at 24 h of reperfusion; 36 h: sacrificed at 36 h of reperfusion.