Protective Effect of Crocin against Cerebral Ischemia in a Dose-dependent Manner in a Rat Model of Ischemic Stroke

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Background: Crocin is a water-soluble carotenoid isolated from the *Crocus sativus L* (saffron) stigma. It has previously been reported that it has protective effects against renal, cardiac, and global cerebral ischemic injury. However its therapeutic effects remain to be clarified regarding ischemic reperfusion injuries, brain edema, and activity of antioxidant enzymes in a transient model of focal cerebral ischemia. *Methods:* Transient focal cerebral ischemia was induced by 60-minute middle cerebral artery occlusion (MCAO), followed by 23-hour reperfusion. Crocin at doses of 15, 30, 60, and 120 mg/kg intraperitoneally were injected at the start of ischemia. Infarct volume and neurologic outcome were evaluated 24 hours after MCAO. For the therapeutic time window measurement, crocin (60 mg/kg) was given 1, 3, and 6 hours after ischemia; 24 hours later brain edema and antioxidant enzyme activity were assessed. *Results:* The results indicated that treatment with crocin at doses of 30, 60, and 120 mg/kg significantly decreased infarct volume by 64%, 74%, and 73%, respectively. Administration of crocin (60 mg/kg) 1 hour before, at the start, or 1 hour after ischemia reduced brain edema by 48%, 52%, and 51%, respectively. Moreover, crocin (60 mg/kg) significantly reduced malondialdehyde (MDA) content and increased activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the ischemic cortex ($P < .001$). *Conclusions:* Our findings indicate that crocin has protective effects against ischemic reperfusion injury and cerebral edema in a rat model of stroke. These effects of crocin may have been exerted primarily by suppression of the production of free radicals and increased antioxidant enzyme activity. **Key Words:** Crocin—brain edema—ischemic reperfusion injuries—antioxidant enzyme activity—focal cerebral ischemia—rat.

Crocin is a water-soluble carotenoid isolated from *Crocus sativus L* (saffron) stigma.[1] Crocin is the most abundant constituent of saffron with a high amount of antioxidants[2-4] Evidence obtained from recent research indicates that crocin has a wide range of activities, including antidepressant,[5] anti-inflammatory,[6] anticarcinogenic,[7] antiatherosclerotic,[8] antioxidant,[4] and antihyperlipidemic effects.[1] New research has demonstrated the neuroprotective activities of crocin in various experimental models of brain disorders, such as memory impairment,[9,10] Alzheimer disease,[11] and cerebral ischemia.[3,4,12] A recent study showed that crocin effectively suppressed ischemic reperfusion–induced vascular injury to cerebral microvessels after global cerebral ischemia in mice.[13] In general, these studies show that crocin has potential therapeutic effects in reducing neuronal and brain injury in various experimental models. However its therapeutic effect in reducing brain edema is not yet clear in experimental focal cerebral ischemia. Moreover, there are very few studies on the effect of crocin on ischemic reperfusion injuries in a transient model of focal stroke. Therefore the aim of

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this study was to determine the effects of various doses of crocin on brain injuries, cerebral edema, and activity of antioxidant enzymes (glutathione peroxidase [GPx] and SOD) as well as malondialdehyde (MDA) content in a transient focal cerebral ischemia model in rats.

Material and Methods

Animals

Adult male Wistar rats (320 ± 20 g) were obtained from the breeding colony of Semnan University of Medical Sciences, Semnan, Iran. All rats were housed in cages in a 12-hour light/dark cycle at 22 to 24°C, with food and water ad libitum. All protocols of the study were performed in accordance with the National Institutes of Health guidelines for Care and Use of Laboratory Animals.

Focal Cerebral Ischemia

Using chloral hydrate (400 mg/kg, Merck Group, Darmstadt, Germany) anesthesia, we induced focal cerebral ischemia in rats, as previously described.[14] Using laser Doppler flowmetry (LDF) guidance (DRT4 laser Doppler perfusion and temperature monitor; Moor Instruments Devon, UK), a 3-0 nylon suture was introduced into the internal carotid artery and gently advanced until LDF showed a sharp decrease in the ipsilateral cerebral blood flow to less than 20% of baseline, indicating adequate occlusion of the middle cerebral artery (MCA). After 60 minutes of middle cerebral artery occlusion (MCAO), reperfusion was started by withdrawing the nylon thread for 23 hours. To monitor the regional cerebral blood flow, an LDF probe was positioned in direct contact with the right temporal bone after a limited dissection of the temporal muscle at the middle distance between the eye and the ear.[15] To fix and prevent the displacement of the LDF probe, a bur hole (2-mm diameter) was drilled 5 mm lateral and 1 mm posterior to the bregma without injury to the dura mater. Local right cortical cerebral blood flow was continuously monitored 15 minutes before, during the occlusion, and up to 15 minutes after the reperfusion.

Infarct Volume

Twenty hours after ischemia, rats were decapitated and the brains rapidly removed and cooled in saline at 4°C for 10 minutes. They were then sectioned coronally into 7 2-mm-thick slices using a Brain Matrix (Zivic Instruments, Pittsburgh, PA). The slices were immersed in 2% 2,3,5-triphenyltetrazolium chloride solution (Sigma Aldrich, Munich, Germany), and kept at 37°C in a water bath for 15 minutes. These slices were then photographed separately using a digital camera (Cannon, Melville, NY) connected to a computer. Unstained areas were defined as infarct and measured using image analysis software (NIH Image Analyzer). The infarct volume of each slice was calculated by multiplying the infarcted area of the slice by its thickness. The total infarct volume of each brain was calculated as the sum of the infarct volumes of the 7 brain slices. The contribution of the edema to the infarct volume was corrected using the following formula, as previously described[16]: corrected infarct volume = left hemisphere size – (right hemisphere size – measured infarct size).

Cerebral Edema

Cerebral edema was evaluated by determining brain water content (BWC).[14] Twenty-four hours after MCAO, the rats were killed and the brains were removed. Afterward, the pons and olfactory bulb were removed and the brains were weighed to obtain their wet weight. Subsequently, brains were dried at 110°C for 24 hours to determine their dry weight. BWC was calculated using the following formula: (wet weight – dry weight)/wet weight × 100.

Preparation of Tissue Homogenates

Brain was removed and ischemic cortex carefully isolated; subsequently, cortex was washed in cold .9% control and kept at −70°C. A fraction of cortex tissue was homogenized (1:10 w/v) in cold 1.15% KCl. The supernatants obtained after centrifugation at 20,000× *g* for 10 minutes at 4°C were used for biochemical analyses. The level of total protein in supernatants was determined by the Bradford method using bovine serum albumin as standard.[17]

Measurement of Malondialdehyde

The MDA content or thiobarbituric acid–reactive substances in ischemic brain cortex homogenates were measured using the tiobarbituric acid method as described previously.[18] The initial sample of 250 mL of supernatant with 1.5 mL of 1% phosphoric acid and thiobarbituric acid .6% were mixed and incubated in a water bath for 45 minutes. After cooling, 2 mL of n-butanol was added to the mixture and mixed in a vortex mixer for 1 minute followed by centrifugation at 3000 rpm for 10 minutes. The organic layer was transferred to a fresh tube and its absorbance was measured at 532 nm using a spectrophotometer (Spectronic 1201, Milton Roy, Ivyland, PA). The standard calibration was plotted using 1,1,3,3-tetramethoxypropane. MDA concentration was expressed in nanomoles/milligram protein.

Measurement of Superoxide Dismutase and GPx Activity

Superoxide dismutase (SOD) and GPx activity in ischemic brain cortex homogenates were measured using a commercial kit (Randox Laboratories Ltd, Crumlin, UK) according to the manufacturer's instructions.