Quercetin Improves Cognitive Deficits in Rats with Chronic Cerebral Ischemia and Inhibits Voltage-dependent Sodium Channels in Hippocampal CA1 Pyramidal Neurons

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Quercetin is a flavonoid compound found in a number of medicinal plants that are often prescribed in Chinese clinics for the treatment of cardiovascular diseases. The present study investigated the effects of quercetin on chronic cerebral ischemia in rats produced through bilateral occlusion of the carotid arteries. Treatment of quercetin (5 mg/kg i.p. for 14 days) was found to improve the performance of learning and memory of ischemic rats in the Morris water maze. Additionally, in electrophysiological experiments, quercetin attenuated the inhibition of long-term potentiation (LTP) in ischemic rats. Also, in acutely isolated rat hippocampal CA1 pyramidal neurons, quercetin (0.3, 3 and 30 μM) decreased the amplitude of voltage-dependent sodium currents in a dose- and voltage-dependent manner. Taken together, these data lend further support for the neuroprotective effects of quercetin and suggest that a therapeutic effect on cerebral ischemia and vascular dementia of quercetin could be due to its inhibition of sodium channels. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: quercetin; ischemia; LTP; voltage-dependent sodium channels.

INTRODUCTION

Vascular dementia (VD) has gained much attention in recent years. VD is a progressive deterioration in cognitive capacity that emerges as various areas of the brain are damaged by loss of blood supply (Kalaria et al., 2004). Reduction in cerebral blood flow and the concomitant abnormalities of energy metabolism that arise in chronic cerebral ischemia can lead to selective neuronal injuries in vulnerable regions of the brain, particularly the hippocampus and cerebral cortex (Choi, 1996). This injury is accompanied by a progressive cognitive decline (Alagona et al., 2004). Spatial learning and memory are dependent on the integrity of the hippocampus. Long-term potentiation (LTP), displayed in the hippocampus as a plastic change in synaptic strength, is widely believed to hold the key to understanding how memories are formed in the brain. Additionally, LTP is a widely accepted model for learning and memory at the cellular level (Malenka and Nicoll, 1999).

Voltage-gated cation channels regulate the transmembrane flux of calcium, sodium and potassium. Ischemia produces a breakdown in the normal function of these ion channels, contributing to a series of pathologi-
the first study presenting results of the effects of quercetin on hippocampal neuronal ion channels.

MATERIALS AND METHODS

Animal model and drug administration. Male Wistar rats (6 weeks, 250 ± 10 g) were obtained from the Chinese Academy of Medical Sciences and were randomly divided into three groups, i.e. vehicle group, sham-operated group and quercetin-treated group. Rats in the vehicle and quercetin-treated groups were subjected to permanent bilateral occlusion of their carotid arteries to induce chronic cerebral ischemia and model the effects of VD. In the sham-operation group, the carotid arteries were exposed but not occluded. The experimental protocols were approved by the Committee for Animal Care at Nankai University and were in accordance with the practices outlined in the NIH Guide for the Care and Use of Laboratory Animals. Quercetin was purchased from Shanxi Huike Pharmaceutical Co., Ltd and dissolved in saline. Fifteen days after surgery, the rats in the quercetin-treated group were administered a saline solution containing quercetin (5 mg/kg, i.p., once a day). The sham-operated group and the vehicle group were injected with the same volume of saline solution alone. This drug administration regimen was conducted for 14 days.

Morris water maze test. The spatial learning performance of the rats was evaluated using the Morris water maze as described previously (Su et al., 2008). For descriptive data collection, the pool was divided into four zones by the software (Ethovision 2.0, Noldus, Wagenigen, Netherlands), with a hidden platform placed in one of the zones. Each rat received two trials every day and the test lasted for 5 days. The escape latency (time to reach the platform) was used to assess performance of learning and memory of the animals. The retention of spatial memory was assessed on day 6 by the spatial probe test with the platform removed. The swimming trace and distance in the target zone where the platform had been set were used to evaluate the cognitive performance.

LTP recording. At the end of the Morris water maze test, LTP was recorded in vivo as described previously (Su et al., 2009). Briefly, the rats were anesthetized with urethane (1.5 g/kg), and then placed in a stereotaxic frame (Narishtige, Japan). Small holes were drilled in the skull for inserting stimulating and recording electrodes (Advent Co., UK). The tip of the recording electrode was positioned in the stratum radiatum of area CA1 (3.4 mm posterior and 2.5 mm lateral to bregma), and the stimulating electrode was inserted 4.2 mm posterior to bregma and 3.8 mm right of the midline. In all experiments, extracellular field excitatory post-synaptic potentials (fEPSPs) were evoked by stimulating with a square-wave constant current pulse of 50 ms duration at a frequency of 0.033 Hz and amplified by a conventional amplifier (AD instruments Ltd, Australia). Baseline fEPSPs were monitored for at least 30 min prior to the induction of LTP to ensure a steady state response. The slope and amplitude of fEPSP was measured and averaged every 5 min. LTP was induced by high frequency stimulation using 20 pulses at 200 Hz, repeated three times at 30 s intervals. All recording and stimulation was performed using an online computerized oscilloscope stimulator and data analysis interface system.

Sodium channel recording. Hippocampal CA1 neurons were acutely isolated by enzymatic digestion and mechanical dispersion from 7–10 day old Wistar rats as described previously (Yao et al., 2007). The neurons were placed in a recording chamber mounted on the stage of an upright microscope (Olympus, Japan) and perfused with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 134, KCl 5, NaH2PO4 1.5, MgSO4 2, CaCl2 2, NaHCO3 25, glucose 10, HEPES 10, PH 7.25 with NaOH). Quercetin was dissolved in ACSF to the final desired concentration (0.3 μM, 3 μM or 30 μM) and applied 5 min after rapture of the membrane. Whole-cell patch experiments were performed using an EPC10 (HEKA Electronics, Lambrecht, Germany) amplifier with pulse software (HEKA, Germany). Glass pipettes with a resistance of about 3–5 MΩ were filled with pipette solution containing (in mM): 120 CsCl, 20 tetra-ethyl ammonium chloride (TEA-Cl), 2 MgCl2, 10 EGTA, 2 Na2-ATP, 10 HEPES (pH 7.25 with CsOH). Data were acquired at a sampling rate of 5 kHz, filtered at 2 kHz, analysed off-line using the Pulsefit analysis software package (HEKA, Germany).

Statistics. Data are reported as mean ± SEM. Student’s paired t-test was used in patch clamp experiments. ANOVA with post hoc Scheffe’ test for multiple comparisons was performed to determine statistical significance of quercetin treatments in the water maze test and LTP recording analysis. Significance levels were established at a level of p < 0.05.

RESULTS AND DISCUSSION

In the Morris water maze, animals from all groups became more efficient at locating the platform on successive trials. The escape latency became progressively shorter in all groups in a day-dependent manner. There were no significant differences among the three groups in the escape latency on day 1 (vehicle: 56.9 ± 13.4 s; sham: 57.5 ± 14.4 s; quercetin: 56.8 ± 11.5 s, p > 0.05 ANOVA) and day 2 (vehicle: 36.7 ± 6.6 s; sham: 26.0 ± 8.5 s; quercetin: 29.1 ± 10.4 s, p > 0.05 ANOVA). On day 3, group comparisons revealed that animals in the sham-operated group displayed a shorter latency in finding the platform when compared with the vehicle group (vehicle: 33.1 ± 5.3 s; sham: 23.1 ± 4.6 s, p < 0.05) and similarly on day 4 (vehicle: 26.2 ± 4.7 s; sham: 10.6 ± 2.8 s, p < 0.01) and day 5 (vehicle: 22.7 ± 3.0 s; sham: 9.7 ± 2.7 s, p < 0.01) (Fig. 1A). These results indicate that the experimentally induced chronic cerebral ischemia results in a significant impairment of memory acquisition, and confirm the utility of this model in the investigation of quercetin effects (de la Torre and Aliev, 2005). In the quercetin group the escape latency was similar to sham (24.2 ± 5.0 s) on day 3 but became significantly shorter compared with the vehicle group by day 4 (quercetin 16.6 ± 2.7 s, p < 0.05) and also by day
5 (quercetin: 16.9 ± 2.2 s, p < 0.05) (Fig. 1A). No significant difference was observed in the mean swim time among all the groups, indicating that the motor ability of the animals in the vehicle group was not impaired by the surgery (Fig. 1C). On day 6, animals in the vehicle group spent less time in the target quadrant (vehicle: 24.7 ± 3.8%; sham: 37.8 ± 6.1%; quercetin: 34.0 ± 4.1%), whereas the sham-operated (p < 0.05, vehicle vs sham) and the quercetin-treated (p < 0.05, vehicle vs quercetin) animals spent significantly longer times, respectively (Fig. 1B). The performance of quercetin-treated rats was not as good as that of sham-operated rats on day 4 (p < 0.05, sham vs quercetin) and day 5 (p < 0.05, sham vs quercetin). Thus, quercetin partially reversed the learning and memory impairment.

Quercetin protects against LTP changes in the hippocampus induced by chronic cerebral ischemia. In the vehicle group chronic ischemia significantly reduced the normalized slope of the fEPSP (vehicle: 114.0 ± 3.1%; sham: 129.8 ± 4.5%, p < 0.05) but in the quercetin treated group there was no significant impairment of the fEPSP slope this being similar to sham levels (quercetin: 127.3 ± 2.0%, p > 0.05 sham vs quercetin) (Fig. 2A, C). Similarly, chronic cerebral ischemia significantly impaired normalized EPSP amplitude, but quercetin treatment also recovered the changes to sham levels (vehicle: 174.0 ± 6.3%; sham: 213.4 ± 6.8%; quercetin: 201.2 ± 6.2%, p < 0.05 vehicle vs sham; p < 0.05 vehicle vs quercetin; p > 0.05 sham vs quercetin) (Fig. 2B, D). These results suggested that quercetin treatments reduced memory impairment induced by chronic cerebral ischemia, which agreed with previous findings using a different approach. Thus Pu et al. (2007) showed that quercetin can alter the cognitive deficits induced by different methods including transient cerebral ischemia. In concert with these behavioural and neuronal changes, analysis of the membrane events showed that quercetin decreased the amplitude of the voltage-dependent sodium channel currents in a dose-dependent way. Inward currents were evoked by depolarizing pulses.
and were abolished by application of 0.5 μm tetrodotoxin (TTX) and thus considered as VGSC currents (INa). The sodium currents were evoked by a series of 40 ms depolarizing voltage steps from −100 mV to −10 mV in hippocampal CA1 neurons (Fig. 3A). Quercetin at 3 μM decreased the current peak to 85.2 ± 6.1% (n = 6, p < 0.05) (Fig. 3B). At the concentration of 0.3 μM and 30 μM quercetin also decreased the current peak to 92.1 ± 2.9% and 81.0 ± 5.3% of the control, respectively, but there were no significant differences between the groups (Fig. 3D). For analysis of the current-voltage (I–V) relationship, neurons were held at −80 mV, sodium currents were obtained by depolarizing steps from a command potential of −100 to +60 mV at 10 mV steps. Quercetin (3 μM) decreased the amplitudes of sodium currents at different membrane potentials, which indicated that quercetin decreased the amplitudes of Na+ currents in a voltage-dependent manner (Fig. 3C). Sodium channels are known to underlie axonal and somatic action potentials and actively propagate information within the dendritic tree of pyramidal neurons (Stuart and Sakman, 1994). Hypoxia led to a small and slow depolarization, which was followed by a rapid complete depolarization; this rapid depolarization has been associated with neuronal damage (Raley-Susman et al., 2001). Under hypoxia, Na+ current is increased. Many voltage-dependent sodium channel blockers (TTX and Lidocaine) have been shown, both in vivo and in vitro, to have neuroprotective effects (Ates et al., 2007; Brahma et al., 2009). Ginkgo biloba extract, which contains some quercetin, was reported to inhibit ion channels and hyperpolarize the membranes in ventricular myocytes (Chen et al., 2005). It is suggested that blockade of sodium currents and inhibition of their activation process by quercetin could reduce sodium influx and delay depolarization, which may have contributed to its neuroprotective actions observed in the present study and as previously suggested by Taylor and Meldrum (1995).

In summary, this work showed that quercetin improved the performance of learning and memory of chronic cerebral ischemic rats, reversed the LTP deficit induced by ischemia and inhibited voltage-dependent sodium channels. These results could be helpful in understanding the mechanism of quercetin’s neuroprotective effect.

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