EFFECTS OF GARLIC AND NIMODIPINE ON CEREBRAL BLOOD FLOW AND THEIR NEUROPROTECTIVE EFFECTS AFTER BRAIN ISCHEMIA IN RABBIT

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Abstract
Background-Nimodipine has been shown to have a neuroprotective effect. Garlic also displays features that are potentially effective in inhibiting ischemic damage. In this study the efficacy of both garlic and nimodipine in preventing ischemic brain injury were assessed.

Methods-Twenty-four rabbits were divided into four groups: nimodipine-treated group (NP), garlic-treated group (GR), normal saline group (NS), and polyethylene glycol-treated as the vehicle group (VH). All animals were subjected to 15 minutes of bilateral common carotid artery (CCA) occlusion. NP and GR groups received garlic and nimodipine 60 minutes before occlusion of the CCA respectively. Cerebral blood flow (CBF) was measured by Laser Doppler flowmetry, during and after the occlusion. Histopathology of the brain was blindly evaluated. The percentage of degenerated cells in the hippocampus was estimated and vascular congestion was graded on a 3-point scale (0, I, II).

Results-After reperfusion, nimodipine and garlic increased the cerebral blood flow (CBF) by 41% and 24% respectively. Comparing these values to the extent of CBF increase in the VH and NS (2% in both) groups, the differences were statistically significant (p<0.05). The percentage of degenerated neuronal cells in zone CA1 was 23%, 31%, 43% and 44% in NP, GR, VH and NS groups, respectively. NP and GR showed a higher grade of congestion compared to control groups (VH and NS)(p<0.05).

Conclusion-This study demonstrates that neuronal damage to the rabbit hippocampus is reduced by garlic administration, although nimodipine can increase CBF more effectively.

Keywords • Garlic • cerebral ischemia • cerebral blood flow • nimodipine

Introduction

Garlic (Allium sativum L.) is thought to decrease blood pressure, inhibit platelet aggregation, reduce blood triglycerides, and cholesterol and to have antibacterial and antifungal properties.1,2 Garlic displays an antihypertensive effect and a dose-dependent angiotensin-converting enzyme inhibitory effect is thought to mediate this blood pressure lowering property.2,3 In addition, garlic has a fibrinolytic activity and it appears that in this way, it exerts a protective effect against atherosclerosis. Some reports have suggested that garlic has free radical scavengering and protective effects against stroke, coronary thrombosis and atherosclerosis.4-7 In the authors’ preliminary study, preischemic gavage of garlic in rabbits was shown to increase cerebral blood flow (CBF).

The effect of nimodipine on CBF is controversial. Its effects are related to the type of ischemia, the time (pre or post-ischemia) and mode of administration8-13, but a potential ability in reducing the amount of ischemia by its neuroprotective effects has been noted.10,13-15

The purpose of the present study was to
evaluate the effect of garlic and nimodipine on CBF in the rabbit model of ischemia and to compare their neuroprotective effects on neuronal damage in the hippocampal region.

**Materials and Methods**

Twenty-four male albino rabbits weighing between 1500 and 2000 g were divided into four groups; nimodipine-treated group, (NP); garlic treated group, (GR); normal saline group (NS) and polyethylene glycol-treated group (VH). The two latter groups were considered as controls. All the animals were housed under diurnal light conditions with adequate access to food and water. All procedures were in accordance with animal care guidance at Kerman University of Medical Sciences.

**Nimodipine administration**

Nimodipine stock solution (5mg/mL) was prepared using polyethylene glycol-400 as solvent. Solutions containing nimodipine were carefully protected from light. Because the peak plasma level of nimodipine is attained 40-60 minutes after administration of a single oral dose, it was administered at the dose of 5 mg/kg 60 minutes before the onset of induced ischemia.

**Preparation of garlic juice**

Fresh garlic (product of the Mahan field, Kerman, Iran) was purchased from the local suppliers. Garlic extract was prepared according to the method reported by Foushee, et al in which the peeled garlic cloves were weighed (1000 g), finely chopped with a stainless meat grinder and garlic paste was squeezed out through double cheesecloth to obtain a pale extract. This process yielded 200 mL of garlic juice which was divided into 5 mL aliquots and immediately stored at -20°C. Considering the variation in the effect of garlic 30-60 minutes after its administration and the variety of doses that have been used in experimental studies, we administered the garlic at the dose of 1 mL/kg by feeding tube, 60 minutes prior to induction of ischemia.

**Blood flow measurement**

Cortical CBF was measured by laser doppler flowmetry (Laser Doppler MBF 3/D Moor Instrument, England) in all groups before (as baseline value) and during the 15 minutes of CCAs occlusion and within 15 minutes of reperfusion. A single laser doppler flow probe was positioned just above the dural surface. Saline was applied to moisten the dura and to fill the space between the dura and the probe. For the measurement of CBF, a point was chosen 5 mm posterior to the bregma and 5 mm lateral to the midline. We drilled the skull at this point and a segment of bone was carefully removed. Steady-state baseline values were recorded before occlusion and the CBF was measured during occlusion and reperfusion as a percentage of baseline values.

**Pathological findings**

At the end of the surgical procedure the animals were sacrificed with intraarterial infusion of formalin. The brain tissues were removed, embedded in paraffin and sectioned coronally into thin slices. Then, the slices were stained with hematoxylin and eosin. In this study, because the animals were sacrificed within one hour of CCA reperfusion and regarding the time-consuming cascade of ischemic cell death, the histologically ischemic cells were not considered as dead cells. With respect to the ischemic changes of the CA1 region, the following parameters were measured: 1) Hydropic degeneration of neuronal cells as percentage of involved cells as the extent of vascular degeneration of cellular cytoplasm, the
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Figure 1. Effect of nimodipine and garlic on cerebral blood flow during ischemia and reperfusion compared to control groups (NS, VH).

before ischemia attenuates CBF and decreases neuronal damage in the hippocampal CA1 region. We performed our experiment on rabbits using a two-vessel occlusion of the CCAs, which induced almost complete ischemia. This model has been introduced as a useful procedure in the investigation of the mechanism of ischemic neuronal cell injury. In this study preischemic administration of garlic juice increased CBF in the reperfusion state. It was also shown that garlic could decrease the extension of ischemic neuronal damage in the CA1 hippocampus region. Garlic produces vasodilatory effects by means of: 1) inducing a direct relaxant effect, 2) increasing adenosine level, 3) production of NO, and 4) release of prostaglandin I2. Considering the generation of free radicals of oxygen as a major mechanism in the pathogenesis of ischemic injury, the free radical scavenging activity of garlic has been corroborated in many studies.

It seems that garlic has an inhibitory effect on calcium channels. In addition to vasodilatory effects, the prevention of calcium influx induces less degenerative change in the ischemic cells. Garlic compounds are very lipophilic, so they can pass through the blood-brain-barrier and exert their effects on brain tissue.

Considering a dose-dependent hypotensive effect of garlic, these data may suggest that a dose of 1 mL/kg of garlic juice applied in this study, is not sufficient to significantly reduce the blood pressure. As mentioned above, the comparable histopathological results of garlic and nimodipine may be related to the multidirectional protective mechanism of garlic in the ischemic cascade. Garlic is still employed in folk medicine in all parts of the world, for both prophylaxis and treatment of a variety of diseases. The present study may prompt us to perform more research on the neuroprotective effects of this natural product and to elucidate the basic mechanisms underlying this effect.

References


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