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ORIGINAL ARTICLE

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 administration[8-13], 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

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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.[8] 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[10,15], 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[2] 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.[4] Considering the variation in the effect of garlic 30-60 minutes after its administration[2] and the variety of doses that have been used in experimental studies[2, 5, 7], we administered the garlic at the dose of 1 mL/kg by feeding tube, 60 minutes prior to induction of ischemia.

Ischemia study

Rabbits were deprived of food for 12 hours prior to surgery. Reversible ischemia was induced by occlusion of the common carotid arteries (CCA) in six of NP and six of GR rabbits. The animals were anesthetized by the co-administration of intraperitoneal ketamine hydrochloride (75 mg/kg) and xylasine (4 mg/kg) with subsequent administration of phenobarbital (50 mg/kg) after 15 minutes, followed by maintenance intravenous dose of 5 mg/kg. The animals were allowed to breathe spontaneously, however tracheostomy was performed for artificial respiration if needed. The right femoral artery was cannulated for continuous monitoring of arterial blood pressure as well as for sampling blood for the measurement of pH, PaO_2 and $PaCO_2$, (ABL30 Acid-Base Analyzer). A vein was cannulated for obtaining blood for hematocrit and blood glucose measurement. Both CCAs were exposed through a midline cervical incision. After 15-min occlusion of the CCAs by temporary clips, the brain was allowed to reperfuse by removal of the clips.

Blood flow measurement

Cortical CBF was measured by laser doppler flowmetery (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 hematoxyllin 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

Table 1. Physiological variables in each study group (mean ± SEM).

Treatment group	N	MAP* (mmHg)	Po2 (mmHg)	Pco2 (mmHg)	pH	Plasma glucose (mg/dL)	Hb (g/dL)
Normal saline	6	85±8	89±3	32±4	7.2±7.8	272±21	15±3
Garlic	6	85±16	87±5	32±7	7.2±6.2	249±36	14±1
Nimodipine	6	72±1	87±7	31±4	7.3±8.2	229±34	14±1
Vehicle	6	72±9	89±1	30±3	7.3±6	238±71	14±1

Data are mean±SEM. There were no significant differences between the groups ($p>0.05$), *Mean Arterial Pressure

pallor of nucleoplasmic and cytoplasmic nucleic acids. 2) Vascular events such as no congestion, vascular collapse (grade 0), uniform dilatation of the vessels (grade 1), and circular dilatation of the vessels along with congestion (grade 2).

Statistics

The one way ANOVA model was used to compare the mean value and the percentage of changes with respective baseline values. When ANOVA showed significant ($p<0.05$) differences, the Tukey test was used for intergroup comparison of the means of the values. Data were presented as mean±SEM.

Results

With the exception of the significantly lower mean arterial blood pressure (MAP) during the preocclusion and reperfusion periods in NP as compared to the control group, no difference was noted in the physiological variables of the treatment groups (Table 1). Nimodipine reduced the MAP from a baseline value of 72.76±4 mmHg to a preischemic value of 66.11±6 mmHg. Deviation of MAP from its baseline was not significant in both the GR and VH groups. Fifteen minutes after of CCAs ligation, the CBF decreased to 35%, 44%, 49% and 56% of the preocclusion state in the NP, GR, VH and NS groups, respectively. Fifteen minutes after reperfusion CBF increased to 41%, 24%, 2% and 2% of the preocclusion state in NP, GR, VH and NS groups, respectively (Figure 1). All groups showed increased blood flow (post-ischemic hyper-perfusion) 5 and 10 minutes after reperfusion. A significant difference was not found between the groups until 15 min after reperfusion. The percentage of ischemic damage in zone CA1 hippocampus was 23% for NP, 31% for GR, 43% for VH and 44% for NS. The percentage of degenerated cells in NP and GR was significantly lower than that of controls (Figure 2). Comparing with the control groups, NP and GR showed a higher grade of congestion (Table 2).

Table 2 .Effects of nimodipine and garlic on grade of congestion compared to control (NS, VH).

Group	Grade of congestion			Mean±SE
	0	1	2	
NS (n=6)	3	3	---	0.5±0.2
GR (n=6)	---	3	3	1.5±0.2
NP (n=6)	---	3	3	1.5±0.2
VH (n=6)	3	3	---	0.5±0.2

Discussion

A variety of drugs have been proposed to prevent cellular injury resulting from ischemia and to improve CBF. Among them, calcium antagonists have recently gained popularity. Nimodipine is widely used clinically for the prevention of ischemic damage.[8, 9, 16-18] Almost all literature reports reveal that nimodipine has neuroprotective effects and some studies suggest that it increases vascular perfusion in the ischemic brain[8,9,19], however other studies show no improvement in the cerebral blood flow [10-12, 14,15, 20] and even no neuroprotective effect followed by nimodipine administration. The effects of many pharmacologic therapies on CBF are the indirect consequence of alteration in the cerebral metabolism provoked by the drug, rather than a direct action of the agent on the cerebral vessels. In addition to the indirect actions on cerebral vessels, nimodipine may directly act on cerebral tissues. We documented that nimodipine not only increased the CBF, but exerted a neuroprotective effect as well.

It was previously demonstrated that like nimodipine the administration of garlic juice

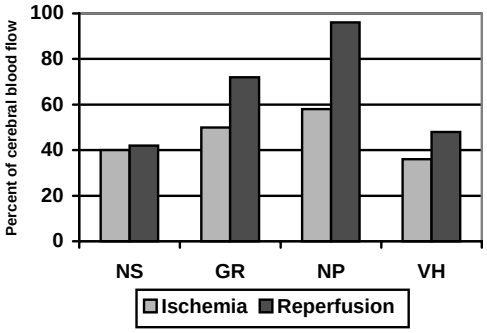

Figure 1. Effect of nimodipine and garlic on cerebral blood flow during ischemia and reperfusion compared to control groups (NS, VH).

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.[21,22] 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[4], 2) increasing adenosine level[23,24], 3) production of NO^{1}, and 4) release of prostaglandin I2. [25,26] 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.[3,6,28] It seems that garlic has an inhibitory effect on calcium

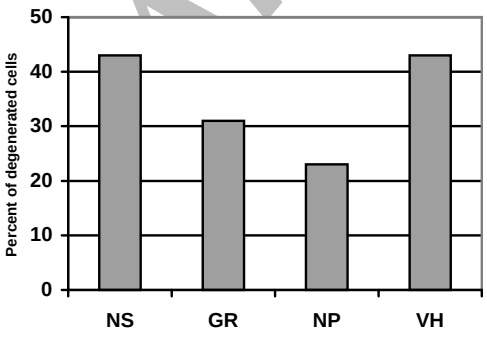

Figure 2. Protective effects of nimodipine and garlic on neuronal degeneration in CA1 hippocampus compared to controls (NS, VH).

channels.[28,29] In addition to vasodilatory effects, the prevention of calcium influx induces less degenerative change in the ischemic cells. Garlic compounds are very lipophillic[14], so they can pass through the blood-brain-barrier and exert their effects on brain tissue.

Considering a dose-dependent hypotensive effect of garlic[2], 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.

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