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흰쥐에서 藕節 추출물의 국소 뇌혈류량 조절 효과

김영균[#1], 권미정[1], 조수인[*2]

1: 동의대학교 한의과대학 내과학교실, 2: 동신대학교 한의과대학 본초학교실

Effect of Nodus Nelumbinis Rhizomatis Extract on the Regulation of Regional Cerebral Blood Flow in Rats

Young-Gyun Kim[#1], Mi-Jung Kwon[1], Su-In Cho[*2]

1: Dept. of Internal Medicine, College of Korean Medicine, Dong-Eui University
2: Dept. of Herbology, College of Korean Medicine, Dongshin University

ABSTRACT

Objectives : In brain disorders such as ischemic stroke, the final outcome depends largely on the duration and the degree of the ischemia as well as the susceptibility of various cell types in the affected brain region. In the present study, the effects of Nodus Nelumbinis Rhizomatis Extract(NNRe) were tested for the anti-oxidative action of rCBF.

Methods : Regional cerebral blood flow(rCBF) were determined by LDF methods. LDF allows for real time, noninvasive, continuous recordings of local CBF. The LDF method has been widely used to trace hemodynamic changes in the superficial or the deep brain structures in experimental stroke research.

Results : NNRe treatment showed no change on rCBF in methylene blue, ODQ and L-NNA pretreated rats. 120 minutes of MCAO and followed reperfusion, 0.1% concentration of NNR treatment improved the altered cerebral hemodynamics of cerebral ischemic by increasing rCBF.

Conclusions : The ischemia/reperfusion induced oxidative stress may have contributed to cerebral damage in rats, and the present study provides clear evidences for the beneficial effect of NNR on ischemia/reperfusion induced brain injury.

Key words : Nodus Nelumbinis Rhizomatis, Regional Cerebral Blood Flow.

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*교신저자 : 조수인. 전라남도 나주시 대호동 동신대학교 한의과대학 본초학교실.
· Tel : 061-330-3513 · Fax : 061-330-3519 · E-mail : sicho@dsu.ac.kr
#제1저자 : 김영균. 부산광역시 부산진구 양정동 동의대학교 한의과대학 제2내과학교실
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Introduction

In oriental medicine, Nodus Nelumbinis Rhizomatis(NNR) has the effect of hemostasis with astringents and promoting blood circulation by removing blood stasis[1]. In the present study, the effects of NNR extract were tested for the changes of rCBF was determinated by Laser-Doppler Flowmetry(LDF).

Cerebral ischemia results in a time-dependent cascade of molecular events including the rapid depletion of intracellular energy stores, anaerobic glycolysis, lactic acidosis, membrane depolarization, glutamate excitotoxicity, intracellular calcium overload, activation of calcium-stimulated enzymes (phospholipases, proteases, protein kinases, nitric oxide synthase or NOS, endonucleases), mitochondrial dysfunction, free radical production, activation of the immune system(neutrophils, monocytes/macrophages), overexpression of genes and neuronal death[2-4].

Free radicals are believed to be implicated in stroke[5-10]. Mitochondria generate basal amounts of superoxide anions under physiological conditions and these are rapidly scavenged by endogenous antioxidant systems(catalase, superoxide dismutase, glutathione peroxidase). Reperfusion after ischemia provides oxygen as a substrate for numerous enzyme oxidation reactions that produce free radicals(superoxide anions, hydroxyl radicals and hydrogen peroxide) to such extent that antioxidant systems are overwhelmed[7,11,12]. This oxidative stress results in oxidative damage, including lipid peroxidation, protein oxidation and DNA damage, which can lead to cell death[8,9,13-15].

In transient cerebral ischemia, evidence has accumulated during the past two decades that an overproduction of nitrogen and oxygen free radicals occurs in the early reperfusion and is deleterious by damaging cellular macromolecules such as lipids, proteins and nucleic acids[8]. Thus, in a rat model of focal cerebral ischemia/reperfusion, oxidative injury to DNA was detected as early as 1 min after the onset of reperfusion[16].

LDF allows for real time, noninvasive, continuous recordings of local CBF. The LDF method has been widely used to trace hemodynamic changes in the superficial or the deep brain structures in experimental stroke research[17]. It has also been employed as a useful tool for imaging the instantaneous changes of cortical CBF related to cortical spreading depression[18] or tracing stimulation elicited by a local vascular response[19]. The present study thus was carried out to determine the mechanism of action of NNR on rCBF by using rancimat and LDF methods.

Materials and Methods

1. NNR extract preparation

NNR were purchased in the special herb market(Songsan Herb, Gwang-Ju, Korea) and good samples carefully selected. To fractionate the aqueous extract, 250g of the dried herb of NNR was boiled with 4300㎖ of pure water at 100℃ for 2hours. After filteration, the filtrate was evaporated under reduced pressure and then freeze-dried to yield the aqueous extract. And the total crude extractive powder was 41.9g. The extract stored in deep freezer when unused, and freshly diluted for experiment.

2. Animals and chemicals

Adult male Sprague-Dawley rats at the body weight of 202±20g were obtained commercially(Daehan experimental animal, Korea) and used. All animals were housed under standard conditions of lights and controlled room temperature, and received food and water ad libitum. All chemicals were purchased from Sigma Chemical Corporation(Sigma-Aldrich Korea, Korea).

3. Measurement of rCBF in rats

Rats were anesthetized with urethane(750mg/kg, i.p.) and placed on a stereotaxic frame. Tracheotomized and entubated, and body temperature was maintained at 37℃ with a servo-controlled heating lamp and a rectal thermistor. One femoral

artery was used for the monitoring of blood pressure with PE-50 polyethylene catheters and were recorded on a polygraph. The skull was exposed and a hole 5 ㎜ in diameter was drilled in the left side at a site 5 ㎜ lateral and 2㎜ posterior to the bregma. A laser-Doppler flowmeter(Transonic Instrument, USA) with a 0.8㎜ needle probe was used to determine changes in rCBF. The probe tip was positioned above the surface of the intact dura and fixed to a support attached to the skull.

4. Middle cerebral artery occlusion (MCAO)

Middle cerebral artery occlusion was carried out according to the method of Zea-Longa et al.[20]. Male Sprague-Dawley rats were anaesthetised and the left middle cerebral artery(MCA) was occluded by an intraluminal filament at the origin from the Circle of Willis. Briefly, the left common carotid artery(CCA) was exposed through a midline incision. The internal carotid artery(ICA) was then isolated and its branch, the pterygopalatine artery, was ligated close to its origin. Then a 2㎝ length of 5-0 rounded dermalon suture with a slightly larger tip was gently advanced from the external carotid artery through the CCA and then up to the ICA for a distance of 11±0.5㎜. The production of ischemia was confirmed by rCBF measurement using a LDF. Following 120minutes of MCAO, cerebral blood reperfused.

5. Data analysis

The data are expressed as the mean±SE. The differences between groups were analyzed by Student's t-test. The significance level was set at $p<0.05$.

Results

1. Effect of NNR on rCBF in propranolol pretreated rats

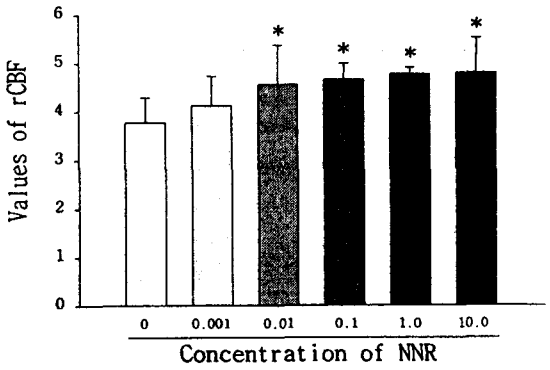

Fig. 1. Effect of NNR extract on rCBF in propranolol pretreated rats. The mean with standard errors were obtained from 6 experiments. If the value of control make 100, the rCBF of NNR treated rats can be 108, 117, 120, 123 and 123 with the dosage of 0.001, 0.01, 0.1, 1.0 and 10. *, statistically significant compared with controlled group.

Propranolol was pretreated and changes of rCBF were monitored. Propranolol pretreated rCBF showed 3.78±0.51 in the 0.5㎖ volume of vehicle, and NNR extract from 0.001 to 10 ㎎/㎏(i.v.) treatment showed as Fig. 1. In detailed numerical values, the rCBF were 3.78±0.51, 4.12±0.61, 4.55±0.82, 4.66±0.33, 4.77±0.13 and 4.80±0.72 at the i.v. infusion concentration of 0(control), 0.001, 0.01, 0.1, 1.0 and 10.0 ㎎/㎏. Statistical significance shown from the concentration of 0.01. In brief, NNR extract i.v. administration showed statistical significance on rCBF in propranolol pretreated rats.

2. Effect of NNR on rCBF in methylene blue pretreated rats

Methylene blue was pretreated and changes of rCBF were monitored. Methylene blue pretreated rCBF showed 4.06±0.41 in the 0.5㎖ volume of vehicle, and NNR extract from 0.001 to 10 ㎎/㎏(i.v.) treatment showed as Fig. 2. In detailed numerical values, the rCBF were 4.06±0.41, 4.39±0.91, 4.40±0.61, 4.60±0.81, 4.44±0.32 and 4.52±0.20 at the i.v. infusion concentration of 0(control), 0.001, 0.01, 0.1, 1.0 and 10.0 ㎎/㎏. But the values had no statistical significance when compared with control. In brief,

NNR extract i.v. administration showed no change on rCBF in methylene blue pretreated rats.

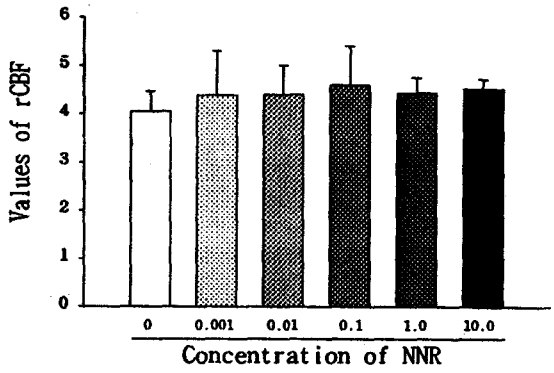

Fig. 2. Effect of NNR extract on rCBF in methylene blue pretreated rats. The mean with standard errors were obtained from 6 experiments. If the value of control make 100, the rCBF of NNR treated rats can be 107, 108, 113, 109 and 111 with the dosage of 0.001, 0.01, 0.1, 1.0 and 10.

3. Effect of NNR on rCBF in ODQ pretreated rats

ODQ was pretreated and changes of rCBF were monitored. ODQ pretreated rCBF showed 3.58±0.18 in the 0.5㎖ volume of vehicle, and NNR extract from 0.001 to 10 ㎎/㎏(i.v.) treatment showed as Fig. 3. In detailed numerical values, the rCBF were 3.58±0.18, 3.44±0.20, 3.45±0.22, 3.53±0.25, 3.45±0.18 and 3.45±0.13 at the i.v. infusion concentration of 0(control), 0.001, 0.01, 0.1, 1.0 and 10.0 ㎎/㎏. But the values had no statistical significance when compared with control. In brief, NNR extract i.v. administration showed no change on rCBF in ODQ pretreated rats.

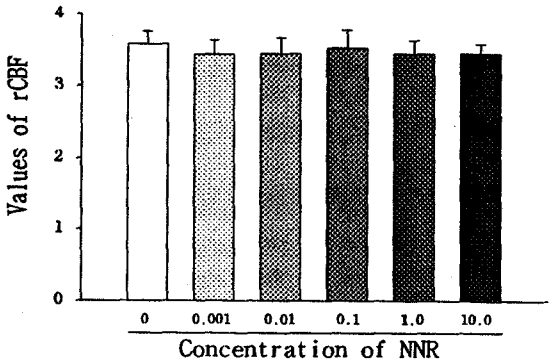

Fig. 3. Effect of NNR extract on rCBF in ODQ pretreated rats. The mean with standard errors were obtained from 6 experiments. If the value of control make 100, the rCBF of NNR treated rats can be 96, 968, 98, 96 and 96 with the dosage of 0.001, 0.01, 0.1, 1.0 and 10.

4. Effect of NNR on rCBF in L-NNA pretreated rats

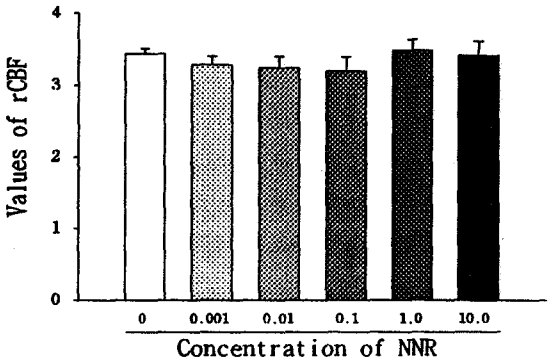

Fig. 4. Effect of NNR extract on rCBF in L-NNA pretreated rats. The mean with standard errors were obtained from 6 experiments. If the value of control make 100, the rCBF of NNR treated rats can be 95, 94, 93, 101 and 99 with the dosage of 0.001, 0.01, 0.1, 1.0 and 10.

L-NNA was pretreated and changes of rCBF were monitored. L-NNA pretreated rCBF showed 3.44±0.07 in the 0.5㎖ volume of vehicle, and NNR extract from 0.001 to 10 ㎎/㎏(i.v.) treatment showed as Fig. 4. In detailed numerical values, the rCBF were 3.44±0.07, 3.28±0.12, 3.24±0.162, 3.20±0.19, 3.48±0.16 and 3.41±0.20 at the i.v. infusion concentration of 0(control), 0.001, 0.01, 0.1, 1.0 and 10.0 ㎎/㎏. But the values had no statistical significance when compared with control. In brief, NNR extract i.v. administration showed no change on rCBF in L-NNA pretreated rats.

5. Time course effect of NNR treatment on the changes in rCBF induced by cerebral ischemia/reperfusion

Middle cerebral artery occlusion(MCAO) was

carried out according to the method of Zea-Longa et al.[20]. Following 120 minutes of MCAO, cerebral blood reperfused. 0.1% concentration of NNR i.v. infusion improved the altered cerebral hemodynamics of cerebral ischemic by increasing rCBF(Fig. 5).

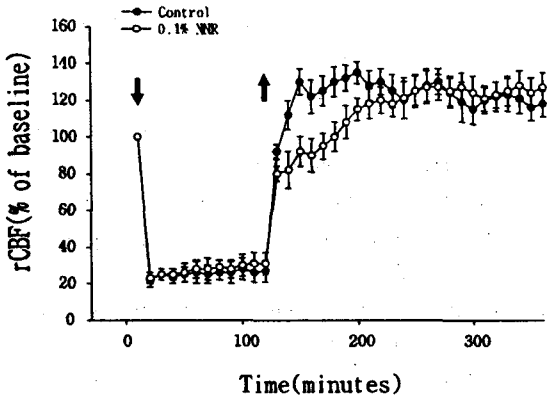

Fig. 5. Time course effect of 0.1% concentration of NNR treatment on the changes in rCBF induced by cerebral ischemia/reperfusion. ⬇, Occlusion of middle cerebral artery; ⬆, reperfusion of blood circulation. The mean with standard errors were obtained from 6 experiments.

Discussion

Nodus Nelumbinis Rhizomatis(NNR) are tuberous roots of the lotus water lily, Nelumbo nucifera GAERTN., family Nymphaeaceae, and has the effect of hemostasis with astringents and promoting blood circulation by removing blood stasis[1].

The use of traditional medicine is widespread and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several antiinflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or antiradical scavenging mechanism as part of their activity[21-23] plants have been extensively studied for their antioxidant activity and radical scavenging activity[24-27].

The nonselective β-adrenoceptor antagonist propranolol was pretreated and changes of rCBF were monitored. rCBF of propranolol pretreated rats showed significant change from the i.v. injection concentration of 0.01㎎/㎏ (Fig. 1). Guanylate cyclase/NO synthase(NOS) inhibitor methylene blue was pretreated and changes of rCBF were monitored, and the rCBF showed no change either(Fig. 2). Soluble guanylyl cyclase(sGC) inhibitor ODQ was pretreated and changes of rCBF were monitored. When ODQ was pretreated and changes of rCBF were monitored, NNR extract from 0.001 to 10㎎/㎏(i.v.) treatment showed no change in ODQ pretreated rats(Fig. 3). An inhibitor of all NO synthetases(NOS) L-NNA was pretreated and changes of rCBF were monitored. NNR extract from 0.001 to 10㎎/㎏(i.v.) treatment showed no change in L-NNA pretreated rats(Fig. 4).

Middle cerebral artery occlusion(MCAO) was carried out according to the method of Zea-Longa et al.[20]. Following 120minutes of MCAO, cerebral blood reperfused. NNR treatment improved the altered cerebral hemodynamics of cerebral ischemic by increasing rCBF(Fig. 5).

In conclusion, the ischemia/reperfusion induced oxidative stress may have contributed to cerebral damage in rats, and the present study provides clear evidence for the beneficial effect of NNR on ischemia induced brain injury. And the action mechanism of elevating effect of NNR on rCBF might be concerned with the role of guanylate cyclase, NOS, sGC, but not with β-adrenoceptor. The action of NNR as an ROS-scavenger might underlie the mechanism. The exact component and mechanism remains for the future study.

Conclusions

This study was carried out to determine whether NNR extract exerts beneficial effect against ischemia induced brain injury. The results are as follows: NNR extract treatment showed significant change from the i.v. injection concentration of 0.01㎎/㎏ in propranolol treated rats. NNR extract treatment showed no change on rCBF in methylene blue, ODQ and

L-NNA pretreated rats. 120minutes of MCAO and followed reperfusion, 0.1% concentration of NNR treatment improved the altered cerebral hemodynamics of cerebral ischemic by increasing rCBF. In conclusion, the present study provides clear evidences for the beneficial effect of NNR on ischemia/reperfusion induced brain injury.

Acknowledgements

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