行政院國家科學委員會專題研究計畫 成果報告

Resveratrol 在於清醒大鼠經過慢性局部大腦缺血傷害的神經保護作用

研究成果報告(精簡版)

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目錄：

報告內容：

前言：

Our previously study showed that resveratrol could suppress infarct volume and exert neuroprotective effect on anesthetized rats subjected to acute focal cerebral ischemia (FCI) injury (Huang et al., 2001b). Resveratrol, a polyphenol found in red wine and grapes, is thought to be a major biologically active ingredient that contributes to the cardioprotective effects of red wine (Renaud and Lorgeril, 1992; Baur and Sinclair, 2006). A recent study showed that dietary resveratrol increased survival and insulin sensitivity in mice who were maintained on a high-calorie diet (Baur et al., 2006). At the cellular and molecular level, resveratrol inhibits the oxidation of low-density lipoproteins (Frankel et al., 1993), reduces platelet aggregation (Szewczuk et al., 2004; Olas and Wachowicz, 2005), promotes vasodilation (Cruz et al., 2006), and enhances endothelial nitric oxide synthase activity (Wallerath et al., 2002). Moreover, resveratrol protects the heart (Hung et al., 2004), spinal cord (Kiziltepe et al., 2004), and kidneys (Giovannini et al., 2001) from ischemia-reperfusion injury.

研究目的：

In the present study, we used conscious Long-Evans rats to investigate the effects of resveratrol on chronic FCI injury, we applied resveratrol daily after right middle cerebral artery (MCA) ligation for 4 weeks. We evaluate the infarct brain volume and functional motor deficits before and after FCI injury for evaluation the therapeutic effect of resveratrol on brain lesions induced by chronic FCI injury. The degree of ischemic brain injury was estimated by infarct volume of right MCA territory at 4 weeks after MCA occlusion. The functional motor deficits were quantified with rotarod test and grasping power test once per week.

文獻探討：

Cerebrovascular disease is the second largest cause of the top ten causes of death here in Taiwan. Therefore, it’s a quite important topic on study for cerebrovascular diseases. There are many studies to report the relationship of ischemia and ischemia-reperfusion injury on the cause of cerebral infarction and the role of free radical on ischemia and ischemia-reperfusion injury (Das et al., 1986; Globus et al., 1995; He et al., 1993). Because of the potent and unstable characters of free radicals, it is easy for free radicals interact with functional groups in living tissue, leading to the results of cellular membrane destruction and even cellular death (Hall et al., 1989). Studying on free radical scavenging medications on issue of ischemia and ischemia-reperfusion induced tissue damage as the strategy to treat cerebrovascular disease are very important (Huang et al., 2001a). Resveratrol, a polyphenol found in red wine and grapes, is thought to be a major biologically active ingredient that contributes to the cardioprotective effects

of red wine (Renaud and Lorgeril, 1992; Baur and Sinclair, 2006). A recent study showed that dietary resveratrol increased survival and insulin sensitivity in mice who were maintained on a high-calorie diet (Baur et al., 2006). At the cellular and molecular level, resveratrol inhibits the oxidation of low-density lipoproteins (Frankel et al., 1993), reduces platelet aggregation (Szewczuk et al., 2004; Olas and Wachowicz, 2005), promotes vasodilation (Cruz et al., 2006), and enhances endothelial nitric oxide synthase activity (Wallerath et al., 2002). Moreover, resveratrol protects the heart (Hung et al., 2004), spinal cord (Kiziltepe et al., 2004), and kidneys (Giovannini et al., 2001) from ischemia-reperfusion injury.

研究方法：

1-1 Animals

All procedures in this present investigation were approved by Chung Shan Medical University Animal Studies Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication NO. 85-23, revised 1996). Male Long-Evans rats (National Lab. Animal Breeding and Research Center) weighing 250-300g were used in this study. The animals were housed in a room with controlled temperature (24±1°C) and humidity (55±5%) under a 12:12 h light-dark cycle. They were allowed free access to food and water.

1.2. Surgical procedure

Our technique was a modification of our previously described method. In brief, In brief, each male Long-Evans rat was anesthetized by inhalation of isoflurane (2%) in air during surgical preparation. Body temperature was maintained during surgery at 37±0.5°C with a heating pad servo-controlled by a rectal probe. The ventral tail artery was cannulated for continuous monitoring of heart rate (HR) and mean arterial blood pressure (MABP) by Statham P23 XL transducer and displayed on a Gould RS-3400 physiological Recorder (Gould, Cleveland, OH, USA) and the pH, P_{O_2} and P_{CO_2} in the blood were tested using blood sampling with Blood Gas Analyzer (GEM-5300 I.L. CO, USA). Measurements were performed before, during, and just after unilateral MCA occlusion.

Focal ischemic infarcts were produced in the right lateral cerebral cortex in the territory of MCA. Both common carotid arteries were exposed by midline anterior cervical incision. The animal was placed in a lateral position, and a skin incision was made at the midpoint between the right lateral canthus and the anterior pinna. The temporal muscle was retracted, and a small (3-mm diameter) craniectomy was made at the junction of the zygoma and squamosal bone using a drill (Dremel Multipro+5395, Dremel com. USA) cooled with saline

solution. Using a dissecting microscope (OPMI-1, ZISS, Germany) and the right MCA was ligated with 10-0 monofilament nylon ties. Both common carotid arteries were then occluded by microaneurysm clips for 1 hr. After removing the clips, return of flow was visualized in the arteries.

1.3. Experimental groups

Resveratrol was purchased from Sigma Chemical Company (St. Louis, Mo. USA). Resveratrol (10 μg/kg) or vehicle (dimethyl sulfoxide-0.9% NaCl, $1:10^4$; v/v) were administrated once per day after FCI injury. Rats injected with vehicle were used as control. No effect of vehicle on FCI injury at such concentration.

After FCI injury, rats were assigned, in randomized sequence, to one of the five different treatment groups (n=10 each): (a) sham group: animals underwent the same described surgical procedures except MCA ligation (b) control group: applied vehicle (i.p.), (c) resveratrol group: applied resveratrol 10 μg/kg (i.p.).

1.4. Infarct volume analysis

After focal cerebral ischemia for 1 hr and reperfusion for 4 weeks, the rats were anesthetized and killed by rapid decapitation. Brains were removed, inspected visually for the anatomy of the MCA and for signs of hemorrhage or infection, immersed in cold saline solution for 10 minutes, and sectioned into standard coronal slices (each 2-mm thick) using a brain matrix slicer (JACOBOWITZ Systems, Zivic-Miller Laboratories INC, Allison park, USA). Slices were placed in the vital dye 2,3,5-triphenyltetrazolium chloride (TTC, 2%; Sigma, USA) at 37°C in the dark for 30 minutes, followed by 10% formalin at room temperature overnight. The outline of right and left cerebral hemispheres as well as that of infarct tissue, clearly visualizable by TTC staining, was outlined on the posterior surface of each slice using an image analyzer (color image scanner, EPSON), connected to an image analysis system (AIS software, Imaging research INC, Canada), which was run on a personal computer (AMD K6-2 3D 400). The area of infarction was measured by subtracting the area of the non-lesioned ipsilateral hemisphere from that of the contralateral side. Infarct volume was calculated as the sum of infarct area per slice multiplied by slice thickness. Both the surgeon and image analyzer operator were blinded to the treatment given each animal.

1.5. Behavioral tests

Behavioral measurements were performed by the rotarod test and the grasping power test at the end of 1st, 2nd, 3rd, and the 4th week after focal FCI injury.

1.5.1. Rotarod test

The accelerating rotarod was used to assess motor deficit following ischemic injury in rats. The rats were placed on rungs of the accelerating rotarod and the amount of time the animals remained on the rotarod was measured. The speed was increased slowly from 4 rev/min to 40 rev/min over the course of 5 minutes. The time, in seconds, at which each animal fell off the rungs was recorded. Each animal received three consecutive trials.

1.5.2 Grasping power test

The grasping power test was a modification of the method of Bertelli et al. For the assessment of grasping strength, a bar of wires was connected to an ordinary electronic balance. Both forepaws were tested, tested one forepaw at a time. The untested forepaw was temporarily prevented from grasping by wrapping it with adhesive tape, and the tested forepaw was kept free. The rats were allowed to grasp the bar while being lifted by the tail with increasing firmness until they loosened their grip and the grasping power was scored.

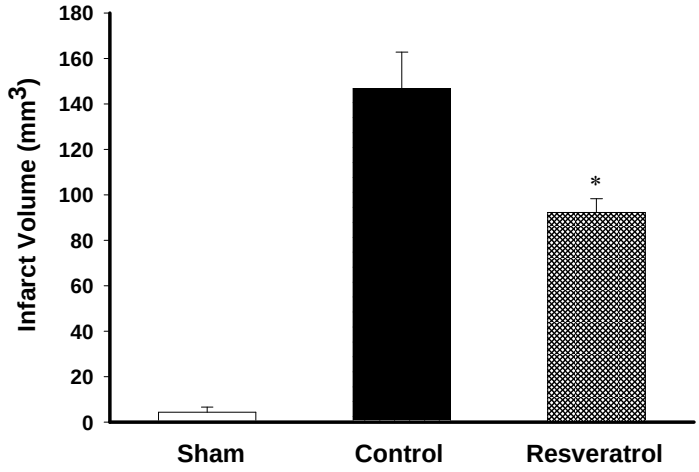
1.6. Statistics

Data are expressed as mean ± standard error of mean (S.E.M.). Statistical analysis of the differences in volume of infarcts and the behavioural deficit scores of rats between control and treatment groups were carried by one-way analysis of variance (ANOVA) followed by unpaired, two-tailed *t*-tests for combined data. A value of $P < 0.05$ was considered to be statistically significant.

結果：

3.1. Infarct volume

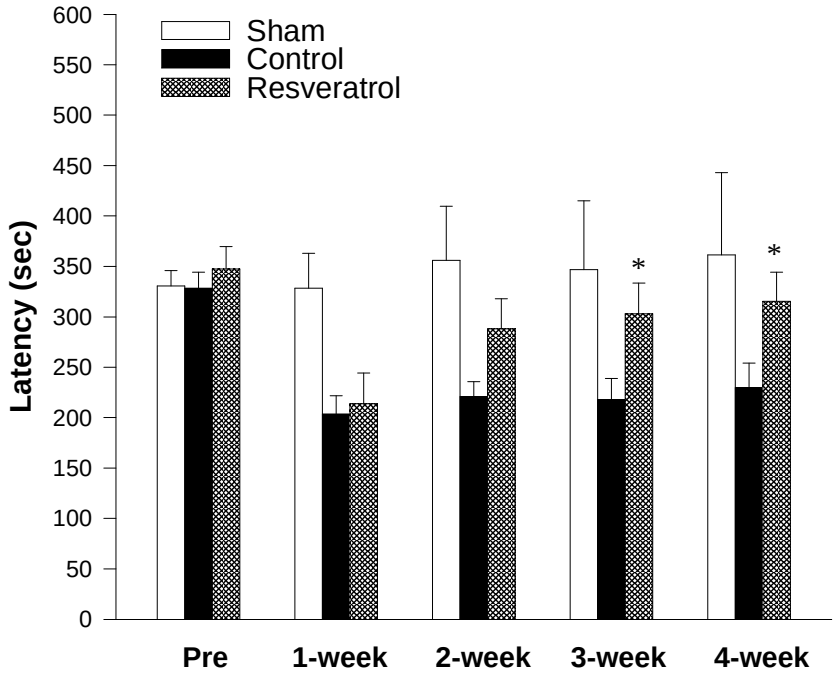
Reproducible brain infarcts were obtained from a territory of right MCA occlusion in the sham group, the resveratrol group, and the control group. The infarct volume at the end of 4th week after FCI injury in the resveratrol group was significantly reduced (92±6 mm^3, $P<0.05$) as compared with the control group (145±16 mm^3) (Fig. 1).The results indicated that intraperitoneal applied resveratrol after FCI injury significantly reduced the total infarcted volume by 37% compared to that of the control group.



3.2. Behavioral results

3.2.1. Rotarod test

The results of the rotarod tests of the three groups are shown in Fig. 2. The duration that the animals stayed on the rotarod was not significantly different among the three groups before surgical preparation. After FCI injury, the mean latencies for rats to stay on the rotarod were 99%, 62% and 61% of baseline, respectively, in the sham, control, and resveratrol groups at the end of 1st week after CFI injury, but 109%, 70% and 91% of baseline at the end of 4th week after FCI injury. The results showed that after FCI injury, the amount of time rats stayed on the rotarod was significantly shorter in the control group than in resveratrol animals at the 4th week. This result indicated that resveratrol could improve the balance and coordination of rats after FCI injury.

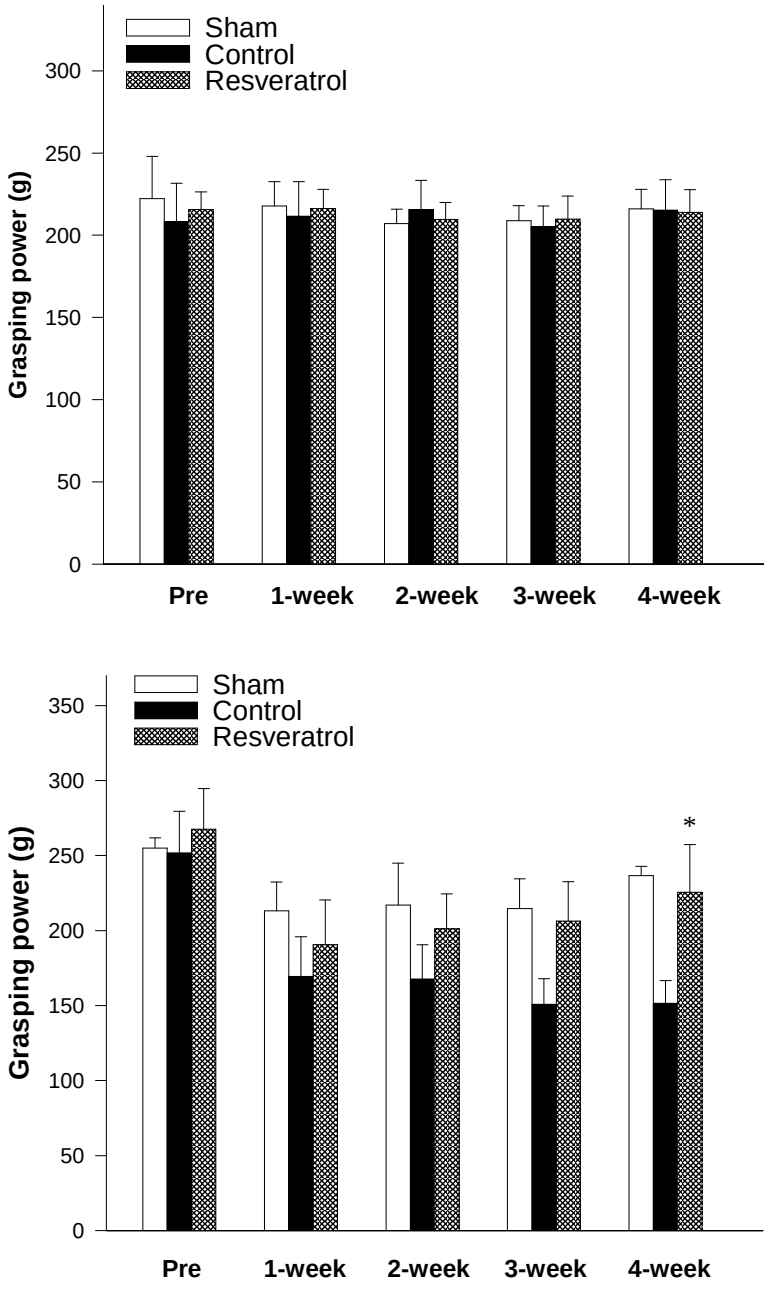


3.2.2. Grasping power test

The effects on grasping power test of the four groups are shown in Fig. 3. Among the three groups, there is no significant difference in the mean grasping power of the left forepaws of rats before the right MCA occlusion, or in the right forepaws of rats before or at the end of the 1st, 2nd, 3rd, and 4th week after right MCA occlusion.

The mean value of grasping powers were 84%, 67 and 71% of baseline, respectively, in the sham, control and resveratrol groups at the end of 1st week after FCI injury, but 93%, 60% and 84% of baseline at the end of 4th week after FCI injury.

This result showed that resveratrol is associated with improvement of grasping strength of rats after FCI injury.



討論：

In order to test the neuroprotective effect of resveratrol on conscious Long-Evans rats subjected to chronic focal cerebral ischemia injury, we daily intraperitoneal injection of 10 μg/kg/day resveratrol for four weeks. Our results showed that application of resveratrol significantly decreased the infarct volume in the MCA occluded rats and significantly improved the motor performance at the end of 4th week after chronic FCI injury.

Occlusion of the MCA in our study not only caused a wide range of infarct volume in rats also but an impairment in motor performance, which was evident from the decreased neurological scores in the rotarod test and the grasping power tests. Our results are consistent with those of other reports, which show that chronic FCI injury induced neuronal damage in experimental animals (Chen et al., 2001; Yonemori et al., 1999). The neuronal damage, in the territory of MCA, i.e caudate putamen, and striatum, will cause motor deficits (Rogers et al., 1997).

Resveratrol is present mostly in the skin and the seeds of grapes and is one of the major components of red wine (Huang et al., 2001b). Several antioxidant activities of resveratrol have been reported. It is known to inhibit the oxidation of low-density lipoproteins and may thereby inhibit atherosclerotic changes (Frankel et al., 1993). In stroke-prone spontaneously hypertensive rats, long term administration of a resveratrol supplement results in decreased levels of urine 8-hydroxydeoxyguanosine and plasma glycated albumin (Mizutani et al., 2001). These results indicate that resveratrol plays a role in suppressing oxidative DNA damage and glycoxidative stress in vivo. Resveratrol also has anti-inflammatory effects. It prevents leukocyte recruitment and endothelial barrier disruption that is induced by several superoxide-dependent proinflammatory stimuli, including ischemia/reperfusion and oxidants generated by the reaction of hypoxanthine/xanthine oxidase or platelet-activating factors (Shigematsu et al., 2003). Resveratrol can also act as a vasorelaxant. In particular, it causes endothelium-dependent vasorelaxation by acting on the nitric oxide (NO)-mediated pathway (Orallo et al., 2002). Other NO-independent dilatory mechanisms may be involved in the resveratrol-induced vasodilatation of arteries during coronary heart disease (Cruz et al., 2006).

In conclusion, in the designed rat model, daily intraperitoneal administration of resveratrol for four weeks significantly reduced the infarct volume in the MCA occluded rats and significantly improved the motor performance. This potential effect may benefit in the treatment of ischemic brain disease, such as stroke, thrombosis or embolization of MCA.

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計畫成果自評：

This study had finished and prepared for publish.