行政院國家科學委員會專題研究計畫 成果報告

白藜蘆醇與胰島素對抗第一型糖尿病鼠遭受急性缺血性中風傷害的機制評估

研究成果報告(精簡版)

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中 文 摘 要 ： 背景：相較於沒有糖尿病的中風病人，具有糖尿病的中風病人有較高的死亡率，較差的神經修復以及較嚴重的不良於行，最近的臨床試驗發現利用胰島素嚴密監控血糖治療糖尿病的中風病人的結果非常矛盾，所以我們希望藉由糖尿病大鼠遭受缺血性中風傷害研究胰島素除了降血糖作用還有甚麼因子參予在其神經保護作用中。
材料和方法： 大花鼠分成三組，分別是 control 組，糖尿病組，以及以胰島素治療糖尿病組，使其經歷局部大腦缺血再灌注損傷。
結果：研究發現高血糖會惡化局部大腦缺血再灌注損傷，增加梗塞體積惡化神經損傷，抑制大腦皮質的葡萄糖攝入以及葡萄糖轉運（GLUT1）活性，減少 Akt 以及 endothelial nitric oxide synthase (eNOS)的蛋白質磷酸化。胰島素治療可以減輕高血糖以及糖尿病症狀，還可以有意義的減少局部大腦缺血再灌注損傷的糖尿病大鼠的大腦梗塞體積以及神經功能損傷，並增加大腦皮質的 Akt 以及 eNOS 的蛋白質磷酸化，然而胰島素治療不能恢復葡萄糖攝入以及葡萄糖轉運（GLUT1）活性。投予 eNOS 抑制劑 N-iminoethyl-L-ornithine 會抑制胰島素改善局部大腦缺血再灌注損傷的糖尿病大鼠的大腦梗塞體積以及神經功能損傷的好處，但是並不影響胰島素的降血糖功能。
結論：此研究提出胰島素必須維持 eNOS 的活性才能夠在於糖尿病遭遇缺血性中風傷害的狀況下表現神經保護作用。

中文關鍵詞： Akt；糖尿病；內皮；eNOS；胰島素；缺血性中風

英 文 摘 要 ： Background and Purpose—Stroke patients with diabetes have a higher mortality rate, worse neurological outcome, and more severe disability than those without diabetes. Results from clinical trials comparing the outcomes seen with intensive glycemic control versus insulin therapy in the treatment of diabetic stroke are conflicting. Therefore, the present study was aimed to identify the key factor involved in the neuroprotective action of insulin beyond its hypoglycemic effects in streptozotocin (STZ)-diabetic rat with ischemic stroke.
Methods—Long-Evans male rats were divided into three groups (control, diabetes, and diabetes treated with insulin) and subjected to focal cerebral ischemia-reperfusion (FC I/R) injury.

Results—Hyperglycemia aggravated FC I/R injuries with an increase in cerebral infarction and neurological deficits, inhibition of glucose uptake and membrane trafficking activity of glucose transporter 1 (GLUT1), and reduction of Akt and endothelial nitric oxide synthase (eNOS) phosphorylation in the cerebrum. Insulin treatment alleviated hyperglycemia and the symptoms of diabetes in STZ-diabetic rats. Insulin administration also significantly decreased cerebral infarction and neurological deficits and increased phosphorylation of Akt and eNOS protein in the cerebrum of FC I/R injured diabetic rats. However, the glucose uptake and membrane trafficking activity of GLUT1 in the cerebrum were not restored by insulin treatment. Co-administration of the eNOS inhibitor, N-iminoethyl-L-ornithine, with insulin abrogated beneficial effects of insulin on cerebral infarct volume and neurological deficits in FC I/R injured diabetic rats without affecting the hypoglycemic action of insulin. Conclusions—These results suggest that eNOS activation is required for the neuroprotection of insulin against ischemic stroke in diabetes.

英文關鍵詞： Akt； Diabetes； Endothelium； eNOS； Insulin； Ischemic stroke

目錄：

報告內容：

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前言：

Diabetes mellitus has been shown to aggravate brain injury induced by ischemic stroke and is associated with higher mortality, worse functional outcome, and increased frequency for recurrent stroke.[1, 2] The mechanism responsible for the aggravating effects of diabetes on ischemic brain injury is not fully understood.[3, 4] Among the various aspects of diabetic pathology, hyperglycemia is likely the most prominent contributor to excessive risk and worse outcome in stroke patients with diabetes.[5-7] However, nearly all of the clinical trials in diabetic patients have failed to show a reduction in cardiovascular disease (CVD) with intensive glycemic control (IGC),[8, 9] suggesting that the pathophysiology of the link between diabetes and CVD is complex, involving more than just hyperglycemia. Moreover, the Action to Control Cardiovascular Risk in Diabetes trial indicates that IGC is associated with an increased risk of cardiovascular death and all-cause mortality.[10] In contrast, the Diabetes Control and Complications Trial demonstrated that IGC reduces CVD outcomes, including risk of nonfatal myocardial infarction, stroke, or death.[11] On the other hand, the UK Prospective Diabetes Study showed insignificant reduction of cardiovascular complications and no obvious benefit of IGC on CVD outcomes such as stroke.[12, 13] Currently, whether IGC reduces risk or improve the outcome of stroke in diabetic individuals is controversial.

研究目的：

The effects of insulin that result in protection against cerebral ischemic insult in diabetes remain undefined. In addition, the inconsistent results from use of IGC to protect cerebral function warrant further investigation. Herein, the streptozotocin (STZ)-induced diabetic rat model was employed to explore the possible mechanisms contributing to the neuroprotective effect of glycemic control with insulin in focal cerebral ischemia-reperfusion (FC I/R) injury.

文獻探討：

In addition to its hypoglycemic effect, insulin exerts neuroprotection in diabetic rats subjected to cerebral ischemia.[14, 15] However, it also has been demonstrated that insulin attenuates brain damage independent of its hypoglycemic effect,[16] even though insulin may have a negative impact on the central nervous system (CNS) either directly[17] or indirectly through the reduction of circulating glucose levels.[18, 19] In the past, the direct effects of insulin on the CNS have largely been ignored, since insulin was considered incapable of crossing the blood brain barrier.[20] However, in recent years both insulin and its receptor have been identified in the CNS and their associated biological functions have been extensively studied.[21] Glucose uptake is known to be a function of CNS activity and insulin has been

shown to increase glucose uptake in the brain.[22] Although recent studies have reported the downregulation of cerebral glucose transporters (GLUT) by ischemic injury and tissue necrosis,[23] the effects of diabetes and insulin treatment on glucose transport and GLUT expression in the cerebral tissue with ischemic injury remain unclear. It is generally accepted that insulin, on binding to its receptor, initiates the tyrosine phosphorylation of cellular substrates including the insulin receptor substrate family members, which in turn modulate the activation of phosphatidylinositol 3-kinase and downstream kinase such as Akt that are responsible for initiating a number of cellular effects.[24] These include the improvement of synaptic plasticity and neuronal survival following cerebral ischemia-reperfusion injury.[24-26] Additionally, insulin also elicits vasodilatation in vivo, a response that can be associated with the reduced sympathetic nerve activity, activation of ATP-dependent K^+ channels,[27] release of vasodilator adenosine,[28] or activation of endothelial Akt for NO production.[29-31]

研究方法：

Animals. This study abides by the rules in 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). Six-week old Long-Evans male rats (National Lab. Animal Breeding and Research Center, Taiwan) weighing 250-280g were used throughout this study and were maintained in the Animal Center of the Chang Gung University, at an ambient temperature of 25 ± 1 ℃ and a light-dark period of 12 hrs. The animals were fed with normal chow and water. All surgical procedures and post-operative animal care were reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung University.

Induction of STZ-diabetic animal model. Rats were fasted for 72 hrs and anesthetized by intraperitoneal injection (i.p.) of pentobarbital at a dose of 65 mg/kg. The animals were randomly divided into two groups: (1) age-matched non-diabetic control (CONT) and (2) STZ-induced diabetes. The animals were subjected to a single intravenous injection of vehicle (normal saline) for CONT or freshly prepared STZ (65 mg/kg) for diabetes induction. STZ has been shown to destroy pancreatic β-cells and induce hyperglycemia during the first 24 hrs after injection.[32] Two weeks later, blood glucose levels were measured using the glucose oxidase method (*ChemWell® 2910 Automated EIA and Chemistry Analyzer* Spectra, GMI Inc., USA) as described previously.[33] Animals with blood glucose levels above 300 mg/dl and symptoms of polyphagia, polyuria, and polydipsia were used for this study. The STZ-diabetic animals were further randomly divided into two groups: (1) STZ-induced diabetic rats treated with saline vehicle (DM) and (2) diabetic rats treated with insulin (DMI). The insulin Monotard® HM (4 IU/rat/day; Novo Nordisk, Bagsvaerd, Denmark) was administered subcutaneously to STZ-diabetic rats for 1 week (i.e., from day 15 to 21 after STZ injection).

Focal cerebral ischemia-reperfusion and infarct volume analysis. After 24 hrs of the last vehicle or insulin treatment, the rats were anesthetized with chlorohydrate (450

mg/kg, i.p.) and body temperature was maintained at 37±0.5°C with a heating pad servo-controlled by a rectal probe. Focal ischemic infarcts in the right lateral cerebral cortex were produced by ligating the right middle cerebral artery (MCA) and occluding both common carotid arteries as described in our previous studies.[1, 2] Blood flow in the common carotid arteries was restored after 1 hr of occlusion but the right MCA ligature was left in place permanently. Our previous studies have shown a consistent formation of cerebral infarct in the right MCA territory following 1-hr FC I/R.

Twenty-four hours after FC I/R-injury, the animals were anesthetized with urethane (1.2 g/kg, i.p.) and killed by rapid decapitation. Brains were removed and inspected visually. Brains without signs of hemorrhage or infection were accepted for further study. Brains were 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 and kept in the dark for 30 minutes, followed by 10% formalin treatment at room temperature overnight. The outline of the right and left cerebral hemispheres as well as that of the infarcted tissue, which lacks TTC staining,[1] was visualized on the posterior surface of each slice using an image analyzer (color image scanner, EPSON GT-9000) coupled to an image analysis system (AIS software, Imaging research INC, Canada). 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 of each animal.

Assessment of neurological functions. Twenty-four hrs after FC I/R injury the modified Bederson score[3] was used to determine global neurological function according to the following scoring system: 0, no deficit; 1, forelimb flexion; 2, decreased resistance to lateral push; 3, unidirectional circling; 4, longitudinal spinning; 5, no movement. Motor function and coordination were evaluated by the grip test.[4] For grip testing, the rat was placed midway on a string between two supports and rated as follows: 0, falls off; 1, hangs onto string by one or both forepaws; 2, as for 1, and attempts to climb onto string; 3, hangs onto string by one or both forepaws plus one or both hind-paws; 4, hangs onto string by fore- and hind-paws plus tail wrapped around string; 5, escape (to the supports). Neurological scores were assessed by an independent and blinded investigator.

Assessment of cerebral glucose uptake. To compare cerebral glucose uptake in control, DM, and insulin-treated DM rats, 2-deoxy-[^{3}H] glucose (2-DG; PerkinElmer, Boston, MA, USA) was administered as an intravenous bolus (1.85 MBq) at the end of FC I/R injury. Brain tissue from the right cerebrum with MCA occlusion (ipsilateral) was harvested for 2-DG uptake and protein analysis. The left cerebrum (contralateral) was used as non-I/R control from the same animal. The method for measurement of cerebral glucose uptake has been described in our previously studies.[5] Briefly, blood samples (0.05 ml) from the femoral artery

were collected for glucose and 2-DG measurement at 5, 10, 20, 40, and 80 min after intravenous injection of 2-DG (1.85 MBq). Brain tissues *were also collected at the last blood sample collection 80 minutes after intravenous injection of* 2-DG. Because 2-DG is a glucose analog that is phosphorylated but not further metabolized in the tissue, glucose uptake in cerebral tissues can be estimated by determining the tissue content of 2-DG-6-P and the plasma 2-DG profile, then fitting to a double exponential or linear curve. The concentration of phosphorylated 2-DG in tissues was calculated as the difference between total ^{3}H-radioactivity of the tissue extracts and the ^{3}H-radioactivity remaining after Somogyi treatment.

Protein extraction and western blot analysis. The membranous and cytoplasmic fraction of cerebral tissue were isolated as described in our previous studies.[2, 5] Protein (30 μg) from cytoplasmic and membranous fractions were subjected to 10% SDS-PAGE and electrophoretically transferred to polyvinylidene difluoride protein sequencing membrane for 2 hrs. The membrane was blocked with 5% non-fat milk in Tris-buffered saline with 0.1% Tween-20. It was then washed and blotted with antibodies against glucose transporter isoforms GLUT1 (Chemicon, USA), GLUT3 (Chemicon, USA) and GLUT8 (Santa Cruz Biotechnology, USA). The membrane was then incubated with HRP-conjugated secondary antibody prior to chemiluminescence detection (Pierce, USA). The expression of nitric oxide synthase (NOS) isoforms nNOS (Upstate, USA), iNOS (Chemicon, USA), eNOS (Chemicon, USA) and phospho-eNOS (ser1177) (Cell signaling, USA), as well as Akt (Santa Cruz Biotechnology, USA) and phospho-Akt (ser473) (Cell signaling, USA), was also analyzed. Tubulin and Na^{+}-K^{+} ATPase were used as internal loading control for the cytosolic and membranous fractions, respectively.

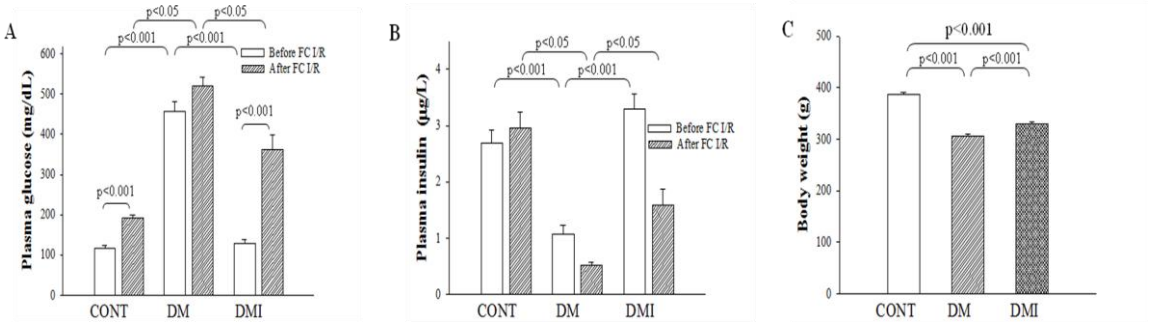
Biochemical Analysis. Blood was collected from the tail vein for biochemical measurements in experimental rats. Plasma was used for the measurements of glucose and insulin. Insulin was measured using a commercial available kit provided by Mercodia (rat insulin ELISA kit, Uppsala, Sweden). Blood glucose levels were determined by the glucose oxidase method (*ChemWell® 2910 Automated EIA and Chemistry Analyzer* Spectra, GMI Inc., USA).

Statistics. Data are expressed as mean ± standard error of mean (SEM). Statistical analysis of differences were carried out by one-way analysis of variance (ANOVA) for combined data and followed by Bonferroni test. $P < 0.05$ was considered to be statistically significant.

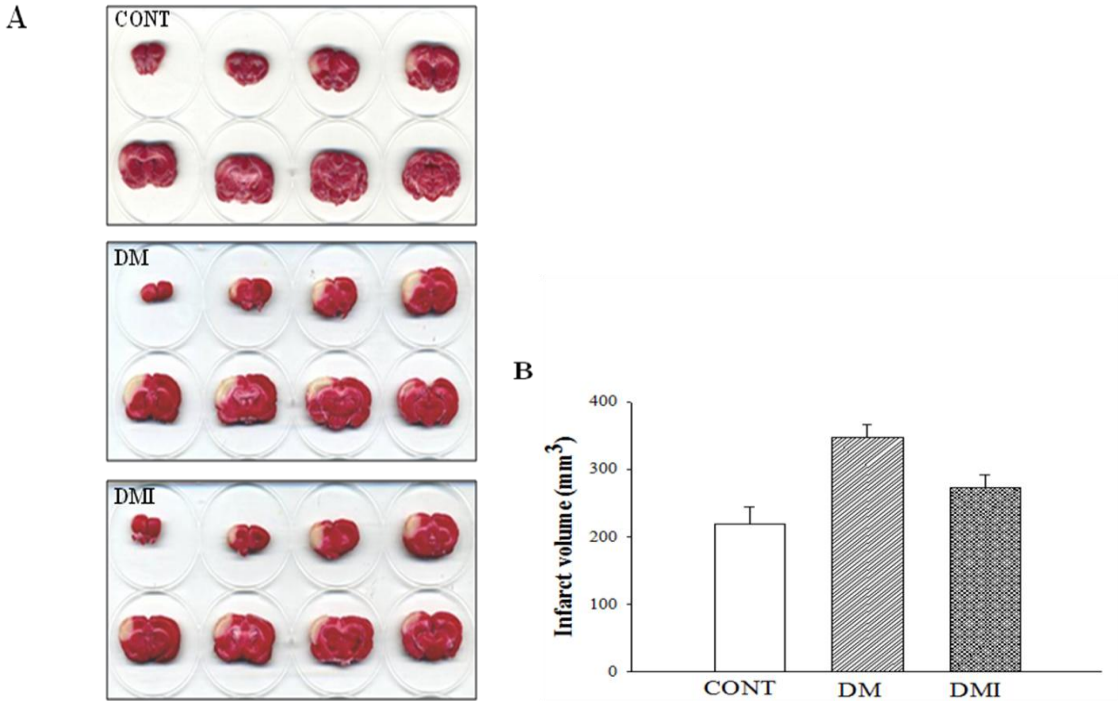
結果：

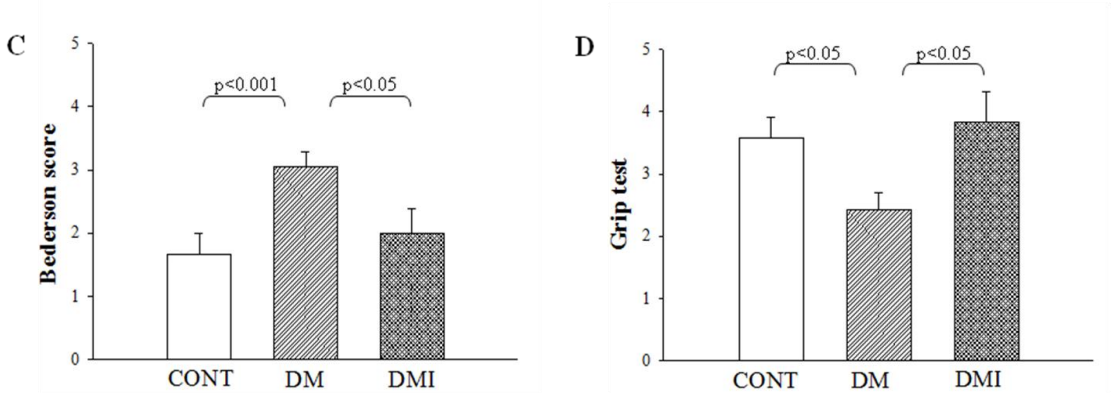
Body weight and plasma levels of glucose and insulin. Animals treated with STZ consistently developed hyperglycemia and hypoinsulinemia that persisted throughout a 3-week period of study. Plasma glucose and insulin levels in diabetic rats were 457±24 mg/dL and 1.08±0.16 μg/L compared with 117±6 mg/dL and 2.70±0.22 μg/L in age-matched

non-diabetic controls, respectively (Figures 1A and 1B). As expected, diabetic rats had lower body weights compared to the age-matched controls (Figure 1C). In contrast, insulin treatment (4 IU/rat/day) for 1 week significantly attenuated STZ-induced hyperglycemia, hypoinsulinemia, and weight loss (Figure 1).



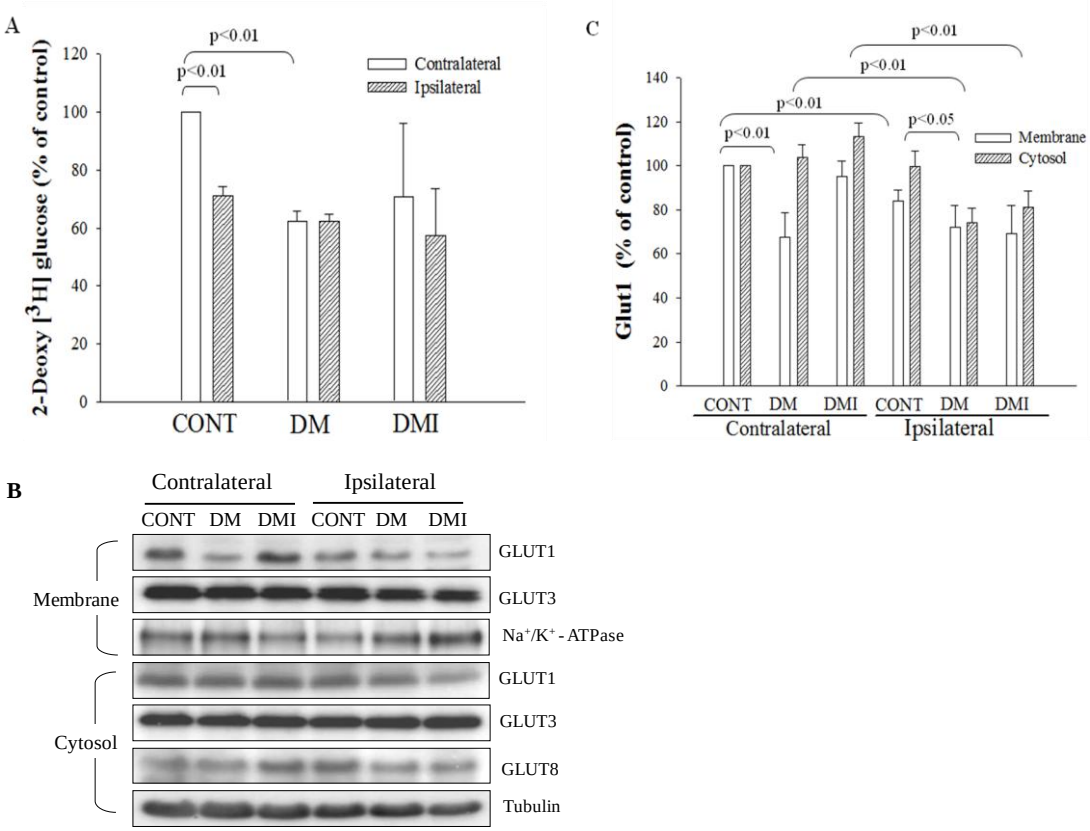
FC I/R-induced brain infarction and neurological deficits. There were no statistical differences in blood gas parameters (PO_2, PCO_2, and pH) of the animals among groups (data not shown) and FC I/R (n=19) generated cerebral infarcts with an average volume of 219.7±24.8 mm^3 in volume Figure 2). The infarct size was significantly increased by about 60% in STZ-diabetic rats ($P < 0.001$, n=21; Figures 2A and 2B). In addition to its hypoglycemic effect, insulin treatment significantly reduced FCI/R-induced infarct volume from 346.9±19.8 to 273.7±17.8 mm^3 ($P < 0.05$, n=12; Figures 2A and 2B). For evaluation of neurological functions, the Bederson and grip tests were performed to examine postural reflexs and muscle strength, respectively. After ischemic stroke, the Bederson and grip scores were 1.6± 0.2 and 3.5± 0.4 in non-diabetic control rats, respectively (Figures 2C and 2D). Both postural reflex and muscle strength were further impaired in diabetic animals (Figures 2C and 2D). The adverse effects of diabetes on neurological function were prevented in animals treated with insulin (Figures 2C and 2D).





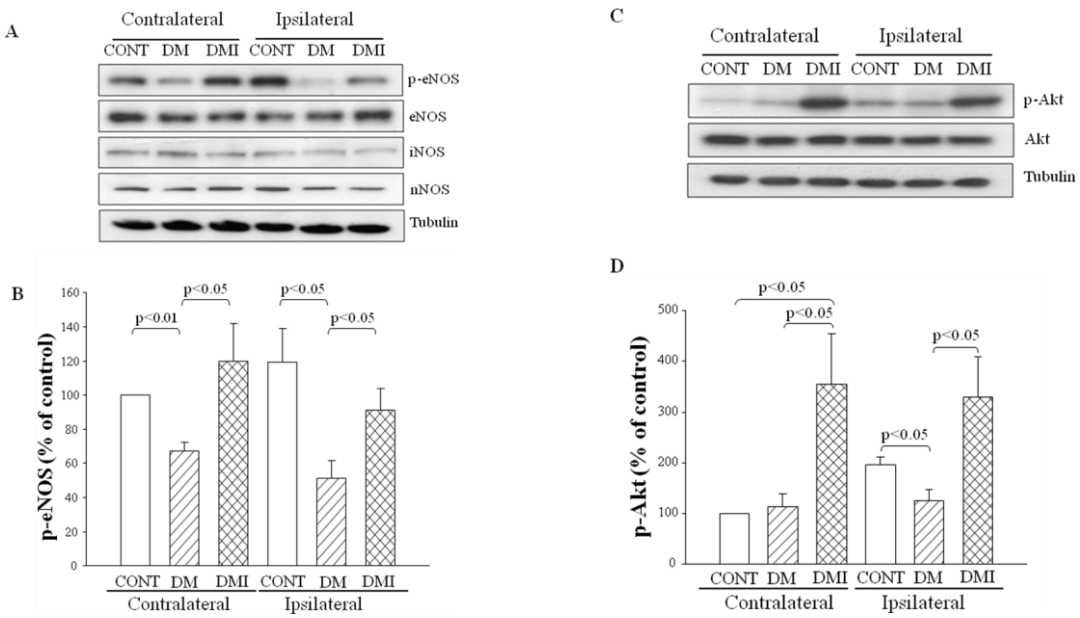
Cerebral glucose uptake and GLUT protein expression. The effect of FC I/R injury on cerebral glucose uptake and GLUT protein expression were examined in control and diabetic animals with or without insulin treatment. The glucose uptake in ipsilateral cerebrum subjected to I/R was reduced by about 29% compared with its contralateral control without I/R (Figure 3A). Diabetes alone also compromised the glucose uptake rate by 38%, but FC I/R injury did not further reduce glucose uptake activity in diabetic animals (Figure 3A). Insulin treatment failed to improve cerebral glucose uptake in diabetic rats with or without FC I/R injury (Figure 3A).

Expression of GLUT proteins (GLUT 1, 3, and 8) in contralateral and ipsilateral brain tissue was examined by Western blotting. For membranous proteins, the GLUT1 expression was significantly reduced by diabetes in contralateral non-I/R tissue (Figures 3B and 3C). Insulin treatment has a tendency, but not in a significant manner, to improve GLUT1 expression (Figures 3B and 3C). FC I/R injury on the ipsilateral tissue significantly reduced GLUT1 expression, and diabetes did not further reduce the expression. Insulin treatment had no effect on the suppressed GLUT1 expression (by FC I/R injury) in diabetic animals (Figures 3B and 3C). For cytosolic proteins, GLUT1 expression in contralateral non-I/R tissue was not altered in diabetic animals with or without insulin treatment (Figures 3B and 3C). FC I/R injury also had no effect on the cytosolic GLUT1 expression, but this insult significantly reduced GLUT1 expression in the diabetic animals in a manner insensitive to insulin treatment (Figures 3B and 3C). In contrast, the GLUT3 and GLUT8 protein expressions were not significantly different among the groups (Figure 3B).

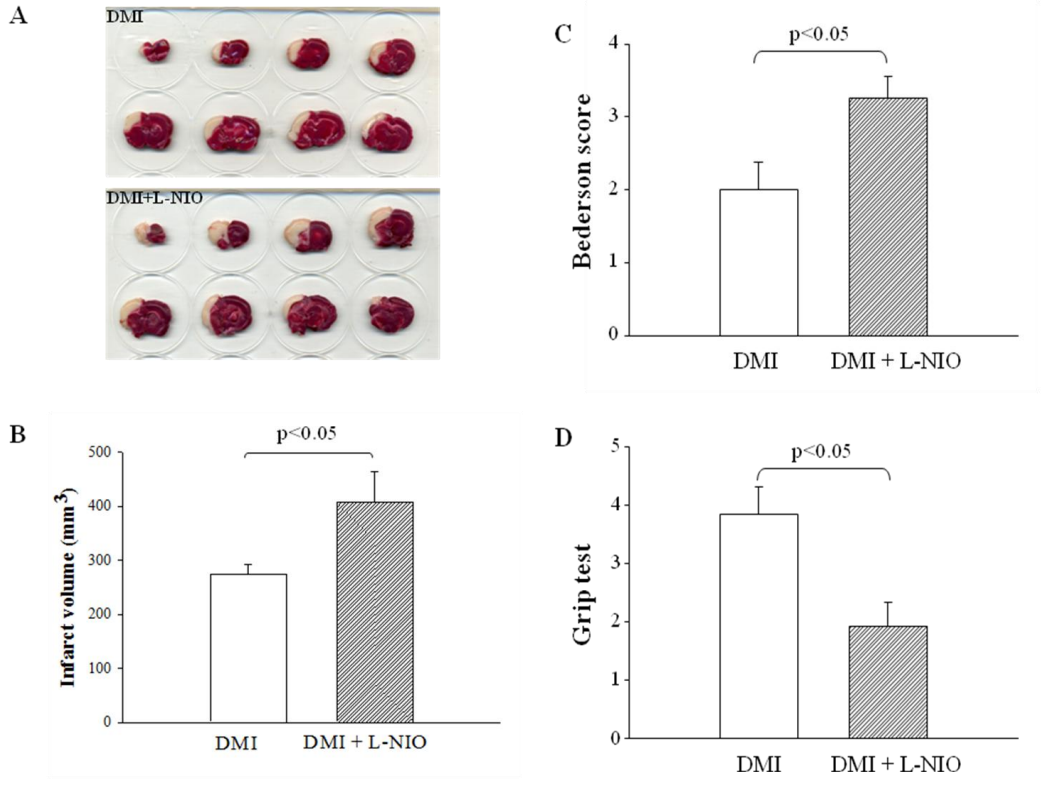


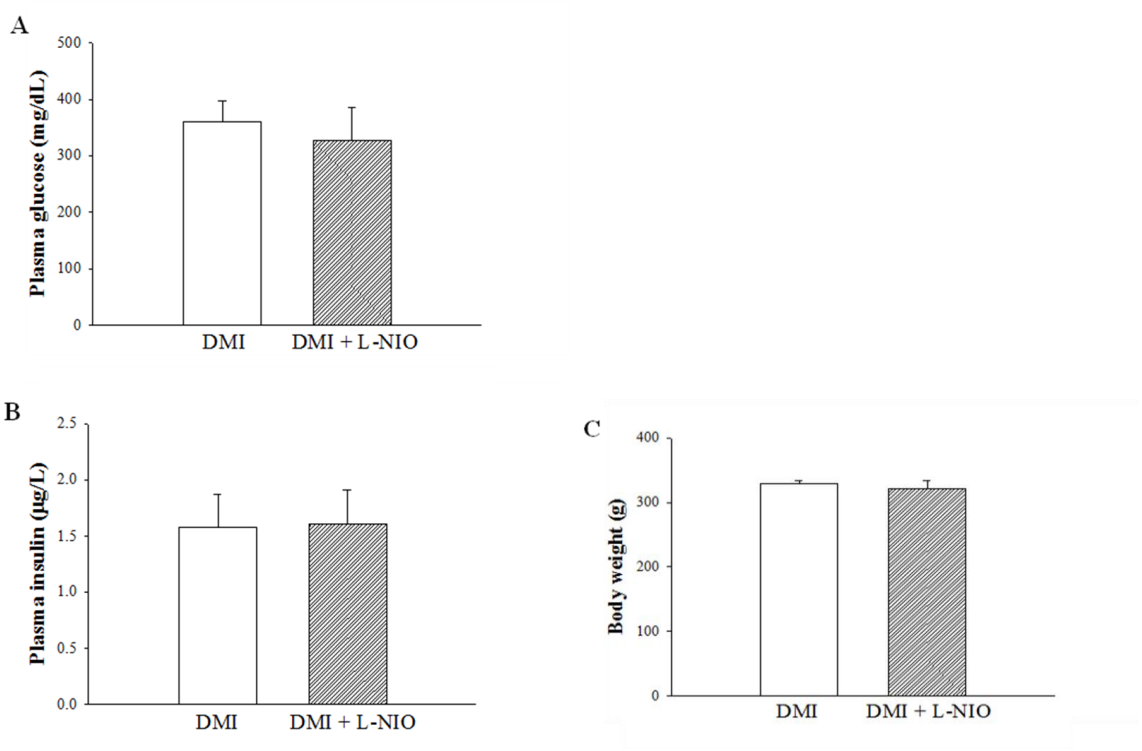
Cerebral Akt and eNOS phosphorylation. In parallel with the glucose transporter expression studies, cerebral Akt and eNOS protein expression and phosphorylation levels were examined. The expression of NOS protein isoforms (i.e., eNOS, iNOS, and nNOS) was not altered by either FC I/R injury or diabetes (Figure 4A). However, the level of phosphorylated eNOS (p-eNOS) protein was reduced by diabetes in an insulin-sensitive manner (Figure 4B). FC I/R injury slightly increased p-eNOS expression in non-diabetic animals, but this insult caused a marked reduction of p-eNOS expression in diabetic animals that was prevented by treating the animals with insulin (Figures 4A and 4B).

The expression of total Akt protein was not altered by either FC I/R injury or diabetes (Figure 4C). Although the level of phosphorylated Akt (p-Akt) in non-I/R contralateral tissue was not altered by diabetes, it was increased by more than 3-fold in diabetic animals treated with insulin (Figures 4C and 4D). FC I/R injury in ipsilateral tissue caused a significant increase in p-Akt expression, which was not observed in diabetic animals. Similar to the effect of insulin on contralateral cerebral tissue, the p-Akt expression level was significantly elevated in insulin-treated diabetic rats subjected to FC I/R insult (Figures 4C and 4D).



Effect of eNOS inhibition on infarct size and neurological function. To elucidate the role of eNOS and its relation to insulin in FC I/R injury in diabetes, we examined the effects of the eNOS inhibitor L-NIO on FC I/R-induced infarct volume and neurological deficits in diabetic rats treated with insulin. Administration of L-NIO 24 hrs prior to FC I/R in insulin-treated diabetic rats significantly increased infarct size (Figures 5A and 5B) and reduced the neuroprotective effect of insulin, with a resultant increase in the Bederson score (Figure 5C) and a reduction in grip capability (Figure 5D). Notably, the plasma level of glucose and insulin and the body weight in insulin-treated diabetic rats were not affected by L-NIO (Figure 6)





討論：

Despite major advances in primary and secondary prevention in the past 50 years, patients with diabetes are still at increased risk for CVD relative to those without diabetes. Stroke is a common complication in diabetic patients and it is important to understand the pathogenesis of acute ischemic stroke in diabetes because the diabetic state itself, particularly hyperglycemia, is likely to contribute to an increased cardiovascular risk. However, it seems that hyperglycemia is only a part of the pathophysiology of the development of diabetic CVD. Today, the major questions and controversies in the field of diabetes as it relates to CVD are in regards to our inability to reconcile observational study data with clinical trial data. Nearly all of the clinical trials among patients with diabetes have failed to show a reduction in CVD events with intensive glycemic control.[8, 9] The main findings of the present study are as follows: (1) Diabetes impaired glucose uptake and membrane trafficking activity of GLUT1 in the cerebrum and this adverse effect was neither aggravated by FC I/R injury nor reversed by insulin treatment. (2) Hyperglycemia exacerbated FC I/R-induced neurological dysfunction. (3) The FC I/R-induced Akt and eNOS phosphorylations were blunted in animals with diabetes. (4) In parallel with its hypoglycemic effects, insulin also increased cerebral Akt/eNOS phosphorylations and improved neurological function in diabetes. (5) Inhibition of eNOS abrogated the insulin-induced neuroprotective effects of reduced cerebral infarct volume and neurological deficits in diabetic rats without affecting insulin-induced hypoglycemia.

The brain uses glucose as its primary substrate for energy production. Delivery of glucose from the blood to the brain requires transport across the endothelial cells of the blood-brain barrier and into the neurons and glia cells. Facilitative glucose transporter proteins mediate

these processes. The three primary glucose transporter isoforms which function in cerebral glucose metabolism are GLUT1, GLUT3 and GLUT8.[34, 35] In the present study, we showed that cerebral glucose uptake and membrane trafficking activity of GLUT1 were compromised in STZ-diabetic rats. The ultimate consequence of these reductions may make the diabetic animal susceptible to FC I/R injury and worsen the neurological outcomes. However, the impairment of glucose uptake and GLUT1 membrane trafficking activity seen with diabetes were not exacerbated by FC I/R, suggesting there is no causal relationship between those two events. On the other hand, insulin treatment significantly ameliorated hyperglycemia, hypoinsulinemia, infarct volume, and neurological deficits but did not improve membranous GLUT 1 protein level and glucose uptake. It appears that the beneficial action of insulin upon FC I/R injury is independent of cerebral glucose uptake.

Recently, endothelial dysfunction has been demonstrated to be one of the earliest detectable events in diabetes-associated ischemic stroke.[36] Impairment of eNOS-dependent vascular function may contribute to a reduction of cerebral blood flow in stroke.[37, 38] A previous study also showed that abnormalities in eNOS phosphorylation are an important common pathway that links diverse cardiovascular risks (such as diabetes, obesity, and metabolic syndrome) with endothelial dysfunction to increase the propensity to ischemic stroke (Atochin et al., 2007). In addition, insulin can activate eNOS through Akt-mediated S1179 phosphorylation, thereby increasing blood flow and cell survival.[39, 25] In the present study, we found that the phosphorylation level of eNOS and Akt in the cerebral tissue was increased after FC I/R injury. This may exert compensatory protection mechanisms against neurological injury by I/R. This context is supported by the finding that diabetes attenuated the increased cerebral eNOS/Akt phosphorylation in parallel with a worse neurological outcome in FC I/R injury. Interestingly, insulin enhanced the cerebral Akt and eNOS phosphorylation levels and also alleviated FC I/R injury in STZ-diabetic rats. To examine whether eNOS was responsible for the neuroprotective effect of insulin in diabetes with FC I/R injury, we administered a selective eNOS inhibitor L-NIO to the STZ-diabetic rats. In agreement with the eNOS/Akt phosphorylation data, inhibition of eNOS significantly antagonized neuroprotective effects of insulin in FC I/R injured diabetic animals without affecting anti-hyperglycemic effect of insulin. Our results suggest that normal vascular endothelial function is essential for insulin protection against FC I/R injury in diabetic rats. Consistent with our findings, Fanne et al. reported that insulin and glucagon exert neuroprotective effects that correlate with their capacity to reduce glutamate, rather than by modifying glucose levels (Fanne et al., 2011).[40]

In summary, glycemic control with insulin treatment may have clinical benefits for stroke patients with diabetes, especially in those with insulinopenic diabetes. Our results point to a role for Akt/eNOS signaling in the detrimental and protective effects of diabetes and insulin, respectively, in the context of ischemic stroke. Our findings indicate that the impact of insulin in diabetes with acute ischemic stroke is concurrently dependent on both eNOS signaling and anti-hyperglycemic effects. When eNOS dysfunction, e.g., vascular endothelial dysfunction,

is developed in diabetes, the beneficial effect of insulin might be diminished in acute ischemic stroke in spite of its preserved anti-hyperglycemic effect. For diabetic individuals, hyperglycemia must be controlled as early as possible, otherwise diabetes-associated endothelial dysfunction could aggravate the damage of ischemic stroke. It is essential to preserve eNOS/endothelial function that can improve clinical outcomes when diabetic individuals develop acute ischemic stroke.

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計畫成果自評：

This study had finished and prepared for publish.

國科會補助計畫衍生研發成果推廣資料表

日期:2012/10/11

國科會補助計畫	計畫名稱: 白藜蘆醇與胰島素對抗第一型糖尿病鼠遭受急性缺血性中風傷害的機制評估
	計畫主持人: 黃相碩
	計畫編號: 100-2320-B-040-007- 學門領域: 中醫藥

無研發成果推廣資料

100 年度專題研究計畫研究成果彙整表

計畫主持人：黃相碩	計畫編號：100-2320-B-040-007-
計畫名稱：白藜蘆醇與胰島素對抗第一型糖尿病鼠遭受急性缺血性中風傷害的機制評估	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	1	100%	篇	
		研究報告/技術報告	0	1	100%		
		研討會論文	0	1	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 ■未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

最近的臨床試驗發現嚴密監控血糖治療糖尿病中風病人的結果非常矛盾，有的臨床試驗提出有幫助，有的臨床試驗提出沒有幫助，所以我們藉由糖尿病大鼠遭受缺血性中風傷害，研究胰島素除了降血糖作用還有甚麼因子參予在其神經保護作用中？本研究計畫提供了關鍵性資訊，胰島素必須在 eNOS 維持活性的狀況下，才能夠在糖尿病遭遇缺血性中風傷害表現神經保護作用。