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Effects of *Scutellarein* on diabetic rat aorta[1]

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KEY WORDS *Scutellarein*；experimental diabetes mellitus；thoracic aorta；vascular endothelium；phenylepherine；acetylcholine；streptozocin；vasoconstriction；vasodilation

ABSTRACT

AIM：To study the effect of *Scutellarein*（*Scu*）on the diabetic rat aorta. **METHODS**：Contractile responses to phenylepherine and endothelium-dependent relaxation responses to acetylcholine（ACh）in rat aorta were investigated after streptozocin-induced 6-wk diabetes，*Scu*-treated streptozocin-induced diabetes，and in age-matched control *in vitro*. **RESULTS**：1）Endothelium-dependent relaxation to ACh in diabetic rats was decreased（$P<0.01$）compared with age-matched control. 2）Contractile responses to phenylepherine were increased（$P<0.01$）in diabetic rats. 3）The dietary supplement of 0.5 % *Scu* starting from 1-wk diabetes induction prevented endothelial dysfunction（$P<0.01$），but the contractile responses to phenylepherine were further increased. **CONCLUSION**：*Scu* prevented vascular endothelial dysfunction in diabetic rats，and also potentiated the contraction induced by phenylepherine

INTRODUCTION

Pathophysiologic changes in blood vessels from experimental diabetic animals have demonstrated that：1）The endothelium-dependent relaxation was impaired[1]，and this was confirmed in both type 1 and type 2 diabetic patients[2,3]；2）Contractile responses of smooth muscle to most agonists appears to be enhanced，in the presence of staurosporine，a protein kinase C（PKC）inhibitor，this increase in response normalized[4]. Alterations in several independent factors may contribute to the impaired endothelium-dependent relaxation of diabetic blood vessels，such as：1）increase in PKC activity[5]；2）increased quenching of nitric oxide（NO）by advanced glycosylation endproducts[6]；3）decreased NO activity because of interaction with increased production of oxygen radicals[7]. Hyperglycemia can activate PKC and the activity of PKC has been increased in diabetic tissues[8,9]. A new theory has suggested that the disturbance of PKC in the diabetic mellitus may be the final intracellular common pathway through which many other factors contribute to the diabetic complications[8].

Traditional PKC inhibitors，such as staurosporine and H-7，are difficult to be used in clinic and chronic experimental animal studies because of their toxicities and nonspecificities[10]. *Scutellarein*（*Scu*）with M_r 462.21，one of the flavonoides isolated from Chinese herb（*Erigeron breviscapus* Handmazz）has been used in the clinic to treat cerebral vascular patients. *Scu* has an inhibitory effect on PKC[11,12]. This study was designed to investigate the preventing effect of *Scu* against the diabetes-associated endothelium dysfunction and increased responses of smooth muscle to phenylepherine.

MATERIALS AND METHODS

General procedures Wistar ♂ rats（Grade Ⅱ，Certificate № 26-98A011）weighing 200－250 g，were injected with streptozocin（STZ，65 mg/kg）dissolved in citrate buffer 0.1 mol·L^{-1}（ip）to induce diabetes. The control rats were injected with a vehicle. A drop of tail blood was obtained at 1 wk after administration of STZ to verify hyperglycemia using a Boehringer Mannheim glucometer［（5.0 ± 0.5）mmol·L^{-1} in control，（22 ± 5）mmol·L^{-1} in diabetic］. The rats were divided into two groups：diabetic group which was given normal drinking water；*Scu* group treated with 0.5 % *Scu* in drinking

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water.

After six weeks, the rats were decapitated, and the descending thoracic aortae were isolated at 37 ℃ in modified Krebs' solution containing NaCl 137, KCl 5.4, $CaCl_2$ 2.0, $MgCl_2$ 1.1, NaH_2PO_4 0.4, glucose 5.6, and $NaHCO_3$ 11.9 mmol·L^{-1}. The solution was aerated with 95 % O_2 + 5 % CO_2. The aortic segments were carefully cleaned of fat and loose connective tissue, and sectioned into 3-mm rings. Two rings were obtained from each aorta. Extreme care was taken in order to avoid damage to the endothelium.

Isolated aortic rings were suspended between parallel hooks in 4-mL modified Krebs' solution baths thermoregulated at 37 ℃. Resting tension was set at a level of 1.5 g. Changes in isometric tension were recorded by a recorder via force-displacement transducers. At the completion of experiment, the rings were blotted dry and weighed.

Individual protocols Each ring was equilibrated for 2 h. During the equilibration, the rings were washed every 20 min. Before recording the data, the rings were precontracted with a submaximal concentration of phenylephrine (0.3 μmol·L^{-1}). When the responses reached plateau, Ach (10 μmol·L^{-1}) was added. If the endothelium was intact, a full relaxation occurred in the control rings. In the denuded rings, Ach caused contraction rather than relaxation. Subsequently, all rings were washed, and the tension was allowed to return to baseline for at least 45 min. Then, cumulative concentration-response curves for endothelium-dependent relaxation response to Ach was studied in the rings precontracted by phenylephrine 0.3 μmol·L^{-1}, and the cumulative concentration-response curve for phenylephrine contraction was also investigated.

Drugs Phenylephrine and acetycholine (ACh) were purchased from Sigma. *Scutellarein* was bought from Medi-world Otsuka Pharmaceutical Co.

Statistics All data were expressed as $\bar{x} \pm s$ and analyzed with t test.

RESULTS

Scu-treatment showed no significant effect on glucose level in nomal rats. Untreated and *Scu*-treated diabetic rats were hyperglycemic [(22 ± 5) mmol·L^{-1} (n = 6), (23 ± 4) mmol·L^{-1} (n = 7) respectively] compared with control [(5.0 ± 0.5) mmol·L^{-1} (n = 12)]. At time of the 6-wk experimentation, diabetic rats lost approximately 15 % body weight [from (225 ± 25) g to (200 ± 21) g], however, control rats gained approximately 30 % body weight [up to (300 ± 18) g].

At the 6-wk duration of diabetes mellitus, concentration-dependent relaxation to ACh was diminished at each concentration of ACh compared with control, and approximately 57.5 % contraction to phenylephrine 0.3 μmol·L^{-1} remained [Ach 10 μmol·L^{-1}, Diabetic 57 % ± 14 % (n = 6) *vs* control 0 % (n = 6), respectively, $P < 0.01$. Fig 1].

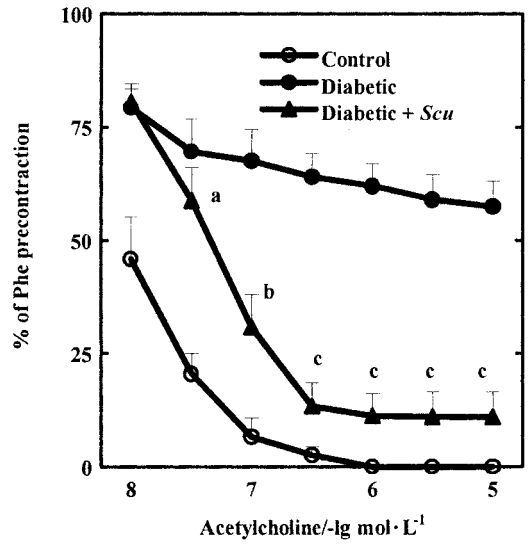

Fig 1. Relaxation response to Ach in Phe 0.3 μmol·L^{-1} precontracted diabetic rat aorta (n = 6), age-matched control (n = 6), and *Scu*-treated diabetic rats (n = 6). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

In some preparations, after the initial relaxation by ACh 0.01 and 0.03 μmol·L^{-1}, the following additions of ACh caused contractions rather than relaxation. In the *Scu*-treated diabetic rat aorta, the relaxation response to ACh was also diminished ($P < 0.01$) at the low concentrations [ACh 0.01 and 0.03 μmol·L^{-1}; *Scu*-treated diabetic 80 % ± 4 % and 59 % ± 7 % (n = 6) *vs* control 45 % ± 9 % and 20 % ± 4 % (n = 6) respectively, Fig 1] compared with control, whereas the endothelial function was also significantly improved compared with diabetic group (diabetic remained 57 % ± 14 %, *Scu*-treated diabetic remained 11 % ± 6 % of contraction, $P < 0.01$).

The cumulative concentration-response curve for phenylephrine contraction was significantly enhanced in diabetic and *Scu*-treated diabetic group further compared

with control [maximal contraction, diabetic (338 ± 27) g/g wet tissue ($n = 5$), *Scu*-treated diabetic (437 ± 69) g/g wet tissue ($n = 6$) *vs* control (242 ± 26) g/g wet tissue ($n = 6$), respectively, $P < 0.01$, Fig 2]. In the *Scu*-treated diabetic, the concentration-dependent contraction of phenylephrine appeared to be more than in diabetic group (increase in diabetic group 39.7 %, increase in *Scu*-treated diabetic group 80.6 %).

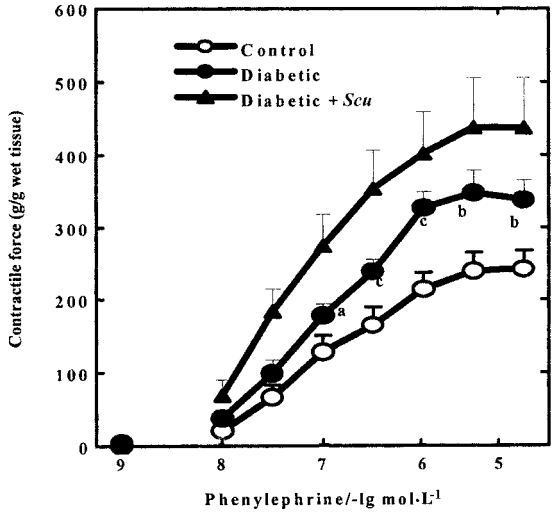

Fig 2. Contractile response of Phe in rat aorta of diabetic ($n = 5$), *Scu*-treated diabetic ($n = 6$), and age-matched control ($n = 6$). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

DISCUSSION

Physiological changes in animal models of diabetes mellitus resemble the abnormalities that develop in human diabetes. Streptozocin which specifically destroys the β cells has been widely used to induce diabetic animal models[13].

Relaxation response of rat aorta to ACh depends on endothelial nitric oxide (NO) production, pretreatment with TEA and indometacin failed to affect this response significantly in both control and diabetic rat aorta, suggesting that prostanoids or endothelium-derived hyperpolarizing factors are not involved[14]. Responses to nitroprusside, which directly supplies NO, were not changed by diabetes, this suggests that NO response to smooth muscle is not changed. Thus the impaired endothelium-dependent relaxation must have resulted from the alterations in endothelial NO synthesis, release, or diffusion to smooth muscle.

In this study, we found that the administration of *Scu* had a preventive effect against the endothelial dysfunction in diabetic rat aorta. It is possible that *Scu* inhibited disturbed PKC activity in diabetes, and prevented endothelium from dysfunction. However, infusion of *Scu* (iv) slightly decreased the blood pressure of anesthetic rat [from (145 ± 2.5) mmHg to (135 ± 3.2) mmHg, $n = 3$, data not shown] (1 mmHg = 133 Pa). This suggests that *Scu* can release NO from endothelium, and improve the endothelial dysfunction in diabetes.

The contractile response to phenylephrine was more enhanced in *Scu*-treated diabetic rat aorta, this may suggest that：1) there may be a different mechanism involved in endothelial dysfunction and smooth muscle dysfunction in diabetes. 2) *Scu* caused contraction in denuded vascular rings, thus, the increased phenylephrine contraction was partly due to *Scu*. In clinic, the contractile effect of *Scu* may be beneficial to cerebral hemorrhage patients, but the possible clinical usage of *Scu* in diabetic complications needs to be investigated further.

In conclusion, *Scu* improved the diabetes-associated deficit of endothelium-dependent relaxation, and potentiated phenylepherine-induced contractions.

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野黄芩甙元对糖尿病大鼠主动脉的影响[1]

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关键词 野黄芩甙元；实验性糖尿病；胸主动脉；血管内皮；苯肾上腺素；乙酰胆碱；链佐星；血管收缩；血管舒张

目的：研究野黄芩甙元对糖尿病大鼠血管合并症的预防作用. 方法：利用平滑肌条离体研究装置. 结果：在第六周的糖尿病大鼠主动脉：1）乙酰胆碱引起的内膜依赖性舒张作用较对照明显减弱（$P<0.01$）；2）苯肾上腺素引起的收缩反应较对照明显增加，最大收缩增加约 40 %（$P<0.01$）；3）糖尿病大鼠服用含 0.5 % 的野黄芩甙元的饮水后，乙酰胆碱引起的内膜依赖性舒张作用较糖尿病组明显增加（$P<0.01$），但是，苯肾上腺素引起的收缩反应增加更显著，最大收缩较对照增加约 80 %（$P<0.01$）. 结论：野黄芩甙元对糖尿病引起的血管内膜功能损害有防护作用，也可增强苯肾上腺素引起的收缩.

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