Antihyperglycemic Effect of Catalpol in Streptozotocin-Induced Diabetic Rats

Wei-Jan Huang,[†] Ho-Shan Niu,[‡,§] Mei-Hsiang Lin,[‡] Juei-Tang Cheng,*[,⊥] and Fen-Lin Hsu*[,†]

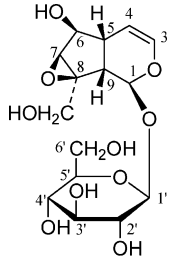
Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei 110, Taiwan, Republic of China, Graduate Institute of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei 110, Taiwan, Republic of China, Department of Nursing, Jente Junior College of Medicine, Nursing and Management, Miaoli, Taiwan, Republic of China, and Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan, Taiwan, Republic of China

Received December 22, 2009

The antihyperglycemic effect of catalpol (**1**) purified from the roots of *Rehmannia glutinosa* was investigated in streptozotocin-induced diabetic rats (STZ-diabetic rats) representing insulin-dependent diabetes mellitus. Bolus intravenous injection of **1** showed antihyperglycemic activity in a dose-dependent manner in STZ-diabetic rats. An effective dose of 0.1 mg/kg **1** significantly attenuated the increase of plasma glucose induced by an intravenous glucose challenge test in normal rats. Catalpol enhanced the uptake of radioactive glucose in the isolated soleus muscle of STZ-diabetic rats in a concentration-related manner. Moreover, an effect by **1** was established on glycogen incorporation in hepatocytes isolated from STZ-diabetic rats. Catalpol was found to increase glycogen synthesis in STZ-diabetic rats. These results suggest that **1** can increase glucose utilization to lower plasma glucose in diabetic rats lacking insulin.

Insulin-dependent diabetes mellitus (IDDM) and noninsulin-dependent diabetes mellitus (NIDDM) rank high among the top 10 causes of mortality throughout the world. Diabetes often leads to disability from vascular complications of coronary artery disease and cerebrovascular disease, renal failure, blindness, and limb amputation, in addition to neurological complications and premature death.[1,2] With rapid therapeutic advancements, novel treatments with fewer side effects have became more feasible for the long-term management of this disorder.

Rehmannia glutinosa Steud. (Scrophulariaceae) is a widely used traditional Chinese herb, from which more than 70 compounds have been reported, including iridoids, saccharides, amino acids, inorganic ions, and other trace elements.[3] An oligosaccharide from *R. glutinosa* has been documented as exerting a significant hypoglycemic effect in normal and alloxan-induced diabetic rats.[4] Rehmannioside D from this plant when given to alloxan-induced diabetic mice showed a blood glucose reduction in a diabetes mouse model.[3] Catalpol (**1**), an iridoid glucoside of *R. glutinosa*, has many reported biological effects,[5] including cytotoxic and anti-inflammatory properties.[6] This compound has been documented as protecting neurons from injury[7] and against H_2O_2-induced apoptosis.[8] In the present study, catalpol (**1**) was purified from *R. glutinosa* roots, and we investigated its effects on glucose metabolism.



1

In order to rule out any pharmacokinetic effects, catalpol (**1**) was studied by intravenous injection into animals. It was found that bolus injection of **1** can lower plasma glucose concentrations effectively in streptozotocin-diabetic (STZ-diabetic) rats. As shown in Figure 1, a dose-dependent increase of antihyperglycemic activity was observed in STZ-diabetic rats on the intravenous injection of **1** at the dose range 0.01−0.1 mg/kg. There was no additional effect of **1** with an increase in dosage to 0.15 mg/kg. The minimal and maximal plasma glucose-lowering activities of **1** in STZ-diabetic rats were 8.53 ± 2.85% at 0.01 mg/kg and 24.33 ± 2.94% at 0.1 mg/kg, respectively. Our previous study demonstrated that the maximal glucose-lowering effect of metformin, a clinically used antidiabetic agent, was 32 ± 5%, at an oral dose of 100 mg/kg in STZ-diabetic rats.[9] By comparison with metformin, this indicated that 0.1 mg/kg of **1** is an effective dose for subsequent experiments. In preliminary data observed, it was found that the plasma glucose-lowering activity at the same dose of **1** in normal Wistar rats was slightly lower than that in STZ-diabetic rats (data not shown). Thus, catalpol (**1**) can be considered to have insulin-independent actions.

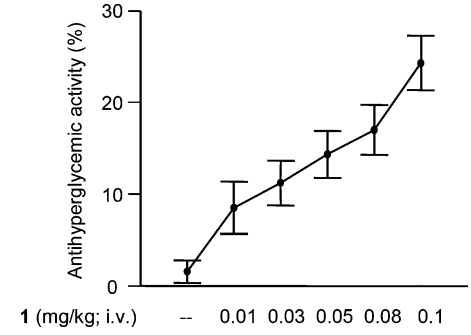


Figure 1. Antihyperglycemic activity of catalpol (**1**) in STZ-diabetic rats. Values (mean ± SE) were obtained from each group of eight animals (i.v., intravenous injection; controls were values from STZ-diabetic rats that received the same volume of vehicle).

An intravenous glucose challenge test (IVGCT)[10] to characterize the ability of rats to clear glucose from the circulation was carried out. Five minutes after glucose injection, the plasma glucose concentration was elevated both in vehicle- and **1**-treated rats (Figure 2). The increase of plasma glucose produced by the glucose injection was significantly lower in the rats pretreated with **1** after 5 min of glucose injection compared to the vehicle-treated rats. The plasma glucose in rats pretreated with **1** remained significantly lower after 20 min of glucose injection compared to the vehicle-treated group (Figure 2). No statistical difference was obtained for

* To whom correspondence should be addressed. Tel: +886-6-238-6548. Fax: +886-6-238-6548. E-mail: jtcheng@mail.ncku.edu.tw (J.-T.C). Tel: +886-2-2737-0903. Fax: +886-2-2737-0903. E-mail: hsu0320@tmu.edu.tw (F.-L.H.).

[†] Graduate Institute of Pharmacognosy, Taipei Medical University.

[‡] Graduate Institute of Pharmacy, Taipei Medical University.

[§] Jente Junior College of Medicine.

[⊥] National Cheng Kung University.

10.1021/np9008317
Published on Web 06/02/2010

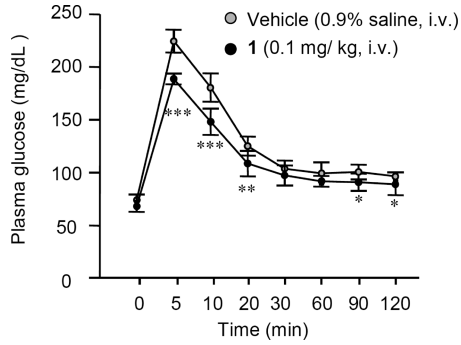


Figure 2. Effect of catapol (**1**) on plasma glucose concentration in normal rats receiving an intravenous glucose challenge test. Catalpol (**1**, 0.1 mg/kg) (open circles) vs saline at the same volume (filled circles) (*$p < 0.05$ vs data from control group; means ± SEM of eight rats in each group).

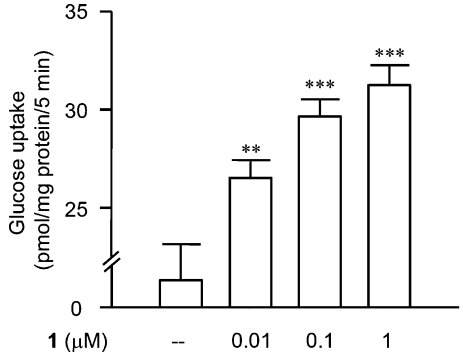


Figure 3. Effect of catalpol (**1**) on glucose uptake in isolated skeletal muscle from STZ-diabetic rats. Values (mean ± SEM) were obtained from each group of eight experiments (**$p < 0.01$, ***$p < 0.001$ vs blank). The basal value of glucose uptake is 171 ± 8 pmol/mg protein/5 min. Blanks were the values from isolated skeletal muscle receiving the same volume of vehicle.

the plasma glucose concentration in rats that received a glucose injection 30 min later between the group treated with **1** and the vehicle-treated controls. This finding shows that **1** can enhance glucose utilization in vivo.

Skeletal muscle is the major site of glucose disposal.[11] Glucose transport, which depends on insulin-stimulated translocation of glucose carriers to the cell membrane, is the rate-limiting step in the carbohydrate metabolism of skeletal muscle.[12] Under basal conditions, the rate of glucose uptake in skeletal muscle is low. Insulin-stimulated glucose utilization is the major site for the regulation of plasma glucose concentrations.[13] IDDM is an abnormal metabolic state characterized by an insulin defect involving muscle tissues and other types of tissues. Thus, glucose uptake stimulated by catalpol (**1**) was investigated to discern the mechanism of changes in glucose utilization. In the present study, specific glucose uptake (2-DG uptake) into soleus muscle of STZ-diabetic rats was measured. After stimulation with **1** for 30 min, the specific glucose uptake into the isolated soleus muscle increased in a concentration-related manner from 10^{-8} M (26.55 ± 0.90 pmol/mg protein/5 min) to 10^{-6} M (33.08 ± 1.62 pmol/mg protein/5 min) (Figure 3). Production of glucose from the liver is the major cause of fasting hyperglycemia in both diabetes mellitus type 1 and type 2.[14,15] Furthermore, mammalian cells store glycogen in the liver for the production of glucose 6-phosphate during glycolysis.[16] Thus, liver samples were used to investigate the effect of **1** on glycogen incorporation into tissues, which can be related to the decrease in plasma glucose by improving glucose utilization. In hepatocytes of STZ-diabetic rats that received a 30 min incubation with **1**, the glycogen synthesis increased in hepatocytes

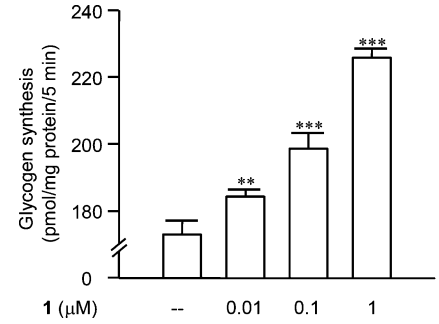


Figure 4. Effect of catalpol (**1**) on glycogen synthesis in hepatocytes from STZ-diabetic rats. Values (mean ± SEM) were obtained from each group of eight experiments (**$p < 0.01$, ***$p < 0.001$ vs blank). The basal value of glycogen synthesis was 12 ± 5 pmol/mg protein/5 min. Blanks were the values of isolated hepatocytes from STZ-diabetic rats receiving the same volume of vehicle.

of STZ-diabetic rats significantly ($p < 0.05$) in a concentration-related manner from 10^{-8} M to 10^{-6} M (Figure 4). It was observed that STZ-diabetic rats treated with **1** may increase the glucose utilization in skeletal muscle (Figure 3). An increase in the responsiveness of **1** in insulin-deficient diabetic rats can thus be considered. These results suggest that **1** can increase the utilization of glucose in peripheral tissue via an insulin-independent mechanism. Further studies are needed to elucidate the molecular mechanisms by which **1** regulates plasma glucose concentrations in the absence of insulin.

In conclusion, the data obtained suggest that intravenous injection of catalpol (**1**) can lower plasma glucose in STZ-diabetic rats through an increase of glucose utilization. Thus, **1** might become a suitable adjuvant for the treatment of diabetic patients in the future.

Experimental Section

General Experimental Procedures. The melting point was recorded on a Büchi B-545 melting point apparatus and is uncorrected. The optical rotation was measured on a JASCO DIP-1020 digital polarimeter. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM-500 (500 MHz) FT-NMR spectrometer in DMSO-d_6 solution, using the solvent as internal standard. The EIMS was determined on a Finnigan TSQ-700 mass spectrometer. Column chromatography was carried out with Diaion HP 20 (100−200 mesh, Mitsubishi Chemical Industries), MCI-gel CHP 20P (75−150 μm, Mitsubishi Chemical Industries), and Cosmosil C_{18}-OPN (75 μm, Nacalai Tesque, Inc.). TLC was conducted on silica gel plates (60 F-254, Merck), and 10% sulfuric acid solution was used as a visualizing agent on heating.

Plant Material. The roots of *Rehmannia glutinosa* were collected in Ping-Tung, Taiwan, in March 2000. The plant material was identified by Dr. Hsian-Chang Chang (Division of Pharmacognosy, National Laboratories of Food and Drugs, Department of Health, Taiwan). A voucher specimen (TMU27308) was deposited in the herbarium of the College of Pharmacy, Taipei Medical University.

Extraction and Isolation. Dried *R. glutionsa* (5 kg) roots were extracted with 60% aqueous acetone (ratio of solvent volume/dry weight about 2 mL/g) three times, each for 2 days, at room temperature. After evaporating the solvents in vacuo at 45 °C, a residue was obtained. This residue was dissolved in H_2O (1.5 L) and then extracted successively with CH_2Cl_2 (1 L × 3) and *n*-BuOH (1 L × 3). The *n*-BuOH extract (22% dry weight) was subjected to column chromatography over Diaion HP20 (15 × 120 cm) and eluted with a step gradient system (H_2O−MeOH, 0−100%) to give fractions A−E. Fraction B (2.37% dry weight) was divided into subfractions B1−B6 by passage over a Cosmosil C_{18} OPN column (10 × 100 cm), eluted with MeOH−H_2O (from 10% to 70%). Catalpol (**1**, 2700 mg) was obtained as colorless needles from subfraction B2 (0.28% dry weight) by MCI-gel CHP 20P column (8 × 100 cm) chromatography, using MeOH−H_2O (from 0% to 40%) as the solvent system. Catalpol (**1**) exhibited mp 202−205 °C (lit. 206−208 °C[17]); $[\alpha]_D$ −104.8 (*c* 0.5, EtOH) (lit. $[\alpha]_D$ −105.0 (*c* 0.8, MeOH)),[18] and gave spectroscopic data (^{1}H NMR, ^{13}C NMR, EIMS) comparable to literature values.[19,20]

Animal Model. Male Wistar rats between the ages of 8 and 10 weeks (200−250 g body weight) were obtained from the Animal Center of National Cheng Kung University Medical College. Diabetic rats were prepared by giving an intravenous injection of streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO) (60 mg/kg) into the fasting rats.[21] Rats with plasma glucose concentrations of 20 mmol/L or greater in addition to polyuria and other diabetic features were considered as having insulin-dependent diabetes mellitus (IDDM). Also, the plasma insulin level in STZ-diabetic rats became 1.33 ± 0.8 pmol/L (n = 8), which was markedly lower than that of normal rats (161.1 ± 3.4 pmol/L; n = 8) showing IDDM. All studies were carried out two weeks after the injection of STZ. Animal procedures were approved by the Institutional Animal Care and Use Committee of National Cheng Kung University and were performed according to the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Institutes of Health, as well as the guidelines of the Animal Welfare Act.

Effect of Catalpol (1) on Plasma Glucose. A solution of catalpol (**1**, purity >99%) was prepared by dissolving with normal saline to a concentration of 5 mg/mL. The fasting STZ-diabetic rats received an intravenous injection of **1** at the desired doses, and blood samples (0.1 mL) were collected under sodium pentobarbital anesthesia (30.0 mg/kg, i.p.) from the tail vein for measurement of plasma glucose. In preliminary experiments, catalpol (**1**) at 0.1 mg/kg was found to produce a maximal plasma glucose lowering effect in STZ-diabetic rats 30 min after intravenous injection. Thus, the effect of **1** on plasma glucose was determined using blood samples collected after 30 min. Control rats received a similar injection of vehicle at the same volume. The antihyperglycemic activity was calculated as decrease percentage of the initial value according to the formula $[(G_iG_t)/G_i] \times 100\%$, where G_i was the initial glucose level and G_t was the plasma glucose concentration after treatment.[21]

Intravenous Glucose Challenge Test. An intravenous glucose challenge test was performed according to a method previously described.[10] Briefly, the basal plasma glucose concentration was obtained from samples from the tail vein of Wistar rats under anesthesia with sodium pentobarbital (30.0 mg/kg, i.p.) before the IVGCT. A solution of **1** at 0.1 mg/kg or the same volume of saline was injected into the tail vein of rats. Then, 30 min later, blood samples (0.1 mL) from the tail vein were drawn and indicated as 0 min. Next, a glucose dose of 60.0 mg/kg was injected through the femoral vein of the rats. Rats receiving a similar injection of saline at the same volume were used as control. Blood samples (0.1 mL) from the tail vein were drawn at 5, 10, 20, 30, 60, 90, and 120 min following the glucose injection for the measurement of the plasma glucose concentrations. Rats were maintained under anesthesia by pentobarbital throughout the procedure.

Determination of Plasma Glucose. Blood samples (0.1 mL) were collected using a chilled syringe containing 10 IU heparin from the tail vein of rats under anesthesia with sodium pentobarbital (30.0 mg/kg, i.p.). Blood samples were then centrifuged at 13 000 rpm for 3 min, and an aliquot (15 μL) of plasma was added to 1.5 mL of Glucose Kit Reagent (Biosystems S.A., Barcelona, Spain) and incubated at 37 °C in a water bath (Yamato-BT-25, Tokyo, Japan) for 10 min. The concentration of plasma glucose was then estimated via an analyzer (Quik-Lab, Ames, Miles Inc., Elkhart, IN), and samples were run in duplicate.

Measurement of Glucose Uptake into the Rat Soleus Muscle. Glucose uptake was determined using the uptake of radioactive glucose analogue, 2-[1-^{14}C]-deoxy-D-glucose (2-DG) (New England Nuclear, Boston, MA), as described previously.[22] Animals were sacrificed by cervical dislocation, and the soleus muscle was quickly excised using a pair of scissors, dissected free of any adjoining connective tissue, blotted, and divided into long longitudinal strips (35−25 mg per strip). Muscles were placed in 3 mL of Krebs-Ringer bicarbonate buffer (KRBB) (37 °C, pH 7.4), containing 1 mmol/L glucose and 1% fatty acid-free bovine serum albumin (BSA) under aeration with 5% CO_2 in O_2. After preincubation for 30 min, the muscle tissue was incubated with catalpol (**1**) at the desired concentrations for 30 min and then with 50 μL of KRBB containing 2-DG (1 μCi/mL) for 5 min at 37 °C in a shaking water bath under aeration. The reaction was terminated by quickly blotting the muscles and dissolving them in 0.5 mL of 0.5 N NaOH for 45 min before neutralization with 0.5 mL of 0.5 N HCl. After centrifugation, 800 μL of each supernatant was mixed with 1 mL of aqueous counting scintillant, and the radioactivity was determined using a β-counter (Beckman LS6000). Nonspecific uptake of 2-DG, assessed after an incubation with 20 μmol/L cytochalasin B (Sigma Chemical Co., St. Louis, MO) for blocking transportation,[22] was subtracted from the total muscle-associated radioactivity.

Measurement of Glycogen Synthesis in Hepatocytes. Hepatocytes were prepared after perfusing isolated liver with collagenase under sterile conditions, as described previously.[23,24] Cell suspensions of >90% viability, as judged by trypan blue exclusion, were used in the experiments. After a 30 min preincubation period in Krebs-Ringer bicarbonate buffer at 37 °C, samples of cell suspension (1−2 mL) containing 40 to 80 mg of cells were transferred to fresh incubation flasks containing [U-^{14}C]-glucose (0.25 μCi/mL) (NEN Research, Boston, MA) with catalpol (**1**) at the desired concentrations for 1 h, the optimal time obtained from preliminary experiments, under continuous shaking. The incorporation of [U-^{14}C]-glucose into glycogen was determined by ethanol precipitation. Label incorporation into glycogen was expressed as pmol per mg of cell protein in 1 h.

Statistical Analysis. The plasma glucose-lowering activity was determined in fasted rats that received intravenous injection of catalpol (**1**) under anesthesia. Data are expressed as the means ± SEM for the number (n) of animals in the group as indicated in the figures. Repeated measures of analysis of variance (ANOVA) were used to analyze the changes in plasma glucose and other parameters. Dunnett range posthoc comparisons were used to determine the source of significant differences, where appropriate ($p < 0.05$ was considered statistically significant).

Acknowledgment. We gratefully acknowledge the financial support from National Science Council in Taiwan for grants NSC97-2320-B-038-010-MY3 and NSC97-2323-B-002-015. We thank Miss Y. F. Ker for her skillful assistance with the experiments.

References and Notes

(1) Goldstein, D. A.; Massry, S. G. *Nephron* **1978**, *20*, 286–296.
(2) Weidmann, P.; Boehlen, L. M.; de Courten, M. *Am. Heart J.* **1993**, *125*, 1498–1513.
(3) Zhang, R. X.; Li, M. X.; Jia, Z. P. *J. Ethnopharmacol.* **2008**, *117*, 199–214.
(4) Zhang, R.; Zhou, J.; Jia, Z.; Zhang, Y.; Gu, G. *J. Ethnopharmacol.* **2004**, *90*, 39–43.
(5) Liu, Y. F.; Zhao, Y.; Wen, X. S.; Dong, Q. T. *Zhongguo Zhong Yao Za Zhi* **2007**, *32*, 1128–1130.
(6) Zhang, J.; Johnston, G.; Stebler, B.; Keller, E. T. *Antioxid. Redox Signaling* **2001**, *3*, 493–504.
(7) Bi, J.; Jiang, B.; Hao, S.; Zhang, A.; Dong, Y.; Jiang, T.; An, L. *Neurochem. Int.* **2009**, *54*, 264–270.
(8) Liang, H. L.; Whelan, H. T.; Eells, J. T.; Wong-Riley, M. T. *Neuroscience* **2008**, *153*, 963–974.
(9) Cheng, J. T.; Huang, C. C.; Liu, I. M.; Tzeng, T. F.; Chang, C. J. *Diabetes* **2006**, *55*, 819–825.
(10) Liu, I. M.; Chi, T. C.; Hsu, F. L.; Chen, C. F.; Cheng, J. T. *Planta Med.* **1999**, *65*, 712–714.
(11) Baron, A. D.; Brechtel, G.; Wallace, P.; Edelman, S. V. *Am. J. Physiol.* **1988**, *255*, E769−774.
(12) Ziel, F. H.; Venkatesan, N.; Davidson, M. B. *Diabetes* **1988**, *37*, 885–890.
(13) Hollenbeck, C.; Reaven, G. M. *J. Clin. Endocrinol. Metab.* **1987**, *64*, 1169–1173.
(14) Rossetti, L.; Giaccari, A.; Barzilai, N.; Howard, K.; Sebel, G.; Hu, M. *J. Clin. Invest.* **1993**, *92*, 1126–1134.
(15) Barzilai, N.; Rossetti, L. *J. Biol. Chem.* **1993**, *268*, 25019–25025.
(16) Wu, G.; Morris, S. M., Jr. *Biochem. J.* **1998**, *336*, 1–17.
(17) Oshio, H.; Inouye, H. *Phytochemistry* **1982**, *21*, 133–138.
(18) Su, B. N.; Yang, L.; Gao, K.; Jia, Z. J. *Planta Med.* **2000**, *66*, 281–283.
(19) Kitagawa, I.; Hino, K.; Nishimura, T.; Mukai, E.; Yosioka, I. *Tetrahedron Lett.* **1969**, *43*, 3837–3840.
(20) Chaudhuri, R. K.; Sticher, O. *Helv. Chim. Acta* **1981**, *64*, 3–15.
(21) Chan, P.; Wong, K. L.; Liu, I. M.; Tzeng, T. F.; Yang, T. L.; Cheng, J. T. *J. Hypertens.* **2003**, *21*, 761–769.
(22) Liu, I. M.; Hsu, F. L.; Chen, C. F.; Cheng, J. T. *Br. J. Pharmacol.* **2000**, *129*, 631–636.
(23) Fry, J. R.; Jones, C. A.; Wiebkin, P.; Bellemann, P.; Bridges, J. W. *Anal. Biochem.* **1976**, *71*, 341–350.
(24) Cheng, J. T.; Liu, I. M.; Chi, T. C.; Tzeng, T. F.; Lu, F. H.; Chang, C. J. *Diabetes* **2001**, *50*, 2815–2821.

NP9008317