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Stimulation of insulin release in rats by Die-Huang-Wan, a herbal mixture used in Chinese traditional medicine

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Abstract

Die-Huang-Wan is a herbal mixture widely used in Chinese traditional medicine to treat diabetic disorders. We have investigated the effect of Die-Huang-Wan on plasma glucose concentration in-vivo. Die-Huang-Wan was administered orally (5.0, 15.0 or 26.0 mg kg^{-1}) to three rat models. Wistar rats were used as the normal animal model, rats with insulin-resistance (induced by the repeated thrice daily injection of human long-acting insulin) were used as the non-insulin-dependent diabetic model, and streptozotocin-induced diabetic rats were used as the insulin-dependent diabetic model. In normal rats, approximately 1 h after oral administration of Die-Huang-Wan the plasma glucose concentration decreased significantly in a dose-dependent manner, from 5 to 26.0 mg kg^{-1}. A similar effect was observed in rats with insulin-resistance. However, this effect was not observed in streptozotocin-induced diabetic rats, even at an oral dose of 26.0 mg kg^{-1}. These results suggested an insulin-dependent action, a view supported by the increase of plasma insulin-like immunoreactivity in normal rats receiving Die-Huang-Wan. The results indicated that Die-Huang-Wan had an ability to stimulate the secretion of insulin and this preparation seemed helpful in improving the diabetic condition, especially hyperglycaemia in type-II diabetes.

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Introduction

Insulin-dependent and non-insulin-dependent diabetes mellitus (Harris et al 1987) is a common and serious disorder throughout the world. This chronic disease often leads to disability from the vascular complications of coronary artery disease and cerebrovascular disease, renal failure, blindness, and limb amputation in addition to neurological complications and premature death (Goldstein & Massry 1978; Weidmann et al. 1993). Treatment of diabetes by insulin and/or oral drugs fails to prevent these complications in many patients, indicating that additional treatment would be helpful.

In Chinese traditional medicine Die-Huang-Wan can be prescribed for the treatment of diabetic disorders, and this mixture of six herbs has been used for a long time in Japan (Ji-Ooh-Gan in Japanese) and other areas of Asia (Suzuki & Kimura 1984; Kamei et al 1987). Scientific data about this herbal mixture is lacking except for its antihyperglycaemic activity in diabetic mice (Suzuki & Kimura 1984). Although Die-Huang-Wan is widely used in Chinese traditional medicine, its

mechanism of action remains unclear. In this study, we have demonstrated that the plasma-glucose-lowering action of Die-Huang-Wan is associated with an increase in insulin secretion and this preparation is helpful for patients with non-insulin-dependent diabetes mellitus.

Materials and Methods

Animals

All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act.

Male Wistar rats, aged 8–10 weeks, were obtained from the Animal Center of National Cheng Kung University. Streptozotocin-induced diabetic rats, used as the insulin-dependent diabetic model, were prepared by an intravenous injection of streptozotocin (60 mg kg^{-1}; Sigma Chemical Co., St Louis, MO) after a three-day fast. Rats with a plasma glucose concentration of 20 mmol L^{-1} or greater in addition to polyuria and other diabetic disorders were considered as having insulin-dependent diabetes. All studies were carried out two weeks after the induction of diabetes. Also, insulin-resistance was induced in eight-week-old rats by the intraperitoneal injection of long-acting human insulin (Monotard HM, Novo Nordisk A/S, Bagsvaerd, Denmark) at 0.5 int. units kg^{-1} three-times daily for 15 days (Chi et al 1998). Rats with insulin-resistance were used as the model of non-insulin-dependent diabetes.

Experimental protocols

Effect on plasma glucose

Granules of Die-Huang-Wan (Cheng-Hoo Pharmaceutical Co., Hsin-Yin City, Tainan Shian, Taiwan) were dissolved in 0.9% (w/v) saline solution for oral administration at 5.0, 15.0 or 26.0 mg kg^{-1} into fasted rats. Effects on the concentrations of glucose and/or insulin in plasma were determined using blood samples collected 1 h after administration. In preliminary experiments this was the time necessary for Die-Huang-Wan to produce maximal action. Wistar rats were used as the normal animal model, rats with insulin-resistance were used as the non-insulin-dependent diabetic model, and streptozotocin-induced diabetic rats were used as the insulin-dependent model. Tolbutamide (10 mg kg^{-1}; Sigma Chemical Co., St Louis, MO) was injected intraperitoneally into Wistar rats for the collection of blood 1 h later.

Effect on insulin metabolism

Streptozotocin-diabetic rats were used to investigate the response to exogenous insulin. The streptozotocin-diabetic rats were divided into two groups. One group received an oral administration of 26.0 mg kg^{-1} Die-Huang-Wan, and the second group received the same volume of vehicle (0.9% saline). An intraperitoneal injection of 1 int. unit kg^{-1} short-acting human insulin (Actrapid HM, Novo Nordisk A/S, Bagsvaerd, Denmark) was used in combination with Die-Huang-Wan or vehicle in each group of streptozotocin-diabetic rats. After 1-h treatment, the plasma glucose lowering activity of short-acting human insulin was compared between the two groups of rats with diabetes.

Laboratory determination

Rats were anaesthetized with sodium pentobarbital (30 mg kg^{-1}, i.p.) and a blood sample (0.2 mL) was collected from the femoral vein by a chilled syringe containing 10 int. units heparin. Blood samples were then centrifuged at 13000 rev min^{-1} for 3 min. The plasma sample (15 μL) was then added to 1.5 mL 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 estimated via an analyser (Quik-Lab, Ames, Miles Inc., Elkhart, IN) run in duplicate (Chang et al 1999). The plasma insulin were determined by radioimmunoassay as described by Morgan & Lazarow (1963) using a commercial kit (RI-13K) of Linco Research Inc. (St Charles, MO). The values obtained were indicated as pmol peptide-like immunoreactivity (L plasma)$^{-1}$.

Statistical analysis

Plasma-glucose-lowering activity was determined in rats receiving Die-Huang-Wan or another agent. Results were calculated as the percentage decrease of the initial value according to the formula:

$$(Gi - Gt)/Gi \times 100\,\%$$

where Gi is the initial glucose concentration and Gt is the plasma glucose concentration after treatment with Die-Huang-Wan or another agent.

Data are expressed as the mean $\pm$ s.e.m. for the number (n) of animals in the group, indicated in legends to Figures 1 and 2. Repeated measures analysis of variance was used to analyse the changes in plasma glucose and other parameters. The Dunnett range post-hoc comparisons were used to determine the source of significant

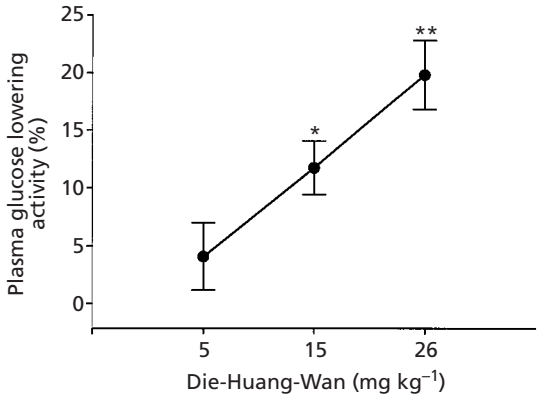

Figure 1 Effect of Die-Huang-Wan on the plasma glucose concentrations in normal (Wistar) rats. Values (mean ± s.e.m.) were obtained from each group of eight animals. *$P < 0.05$, **$P < 0.01$ compared with data from rats receiving 5 mg kg^{-1} Die-Huang-Wan orally.

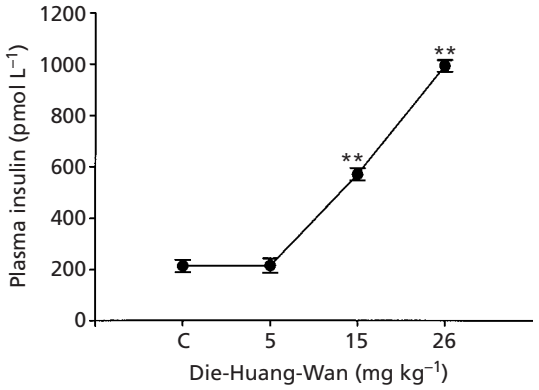

Figure 2 Effect of Die-Huang-Wan on the plasma insulin concentrations in Wistar rats. Die-Huang-Wan was dissolved in vehicle (distilled water containing 0.9% NaCl). Animals receiving the same volume of vehicle only were taken as the control. Values (mean ± s.e.m.) were obtained from each group of 10 animals. **$P < 0.01$ compared with control.

differences where appropriate. The obtained P value of 0.05 or less was considered statistically significant.

Results and Discussion

A dose-dependent decrease of plasma glucose was observed in Wistar rats (n = 8) receiving oral Die-Huang-Wan 5.0–26.0 mg kg^{-1} (Figure 1). The plasma-glucose-lowering activity of 10 mg kg^{-1} tolbutamide in Wistar rats was approximately 26.1 ± 5.1% (n = 8). In diabetic rats with insulin-resistance similar treatment with Die-Huang-Wan also lowered the plasma glucose, except that the effective dose needed to be higher than 26.0 mg kg^{-1}. In rats with non-insulin-dependent diabetes oral treatment with 26.0 mg kg^{-1} Die-Huang-Wan markedly decreased the plasma glucose from 5.9 ± 0.6 to 4.2 ± 0.5 mmol L^{-1} (n = 8) ($P < 0.05$). The plasma glucose lowering activity of 26.0 mg kg^{-1} Die-Huang-Wan was 19.8 ± 4.2% in normal rats and 28.2 ± 5.2% in non-insulin-dependent diabetic rats; two values were without statistical difference. However, Die-Huang-Wan failed to affect the plasma glucose level in streptozotocin-diabetic rats at 26.0 mg kg^{-1} (n = 8). The plasma glucose concentration in streptozotocin-diabetic rats was changed from 25.7 ± 1.0 to 25.4 ± 1.4 mmol L^{-1} ($P > 0.05$) showing a plasma glucose lowering activity (2.4 ± 2.5%; n = 8) without significance. This indicated that treatment with Die-Huang-Wan elicited a marked reduction of plasma glucose concentrations in animal models with better pancreatic function. Thus it can be considered that Die-Huang-Wan has an insulin-dependent action.

To examine the role of insulin in the plasma-glucose-lowering activity of Die-Huang-Wan, plasma insulin levels were measured in normal rats. Values of insulin-like immunoreactivity in the plasma from normal rats were dose-dependently increased by the oral administration of Die-Huang-Wan, as compared with the basal level (214.2 ± 24.2 pmol L^{-1}) (n = 10) (Figure 2). Inhibition of insulin metabolism by Die-Huang-Wan seems unlikely because the response to exogenous insulin was not influenced by Die-Huang-Wan, directly investigated in the streptozotocin-diabetic rats. The plasma-glucose-lowering activity of 1 int. units kg^{-1} short-acting human insulin in streptozotocin-diabetic rats receiving Die-Huang-Wan (26.0 mg kg^{-1}) was 22.7 ± 4.3% (n = 8), not significantly different ($P > 0.05$) from the results produced in the streptozotocin-diabetic rats receiving vehicle (21.4 ± 5.6%; n = 8). Thus, there was no doubt that secretion of endogenous insulin was a mediator in the plasma-glucose-lowering activity induced by Die-Huang-Wan. It also indicated that oral treatment with Die-Huang-Wan would be more effective in the patient whose pancreatic cells still retained partial insulin secretion.

Die-Huang-Wan had an effective benefit in regulating plasma glucose in rats, and so clinical trials of this herbal preparation in man will provide helpful evidence. The capacity of Die-Huang-Wan to modulate insulin secretion was obviously dose-dependent in rats. This was similar to the oral hypoglycaemic sulphonylurea drugs that can augment insulin secretion from β-cells (Shennan et al 1987; Chi et al 1998; Chang et al 1999). However, the action of Die-Huang-Wan is unlike met-

formin, which can lower plasma glucose without the stimulation of insulin secretion (Bailey 1993; DeFronzo 1999; Wiernsperger & Bailey 1999). Therapeutic efficacy of Die-Huang-Wan needed the presence of insulin and this will be useful for patients with non-insulin-dependent diabetes but not for insulin-dependent diabetic patients. In non-insulin-dependent diabetes insulin secretion from the pancreatic β-cell is milder, and in many cases an impairment of insulin action is present (Reaven 1980; DeFronzo 1987). Die-Huang-Wan may be helpful to improve these changes; however, the detailed mechanism of action as to how this herbal mixture stimulates insulin secretion needs further investigation.

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