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Short communication

Hypoglycemic effect of *Rehmannie Radix Preparata* (Sookjihwang) extract in streptozotocin-induced diabetic rats

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Abstract

Rhemannie Radix Preparata (RRP) has been previously employed in traditional oriental medicine as a treatment for diabetic thirst and improving blood flow. The aim of this study was to evaluate its hypoglycemic control by assaying the activities of key enzymes of carbohydrate metabolism in streptozotocin-(STZ)-induced diabetic rats. Further, RRP extracts were prepared in water (RRPW), in 50% ethanol (RRP50), and in 100% ethanol (RRP100), respectively, and compared for their actions in diabetic rats. The oral treatment of RRP (5 mg/kg b.w./d) to diabetic rats for 21 days resulted in a significant decline in blood glucose by 67% compared to diabetic control rats ($P < 0.05$). The altered activities of glucokinase, glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), and acetyl CoA carboxylase (ACC) in the livers of diabetic rats were reversed significantly to near-normal levels by the administration of RRP ($P < 0.05$). Among the three RRP extracts, RRP100 was the most effective in terms of hypoglycemic action. However, the administration of RRP to diabetic rats did not improve insulin production. The modulatory effects of RRP100 on the attenuation of carbohydrate enzyme activities appear to hold promise for widespread use for the treatment of diabetes in the future.

Key Words: Sookjihwang, *Rhemannia Radix Preparata*, streptozotocin-induced diabetes, hypoglycemic action, carbohydrate enzyme activity

Introduction

Rhemannie Radix (RR) has been employed in traditional Asian medicine since approximately 200 B.C. It was classified as high-grade (very safe) medicine [1]. RR exists in three different types; unprocessed, dried, and processed RR. Dried-RR was prepared by peeling and drying of raw RR. The RR preparata (RRP) was prepared via the extraction of raw RR in Korea rice wine, Makgeolli, and nine repetitions of a steaming and drying procedure [2]. Compositional analysis of RRP showed that starch was degraded and total sugar was reduced, but the major marker constituent of RRP, 5-hydroxymethyl-2-furaldehyde (5-HMF) was gradually increased along with repeated processing, as compared with RR [3].

In particular, RRP differs significantly from raw RR in its usage in oriental medicine: Raw RR can reduce heat in the blood and promote the production of body fluid [1], whereas RRP can nourish 'yin', modulate diabetic thirst, and promote blood flow [4]. Based on the empirical clinical findings, a herbal formula containing RR evidenced anti-diabetic effects in neonatal streptozotocin (STZ)-induced rats [5]. Three radix extracts, including Rhemannia, Panax Ginseng, and Scutellariae improved insulin secretion and beta-cell proliferation via the induction of insulin receptor substrate 2 (IRS2) protein [6]. However, these anti-diabetic effects of RRP have been examined as a complex form rather than a single treatment. Moreover, the roles of RRP in glucose metabolism have not been particularly well established.

Therefore, the principal objective of this study was to determine whether RRP extract exerts hypoglycemic activity in STZ-induced diabetic rats by modulating glucose metabolic enzymes. Moreover, we compared the activity of RRP using different extraction solutions including water, 50% ethanol and 100% ethanol.

Materials and Methods

Preparation of RRP extract

The RR preparata (RRP) was obtained from a commercial market (Keumsan, Korea), and prepared via the traditional production method (Fig. 1). The RRP was extracted with three kinds of solutions, or water, 50% ethanol and 100% ethanol,

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Fig. 1. *Rehmannie Radix Preparata* (Sookjihwang)

which could show differen sugar or lipid extraction. The extraction processes were repeated 3 times at 100℃ for 5h with water, and at 25℃ for 24 h with 50% ethanol and 100% ethanol. The extracts were filtered and concentrated via vacuum evaporation and lyophilization (Savant SC 100A, Holbrook, NY, USA).

Induction of experimental diabetes

Male Sprague-Dawley (4 week old, n = 20) rats were obtained from Orient Bio (Seongnam, Korea). Animals were maintained under environmentally-controlled conditions with a 12 h light/dark cycle at 22 ± 2℃ and a relative humidity of 50 ± 5%. They were acclimatized to the laboratory for 7 d before the experiments, and provided with free access to standard pellet chow diets (Purina Korea Inc., Korea) and water.

Experimental diabetes was induced in rats that had fasted for 12 h via intraperitoneal injections of streptozotocin (STZ, 50 mg/kg) dissolved in 0.1 M of cold citrate buffer (5 mM, pH 4.5). Because STZ is capable of inducing fatal hypoglycemia as a consequence of massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6h of STZ administration for the next 24h to prevent hypoglycemia. After a week to allow for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration, > 250 mg/dL) that evidenced glycosuria and hypoglycemia were selected for the experiments. Control littermates received only an injection of citrate buffer. All procedures involving animals were approved by the Experimental Animal Care Committee of Joongbu University, Choongnam, Korea.

Experimental design

After the successful induction of experimental diabetes, the rats were randomly divided into four groups, each comprising a minimum of five rats: Group 1: normal control (NC), Group 2: STZ-induced diabetic control (DC), Group 3: diabetic rats administered with RRP extract in 50% ethanol , Group 4: diabetic rats administered with RRP extract in 100% ethanol. Group 5: diabetic rats administered with RRP extract. After each RRP was diluted with water in ratio of 1 g/L, the amount of 5 mg/kg BW/d was fed through the mouth of rats by catheter for 21 days. The dosage of RRP in this study was adopted from traditional oriental medical prescriptions. Two control groups--a normal control (NC) and a diabetic control (DC)--received equal volumes of distilled water. At the end of the treatment period, the rats were fasted overnight, anaesthetized, and sacrificed via cervical decapitation. The blood and liver tissues were collected.

Biochemical estimations

Blood glucose was estimated via the methods described by Sasaki *et al.* [7]. Insulin levels were measured in plasma using the sensitive rat insulin radioimmunoassay kit (Shibayagi, Shibukawa, Japan). A portion of the liver tissue was dissected out and immediately washed in ice-cold saline, and then homogenized in 0.1 M Tris-HCl buffer (pH 7.4) to identify the key enzymes of carbohydrate metabolism. The homogenates were centrifuged at 10,000 rpm to remove the debris and the supernatants were employed as an enzyme source for the assays of glucokinase, glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate-dehydrogenase (6PGD) and acetyl-CoA carboxylase (ACC), according to the methods described by Joo *et al.* [8].

Statistical analysis

Results were expressed as means ± SD of five rats per group and statistical significance was assessed via one-way analysis of variance (ANOVA) and Duncan's multiple range test using SAS software. Values were considered statistically significant at $P < 0.05$.

Results

The effects of RRP administration on fasting blood glucose and insulin levels in the streptozotocin (STZ)-injected rats are shown in Table 1. The diabetic animals evidenced statistically significant increases in fasting blood glucose as compared to the normoglycemic animals ($P < 0.05$). The treatment of STZ-induced diabetic animals with RRP for 21 days resulted in significant reductions in blood glucose levels compared to those measured in the diabetic control animals ($P < 0.05$). The hypoglycemic effect of RRP was altered slightly according to the extraction solutions, which was higher in the following order: RRP100 (67% of diabetic control), RRP50 (58% of diabetic control) and RRPW (49% of diabetic control).

STZ also induced an apparent decline in insulin levels as shown in Table 1 ($P < 0.05$). The oral administration of RRP ethanol extract maintained the plasma insulin at the level observed for the diabetic control animals. Plasma insulin levels were lowest in the RRPW group among the experimental groups ($P < 0.05$) (Table 1).

We then determined the role of RRP treatment on carbohydrate metabolism enzyme activities in diabetic model animals, as shown in Table 2. The activities of hepatic glucokinase, G6PD,

Table 1. Effects of *Rehmannie Radix* extract on the level of blood glucose and insulin in streptozotocin-induced diabetic rats

Groups	Glucose (mg/dL)	Insulin (ng/mL)
Normal control	62.20 ± 6.80[4,5)c]	3.94 ± 0.79[a]
Diabetic control[1)]	332.40 ± 21.30[a]	1.45 ± 0.30[b]
RRP50[2)]	141.02 ± 6.31[b]	1.57 ± 0.11[b]
RRP100[3)]	111.12 ± 4.42[b,c]	1.45 ± 0.21[b]
RRPW[4)]	168.02 ± 12.52[b]	0.63 ± 0.15[c]

[1)] Streptozotocin (50 mg/kg body weight)-induced diabetic rats
[2)] Streptozotocin-induced diabetic rats treated with RRP extract in 50% ethanol by daily oral administration (5 mg/kg body weight) for 21 days
[3)] Streptozotocin-induced diabetic rats treated with RRP extract in 100% ethanol by daily oral administration (5 mg/kg body weight) for 21 days
[4)] Streptozotocin-induced diabetic rats treated with RRP extract in water by daily oral administration (5 mg/kg body weight) for 21 days
[5)] Data are expressed as mean ± SD (n = 5).
[6)] Values with different superscripts within the column are significantly different at $P < 0.05$ by Duncan's multiple range test.

G6PDH, and acetyl Co A carboxylase in STZ-induced rats were reduced significantly compared to the normal control rats. Both the RRP 50 and RRP 100 groups evidenced significantly improved glucokinase activity in the liver, as observed in Table 2 ($P < 0.05$).

The results of this study demonstrated that the reduction in G6PDH activity in diabetic rats (51 unit) is significantly regulated by treatment with RRP 50 (110 unit), and RRP 100 (190 unit) ($P < 0.05$) as shown in Table 2.

The other HMP enzyme, 6PGD, is an oxidative carboxylase which catalyzes the decarboxylating reduction of 6-phosphogluconate into ribulose 5-phosphate in the presence of NADP. The observed reduction in the 6PGD activity of diabetic rats (5 unit) was reversed significantly by RRP 50 (90 unit) and RRP 100 (60 unit), respectively, whereas normal rats had 40 units, as shown in Table 2 ($P < 0.05$).

The significant reduction in the ACC activity of diabetic rats (0.17 nmole/min/mg protein) was significantly increased to the level of normal rats (0.30 nmole/min/mg protein) by the treatment of RRP 50 (0.38 nmole/min/mg protein) and RRP 100 (0.40 nmole/min/mg protein), respectively ($P < 0.05$, Table 2). RRPW did not stimulate other carbohydrate metabolism enzymes, but rather normalized hepatic ACC activity ($P < 0.05$) in the present study (Table 2).

Discussion

In this study, hypoglycemic action of RRP ethanol extract was determined in the streptozotocin (STZ)-injected rats by altering activities of glucose metabolic enzymes. Because there is a lack of studies on RRP as a single treatment in diabetes, it is not easy to compare the present results with others. However, blood glucose lowering effect was shown by the crude extract administration of dried RR in diabetic nephropathy model [9]. Kim *et al.* [5] proved anti-diabetic effects of herbal formula including RRP. Also, Park *et al.* [6] reported that administration of three radix complexes of RR, Ginseng and Scutellariae lowered plasma glucose in diabetic rats. On the other hand, it has been reported that 7 days of treatment with RR water extract exerted no significant glycemic control effects in neonatal STZ-rats, although a trend toward decreasing plasma glucose levels was noted and it was effective in promoting diabetic foot ulcer healing in rats via inflammation control [4].

The selective destruction of pancreatic beta cells was derived from STZ injection, resulting in an apparent decline in insulin levels [10]. Interestingly, plasma insulin level was not stimulated by the oral administration of RRP ethanol extract, and it was significantly lowered in the RRPW group among the experimental groups ($P < 0.05$) (Table 1). This result strongly indicated that certain lipid soluble substances in RRP should exert a hypoglycemic effect independent of insulin secretion. It needs to be studied whether RRPW has possible toxicity on the pancreas physiology, because RRPW has significantly reduced insulin secretion compared to STZ control group in this study. Also, since there were quite different results in glucose metabolism between RRP 50 and RRPW, the possible effects of RRP extracts in other solvent systems need to be determined. Glucokinase closes the KATP channel, resulting in efficient insulin secretion when serum glucose is elevated. Simultaneously,

Table 2. Effects of *Rehmannie Radix* extract on carbohydrate enzyme activities in streptozotocin-induced diabetic rats

Groups	Glucokinase (unit/mg protein)	G6PD[5)] (unit/mg protein)	6PGD[6)] (unit/mg protein)	ACC[7)] (nmole/min/mg protein)
Normal control	55.12 ± 4.43[6)a7)]	400.02 ± 52.12[a]	40.33 ± 5.12[c]	0.30 ± 0.07[a]
Diabetic control[1)]	17.21 ± 2.21[c]	51.00 ± 4.32[c]	5.12 ± 2.12[d]	0.17 ± 0.03[b]
RRP50[2)]	30.22 ± 1.32[b]	110.34 ± 12.11[b]	90.24 ± 4.23[a]	0.38 ± 0.09[a]
RRP100[3)]	34.12 ± 2.18[b]	190.21 ± 22.32[b]	60.11 ± 4.44[b]	0.40 ± 0.10[a]
RRPW[4)]	15.02 ± 3.24[c]	50.10 ± 2.56[c]	8.09 ± 2.11[d]	0.34 ± 0.05[a]

[1)] Streptozotocin (50 mg/kg body weight)-induced diabetic rats
[2)] Streptozotocin-induced diabetic rats treated with RRP extract in 50% ethanol by daily oral administration (5 mg/kg body weight) for 21 days
[3)] Streptozotocin-induced diabetic rats treated with RRP extract in 100% ethanol by daily oral administration (5 mg/kg body weight) for 21 days
[4)] Streptozotocin-induced diabetic rats treated with RRP extract in water by daily oral administration (5 mg/kg body weight) for 21 days
[5)] glucose-6-phosphate dehydrogenase
[6)] 6-phosphogluconate-dehydrogenase
[7)] Acetyl-CoA carboxylase
[8)] Data are expressed as mean ± SD (n = 5).
[9)] Values with different superscripts within the column are significantly different at $P < 0.05$ by Duncan's multiple range test.

it functions as one of the key enzymes in glucose catabolism, phosphorylating glucose and converting it into glucose-6-phosphate; glucokinase levels have been shown to be altered in diabetes. This results in a decline in glycolysis and reduced utilization of glucose for energy production [6,11,12]. This study demonstrated that the specific activity of hepatic glucokinase, an insulin-inducible enzyme and the major enzyme in the phosphorylation of glucose in the liver, was reduced in diabetic animals. Additionally, the administration of RRP 50 and RRP100 significantly returns glucokinase activity to the similar value of normal control animals. Similarly, another herbal extract of Angelica gigas (AG) radix has hypoglycemic effect in STZ-induced rat [13].

G6PD, a housekeeping enzyme, catalyzes the first and rate-limiting step of the hexose monophosphate shunt (HMP) and generates the NADPH required for the maintenance of reduced glutathione and reductive biosynthesis [14]. The activity of G6PD is also regulated via alternative splicing in response to hormonal and nutritional cues such as glucose and lipids. Modest changes in G6PD itself exert significant effects on cell growth and cell death in a variety of cell types. The observed reduction in G6PD activity in diabetic rats also may suggest a reduction in metabolism via the phosphogluconate oxidation pathway [15-17].

The present study showed that the reduction in G6PDH activity in diabetic rats (51 unit) is significantly regulated to 110 unit by the treatment with RRP 50, and to 190 unit by the treatment with RRP 100, respectively ($P < 0.05$). In comparison, Park *et al*. [13] reported that treatment with AG radix extract in STZ-injected rats resulted in an enhancement of G6PD activity to 38 units (AG in 50% ethanol), and 69 units (AG in 100% ethanol).

The other HMP enzyme, 6PGD, is an oxidative carboxylase which catalyzes the decarboxylating reduction of 6-phosphogluconate into ribulose 5-phosphate in the presence of NADP [18]. The reduced activity of 6PGD in diabetic rats (5 unit/mg) was reversed by RRP 50 administration to 18 fold (90 unit/mg) and by RRP 100 administration to 12 fold (60 unit/mg), respectively, as shown in Table 2 ($P < 0.05$).

On the contrary to this, the administration of AG radix extract did not enhance the 6PGD activity in the previous study [13]. Thus, RRP is a better enzymatic regulator of carbohydrate catabolism than Angelica gigas radix extract, which has been previously employed in complex prescriptions with RRP in oriental medicine.

Acetyl-CoA carboxylase (ACC) catalyzes the biotin-dependent carboxylation of acetyl-CoA, thereby generating malonyl-CoA [19]. This is the first step, and the commitment point, in the biosynthesis of long-chain fatty acids. Simultaneously, a second isoform of ACC, ACC2, is associated with the mitochondrial membrane and produces malonyl-CoA, which regulates fatty acid oxidation by profoundly inhibiting the carnitine palmitoyltransferases (CPT-Is). Mice deficient in ACC2 show elevated fatty acid oxidation and reduced body fat content and body weight, despite the fact that they consume more food. Therefore, inhibitors against ACCs might prove efficacious in treating obesity and diabetes [19]. ACC-specific activity is also rapidly modulated, and increased in response to insulin and reduced after the exposure of cells to catabolic hormones or environmental stress conditions [20]. In our study, RRP ethanol extract showed the significant reversal potential in the ACC activity of diabetic rats to the level of normal rats ($P < 0.05$, Table 2). This result is also comparable to the observed enhancement effect on ACC activity of AG extract (0.29-0.31 nmole/min/mg protein) in STZ-injected rats. Thus, the results of this study demonstrated that simultaneous activations of RRP extracts--particularly 100% ethanol extract--on hepatic glucokinase, G6PD, 6PGD, and ACC contributed to hypoglycemic regulation in STZ-injected rats. It should be considered important to select the appropriate solution to enhance the hypoglycemic control characteristics of the RRP extract.

In conclusion, the administration of RRP ethanol extract to streptozotocin-induced diabetic rats restored the key enzyme activities in glucose metabolism and reversed blood glucose concentration. The findings of this study demonstrate that RRP ethanol extract has complementary potency for widespread use in treating diabetes in the future.

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