Ginsenoside Re of *Panax ginseng* possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats

William C.S. Cho \(^{a,*}\), Wai-Shing Chung \(^{a}\), Sally K.W. Lee \(^{a}\), Albert W.N. Leung \(^{b}\), Christopher H.K. Cheng \(^{c}\), Kevin K.M. Yue \(^{a}\)

\(^{a}\) School of Chinese Medicine, Hong Kong Baptist University, 7 Baptist University Road, Hong Kong, China
\(^{b}\) School of Chinese Medicine and Health Care, Chinese University of Hong Kong - Tung Wah Community College, Hong Kong, China
\(^{c}\) Department of Biochemistry, Chinese University of Hong Kong, Hong Kong, China

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Abstract

Diabetes mellitus is characterized by hyperglycemia and complications affecting the eye, kidney, nerve and blood vessel. We have previously demonstrated the occurrence of oxidative stress of streptozotocin-induced diabetic rats, preceded by a depletion in the tissue level of glutathione. In this study, when diabetic rats were treated with ginsenoside Re of *Panax ginseng* C.A. Meyer, there was a significant reduction in blood glucose, total cholesterol and triglyceride levels. On the other hand, oxidative stress has been implicated in the pathogenesis of diabetes and its complications. It was found that treatment by ginsenoside Re restored the levels of both glutathione and malondialdehyde in the eye and kidney to those found in the control rats. This is the first report demonstrating ginsenoside Re has significant antioxidant efficacy in diabetes, and prevents the onset of oxidative stress in some vascular tissues. Our results demonstrated that ginsenoside Re could lower blood glucose and lipid levels, and exerts protective actions against the occurrence of oxidative stress in the eye and kidney of diabetic rats. Our data also provide evidence that ginsenoside Re could be used as an effective antidiabetic agent particularly in the prevention of diabetic microvasculopathy.

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1. Introduction

Diabetes mellitus is the most common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eye, kidney, nerve and blood vessel. *Panax ginseng* C. A. Meyer (ginseng) has been widely used to treat diabetes in traditional Chinese medicine (Cho et al., 2005; Covington, 2001). Results of clinical studies demonstrated that ginseng could improve the immune response in diabetic patients (Kiefer and Pantuso, 2003). In most cases, it is the root of ginseng that is used to treat diabetes. Recent reports found that the berry extract of ginseng could also be used to treat diabetes in obese mice (Attele et al., 2002; Dey et al., 2002, 2003; Xie et al., 2002), and the beneficial effects could be attributed to ginsenoside Re (Attele et al., 2002; Xie et al., 2005). However, the mechanism of actions of ginsenoside Re remains to be elucidated. In this study, the action of ginsenoside Re on serum glucose, cholesterol and triglyceride of streptozotocin-induced diabetic rats was studied. This natural compound was also assessed of its ability to prevent the occurrence of oxidative stress in the vascular tissues of diabetic rats.

2. Materials and methods

2.1. Chemicals

Streptozotocin was obtained from Sigma Chemical Co (St Louis, MO, USA). Ginsenoside Re of high performance liquid chromatography grade with purity >99% was obtained from Hongjiu Ginseng (Jilin, PR China). The chemical structure of ginsenoside Re is shown in Fig. 1. Unless otherwise stated, all chemicals are of analytical grade.
2.2. Experimental animals

Experiments were in accordance with the recommendations from the Declaration of Helsinki and the internationally accepted principles in the care and use of experimental animals. The experimental protocol used in this study was designed in accordance with the Guide for Animal Experimentation, Hong Kong Baptist University. Wild-type outbred male Sprague-Dawley (SD) rats (Laboratory Animal Service Centre, The Chinese University of Hong Kong) weighing 250–300 g were divided into groups and maintained at the Animal House of the Hong Kong Baptist University for 6 weeks prior to the commencement of ginsenoside Re administration. Pellet diet (Lab Diet, Brentwood, MO, USA) and tap water were provided ad libitum.

2.3. Induction of diabetes

Induction of diabetes in the overnight-fasted SD rats and monitoring of blood glucose concentrations were based on established procedures previously described (Cho et al., 2006a,b). Briefly, two injections of freshly prepared streptozotocin in 0.1 M sodium citrate buffer at pH 4.5 were given intraperitoneally to the rats at a dose of 40 mg/kg on two consecutive days. Control animals received injections of citrate buffer only. Blood samples collected from the tail veins of overnight-fasted rats were allowed to clot for 1 h at room temperature. The samples were then centrifuged at 1500 × g for 10 min before collection of sera. Diabetes was confirmed by the Glucose Diagnostic Kit (Sigma, St Louis, MO, USA) 4 weeks after streptozotocin administration and only rats with blood glucose levels >300 mg/dl were allowed to enter into the study protocol as diabetic animals. Fasting serum glucose concentrations were then measured at three time points (6 weeks, 7 weeks and 8 weeks after streptozotocin administration) with reference to our previous finding that the onset of oxidative stress in diabetic rats starts at week 8 (Yue et al., 2003).

2.4. Drug preparation and administration

Drug preparation and administration were based on established procedures previously described (Cho et al., 2006c). Briefly, ginsenoside Re (150 mg) was first mixed with 50 ml high performance liquid chromatography grade MeOH (Fisher, Hampton, NH, USA), and then dissolved in 1500 mg poly-vinylpyrrolidone-10 (PVP-10) (Sigma, St Louis, MO, USA) in 50 ml MeOH. To avoid oxidization of the materials in the evaporation process, the solution was evaporated under N2 in a 50 °C water-bath to dryness, and then stored in a 4 °C refrigerator. Before each oral administration, the dried ginsenoside Re was re-dissolved in PVP-10 solution (5 mg/ml of water). The diabetic rats were equally divided into four groups each fed with a daily different dose of ginsenoside Re (5, 10 or 20 mg/kg of body weight) or PVP-10 solution (vehicle) for 2 weeks starting from the sixth week after streptozotocin administration, while the control normal rats were equally divided into two groups each fed with 20 mg/kg ginsenoside Re or the vehicle.

2.5. Glycated hemoglobin measurement

Blood samples (0.9 ml) were collected from each overnight-fasted rat and then kept on ice. The glycated hemoglobin (HbA1c) levels were measured by the Glycated Hemoglobin (Total) Diagnostic Kit (Sigma, St Louis, MO, USA) on the same day.

2.6. Serum lipids measurements

The fasting serum total cholesterol and serum triglyceride concentrations were measured by commercial enzymatic assay kits from Stanbio Laboratory (Boerne, TX, USA) and BioSystems (Barcelona, Spain) respectively.

2.7. Tissue sample preparation

Based on our previous protocols (Cho et al., 2006a,b; Yue et al., 2003), tissue samples (kidney, eye and aorta) were excised from the rats on the day of sacrifice, rinsed with ice-cold phosphate buffered saline and then blotted dry. They were then frozen immediately in liquid nitrogen and stored at −80 °C for subsequent use. The samples were randomly divided into two portions, one for glutathione determination and the other for the malondialdehyde determination.

2.8. Glutathione and malondialdehyde measurements

The glutathione and malondialdehyde concentrations of tissue samples were determined by the BIOXYTECH GSH-400 Assay Kit (OxisResearch, Portland, OR, USA) and the BIOXYTECH LPO-586 Assay Kit (OxisResearch, Portland, OR, USA) respectively. Using a mechanic homogenizer, tissue
homogenates (1 g/10 ml) for glutathione determination were prepared in ice-cold metaphosphoric acid at 4 °C, and the homogenates for malondialdehyde determination were prepared in phosphate buffered saline (20 mM, pH 7.4) with butylated hydroxytoluene (10 μl of a 0.5 M solution per ml tissue homogenate) at 4 °C. The homogenates were then centrifuged at 5000 × g (for kidney and eye) or 2700 × g (for aorta) at 4 °C for 10 min. Aliquots of the clear supernatant were taken for the glutathione and malondialdehyde measurements. Protein concentrations in the samples were determined using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA).

2.9. Statistical analyses

The results were analyzed by the Mann-Whitney U test and expressed as the mean values ±S.E.M. P values < 0.05 are considered statistically significant.

3. Results

3.1. Antihyperglycemic action of ginsenoside Re

The fasting serum glucose concentrations were measured at three time points (6 weeks, 7 weeks and 8 weeks after streptozotocin administration). At week 6 and week 7, the fasting serum glucose levels in the diabetic rats were significantly higher than the control normal rats (P < 0.01). At week 8, the fasting serum glucose levels of the Re-treated diabetic rats were significantly reduced by 23% when compared with the vehicle-treated diabetic rats (P < 0.01). The fasting serum glucose levels of the control normal rats were not significantly altered by treatment with ginsenoside Re at all the time points. In the dose-response study involving the administration of different doses of ginsenoside Re to the rats, no significant difference was observed among the four groups at all the time points (Table 1 and Fig. 2).

The HbA1 values in whole blood of both the diabetic and control normal rats were measured at week 6 and week 8. The HbA1 values in the diabetic rats were significantly higher than the control normal rats at week 6 (P < 0.01). After 2 weeks administration of ginsenoside Re or vehicle, the HbA1 values of both the diabetic rats and control normal rats were not affected in response to ginsenoside Re treatment. In the dose-response study, no significant difference was found among the four groups at both time points (Table 1).

3.2. Action of ginsenoside Re on serum lipids (total cholesterol and triglyceride)

The serum total cholesterol level in the diabetic rats was significantly higher than the control normal rats at week 6 and week 8 (P < 0.01). There were significant reductions in the serum total cholesterol in the diabetic rats treated with 10 mg/kg or 20 mg/kg ginsenoside Re, but not for those treated with 5 mg/kg only. One-week treatment of the diabetic rats by 20 mg/kg ginsenoside Re reduced serum total cholesterol by 22% when compared to the vehicle-treated rats (P < 0.05), while two-week

<table>
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<th>Diabetic rats</th>
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*P value: Vehicle-treated diabetic group vs 20 mg/kg ginsenoside Re-treated diabetic group.
treatment normalized the serum total cholesterol by a reduction of 32% when compared to the vehicle-treated rats (P < 0.01) (Table 1 and Fig. 3A).

Similarly, the serum triglyceride levels in the diabetic rats were also significantly higher than the control normal rats at week 6, week 7 and week 8 (P < 0.01). For the serum triglyceride levels in the diabetic rats treated with 20 mg/kg ginsenoside Re, a two-week treatment has resulted in a significant reduction of 44% when compared to the vehicle-treated rats (P < 0.01) (Table 1 and Fig. 4A).

In the dose-response study involving the administration of different doses of ginsenoside Re to the rats, a progressive decreasing trend in the total cholesterol level (from the control vehicle group → 5 mg/kg ginsenoside Re group → 10 mg/kg ginsenoside Re group → 20 mg/kg ginsenoside Re group) was observed in response to the treatment at both week 7 and week 8 (Table 1 and Fig. 3B).

In the ginsenoside Re dose-response study, though no significant difference in the triglyceride level was observed among the four groups after one-week of ginsenoside Re treatment, a significant progressive decreasing trend was found in response to a two-week ginsenoside Re treatment with different doses of ginsenoside Re (Table 1 and Fig. 4B).

3.3. Action of ginsenoside Re on glutathione levels in the kidney and eye

The glutathione levels in the kidney and eye of vehicle-treated diabetic rats were found to be significantly lower than the control normal rats (P < 0.01). There were significant elevations in the glutathione levels of the kidney and eye of the diabetic rats treated with 20 mg/kg ginsenoside Re. Two-week treatment of diabetic rats by ginsenoside Re significantly increased kidney glutathione levels by 28% (P < 0.05) and eye glutathione levels by 34% (P < 0.01) when compared to the control vehicle-treated rats. The glutathione levels in the kidney and eye of the diabetic rats were normalized after treatment with ginsenoside Re (Table 1 and Fig. 5A).

In the ginsenoside Re dose-response study, the glutathione levels in the kidney were not affected by the two lower doses of ginsenoside Re. Only the 20 mg/kg ginsenoside Re-treated rats exhibited significantly higher levels of glutathione. In the eye, all the three ginsenoside Re-treated groups exhibited significant higher glutathione levels than the control vehicle group. However, no significant difference was observed among the three groups treated with different doses of ginsenoside Re (Table 1 and Fig. 5B).

3.4. Action of ginsenoside Re on malondialdehyde levels in the kidney and eye

The malondialdehyde levels in the kidney and eye of the vehicle-treated diabetic rats were significantly higher than the control normal rats (P < 0.01). There were significant reductions in the malondialdehyde levels of the kidney and eye of diabetic rats treated with 20 mg/kg ginsenoside Re. Two-week treatment of diabetic rats by ginsenoside Re significantly reduced kidney malondialdehyde by 11% and eye malondialdehyde level by 39% when compared to the vehicle-treated rats (P < 0.05). The malondialdehyde level in the eye of the diabetic rats was normalized after treatment with ginsenoside Re (Table 1 and Fig. 6A).
In the dose-response study, the malondialdehyde levels in the kidney were not affected by the two lower doses of ginsenoside Re. Only the 20 mg/kg ginsenoside Re-treated rats exhibited significantly lower malondialdehyde level in the diabetic rats than the vehicle-treated rats. In the eye, a significant progressive decreasing trend in the malondialdehyde levels of the diabetic rats was observed after treatment with different doses of ginsenoside Re as compared with those of the vehicle-treated group (Table 1 and Fig. 6B).

3.5. Action of ginsenoside Re on glutathione level in the aorta

The glutathione level in the aorta of vehicle-treated diabetic rats was significantly lower than the control normal rats \((P<0.01)\). Though there was an increase in the aorta glutathione level of the diabetic rats after treatment with 20 mg/kg ginsenoside Re, it did not change significantly as compared with the vehicle-treated rats \((P>0.05)\) (Table 1 and Fig. 5A).

In the dose-response study, no significant difference in the glutathione levels in the aorta was observed among the four diabetic groups (Table 1 and Fig. 5B).

3.6. Action of ginsenoside Re on malondialdehyde level in the aorta

There was a significant elevation of the malondialdehyde level in the aorta of vehicle-treated diabetic rats when compared to the control normal rats \((P<0.01)\). However, the malondialdehyde level of the diabetic rats treated with 20 mg/kg ginsenoside Re was not significantly altered when compared to the vehicle-treated rats \((P>0.05)\) (Table 1 and Fig. 6A).

Although there were some variations among the malondialdehyde levels in the aorta of the four groups of diabetic rats treated with different doses of ginsenoside Re, neither significant difference nor a general trend could be observed among them (Table 1 and Fig. 6B).

4. Discussion

According to World Health Organization, around 171 million people worldwide are suffering from diabetes in 2000. This figure is predicted to double by 2030 (Wild et al., 2004). Diabetic retinopathy, nephropathy and cardiovascular disease are among the most common complications of diabetes. Around 85% of all diabetics eventually develop diabetic retinopathy, which is the commonest cause of blindness in the fourth and seventh decades of life (Tewari and Venkatesh, 2004). Diabetes is also one of the leading causes of kidney failure, whereas heart disease accounts for the majority of deaths among people with diabetes in developed countries.

Ginseng is a herb commonly used to treat diabetes in traditional Chinese medicine. An early study reported that ginseng could prevent alloxan-induced activation of processes of lipid peroxidation in the pancreas and demonstrated definite insulinogenic properties (Davydov et al., 1990), whereas a double-blind placebo-controlled trial found that 200 mg of ginseng per day could improve blood sugar levels in non-insulin-dependent diabetic patients (Sotaniemi et al., 1995). Recent studies found that decreased c-Fos expression in the CA regions of the hippocampus was observed with streptozotocin-induced diabetes,
and administration of ginseng enhanced the streptozotocin-induced inhibition of c-Fos expression both dose- and duration-dependently. These results suggested that hyperglycemia-induced suppression of Fos expression might trigger the diabetes-induced disruption of hippocampal information processing, and that ginseng might alleviate this diabetes-induced disturbance in hippocampal functions (Jang et al., 2003).

In this study, a significant anti-hyperglycemic action on the fasting serum glucose was observed after a two-week treatment of diabetic rats with 20 mg/kg ginsenoside Re. This finding provided a valuable evidence for past reports on the anti-diabetic action of ginsenoside Re (Attele et al., 2002; Xie et al., 2004, 2005). However, no significant trend was observed in the fasting serum glucose of the animals treated with different doses of ginsenoside Re.

As hyperglycaemia leads to non-enzymatic glycation of proteins, measurement of HbA1c is usually carried out to monitor the long-term control of blood glucose levels in subjects with diabetes (Steffes et al., 2005). The HbA1c level of the diabetic rats was found to be increased. No significant difference in HbA1c level was observed between the vehicle-treated diabetic rats and the ginsenoside Re-treated diabetic rats, indicating that a two-week treatment with ginsenoside Re did not produce a sufficiently long enough anti-hyperglycemic action to be reflected in the normalization of HbA1c level.

Heart disease and stroke remains the primary cause of diabetes-associated morbidity and mortality (Ancion et al., 2005). Dyslipidemia is one of the major cardiovascular risk factors. Our results have clearly demonstrated the anti-hypercholesterolemic efficacy of ginsenoside Re on diabetic rats, as a one-week treatment of ginsenoside Re was sufficient to produce a significant reduction in the total cholesterol level in the diabetic rats. The serum total cholesterol in diabetic rats was restored to normal after a two-week treatment period. These results indicate that ginsenoside Re has a cholesterol-lowering effect on the diabetic rats but not on the normal rats. Moreover, the decrease at week 7 and week 8 showed a dose-dependent trend, indicating that the cholesterol-lowering efficacy is proportional to the amount of ginsenoside Re intake.

Apart from hypercholesterolemia, increased serum triglyceride level is also an independent risk factor for heart disease. Diabetic patients have problems in packaging cholesterol and tend to have higher serum triglyceride levels. Our data were in line with this notion as the serum triglyceride levels of the diabetic rats increased from week 6 to week 8 after streptozotocin administration. Although a one-week treatment with ginsenoside Re did not reduce the serum triglyceride of diabetic rats, a two-week treatment did alleviate the hypertriglyceridemia significantly. A dose-dependent serum triglyceride-lowering effect of ginsenoside Re was also observed.

There is increasing evidence showing that diabetes is associated with increased oxidative stress (Lee and Chung, 1999). Persistent hyperglycemia may cause increased production of free radicals which is related to glucose auto-oxidation that has been linked to non-enzymatic glycation, and glycated proteins have been shown to be a source of free radicals (Ceriello et al., 1992). Glutathione, the primary endogenous antioxidant, has a multifaceted role in antioxidant defence and it is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases (Winterbourn, 1995). Malondialdehyde, an index of endogenous lipid peroxidation, often acts as a quantitative marker of the aggression suffered by tissues. Results of the present study clearly demonstrated that glutathione levels in the kidney, eye and aorta of diabetic rats were significantly lower than those of the control animals; whereas the malondialdehyde levels in these organs were significantly higher than those of the control normals. Ginseng extracts have been shown to scavenge reactive oxidative species (Liu et al., 2002, 2003; Zhang et al., 1996) and to attenuate lipid peroxidation (Keum et al., 2000; Surh et al., 2001; Zhang et al., 1996). In this study, the glutathione and malondialdehyde levels in the kidney and eye of the diabetic rats were restored to normal levels after treatment with ginsenoside Re. Not only could ginsenoside Re ameliorate the antioxidant status in the kidney and eye, but it could also provide a reference dose for the drug administration.

Our dose-response results indicated that the glutathione level in the kidney was only significantly improved by 20 mg/kg ginsenoside Re treatment while the glutathione level in the eye already exhibited a significant increase with just 5 mg/kg ginsenoside Re. On the other hand, the malondialdehyde levels in the kidney and eye were only normalized with 20 mg/kg of ginsenoside Re treatment. However, no significant antioxidant efficacy was found on the aorta of the diabetic rats. As the aorta glutathione levels of the diabetic rats were lower than those in the kidney and eye while the malondialdehyde levels were much higher than the other two tissues, a larger dose of ginsenoside Re or a longer period of treatment may possibly be required to combat the increased level of reactive oxidative species generated in the aorta of the diabetic animals. This is the first report demonstrating that ginsenoside Re has significant antioxidant efficacy in diabetes, and this compound could prevent the onset of oxidative stress in some vascular tissues. These results suggest that ginsenoside Re may be beneficial to the treatment in some microvascular complications such as nephropathy and retinopathy.

We have a recent report making use of high-throughput proteomic profiling on the ginsenoside Re treated diabetic rats. Ginsenoside Re treatment of the diabetic rats has resulted in a significant reduction in the C-reactive protein level. This result demonstrated that the intake of ginsenoside Re could reduce the elevation of C-reactive protein in diabetes, implying ginsenoside Re may improve diabetes and its complications by reducing inflammation (Cho et al., 2006c).

Our results demonstrating that the orally administered ginsenoside Re possesses significant anti-hyperglycemic actions and could effectively normalize the impaired oxidative stress in the kidney and eye of the diabetic rats. In addition, ginsenoside Re exhibits definitive actions towards hypercholesterolemia and hypertriglyceridemia associated with diabetes. The promising antioxidant and anti-hyperlipidemic efficacies of ginsenoside Re demonstrated in this study may open new avenues in the treatment of diabetes and its complications.
References


