Antioxidant Protection by American Ginseng in Pancreatic β-Cells

Elaine Lin,*† Yong Wang,* Sangeeta Mehendale,*‡ Shi Sun,*† Chong-Zhi Wang,*‡ Jing-Tian Xie,*‡ Han H. Aung,*† and Chun-Su Yuan*†

*Tang Center for Herbal Medicine Research
†Department of Anesthesia and Critical Care
The University of Chicago
Chicago, Illinois 60637, USA

Abstract: Hyperglycemia in diabetic conditions may cause oxidative stress in pancreatic β-cells, leading to their dysfunction and insulin resistance within peripheral tissues. Previous studies suggest that American ginseng berry extract may have hypoglycemic effects, as well as offer antioxidant protection. We examined effects of American ginseng berry extract and ginsenoside Re in a pancreatic β-cell line, MIN-6, to determine if these two properties are related. Cells were exposed to oxidative stress via hydrogen peroxide incubation and oxidative stress was measured by oxidation of 2′,7′-dichlorofluorescin diacetate. These cells showed a concentration-related response to hydrogen peroxide at 100–500 µM. In acute conditions where cells were treated with the extract for 10 min, we observed reduced oxidant injury suggesting direct scavenging effects. Chronic incubation of cells with the extract for 48 hours also demonstrated attenuation of oxidative stress. At high concentrations, Re showed a mild antioxidant effect in MIN-6 cells. Our insulin release observations also showed that the extract may help to increase insulin secretions from the cells. Our data suggest that the observed ability of ginseng to reduce blood glucose levels may be linked to its antioxidant effects on pancreatic β-cells.

Keywords: Panax quinquefolius; Diabetes; MIN-6 Cell; Antioxidant; Reactive Oxygen Species; ROS; Hydrogen Peroxide.

Introduction

Type 2 diabetes is a heterogeneous disorder in which individuals experience insulin resistance with evidence of progressive loss of β-cell function (Diabetes, 2003). Hyperglycemia is the
widely accepted cause of the difficulties associated with type 2 diabetes, yet there is no consensus regarding the pathogenic link between hyperglycemia and its complications (The Diabetes Control and Complications Trial Research Group, 1993; Lo et al., 2006). Raised glucose levels have been linked to reactive oxygen species (ROS) generation and studies indicate that hyperglycemia may be the cause of oxidative stress in organisms, including in pancreatic β-cells (Mohanty et al., 2000; Ihara et al., 1999). ROS generation and oxidative stress lead to β-cell damage and have been associated with other complications such as atherosclerosis and nephropathy in diabetic patients (Li and Shah, 2003; Maechler et al., 1999). Studies also suggest that increased oxidative stress causes reduced insulin secretion (Kajikawa et al., 2002; Sakai et al., 2003). Thus, pancreatic β-cells are subject to injury via ROS generation while a standard method of protection has yet to be discovered.

Previous animal experiments and clinical trials have shown that ginseng may have anti-hyperglycemic activities (Vuksan et al., 2000; Attele et al., 2002; Cho, 2007; Wang et al., 2007). Other studies have also demonstrated the ability of ginseng to protect against ROS induced damage (Li et al., 1999; Kitts et al., 2000; Chang et al., 2007; Rhyu et al., 2007). However, it is unclear if the antioxidant properties of ginseng are the mechanisms responsible for blood glucose control. The present study sought to evaluate the effects of American ginseng on pancreatic β-cells and explore the relationship between protection against oxidative stress and improvements in insulin secretion.

Materials and Method

Cell Culture

The MIN-6 cell line, a pancreatic insulinoma β-cell line, was used in this study. This cell line has been used in other studies as a possible model for normal pancreatic islets (Dalle et al., 1999; Ishihara et al., 1993; Bell et al., 2003). Dulbecco’s modified Eagle medium (DMEM) was obtained from Gibco (Grand Island, NY). The cells were cultured in DMEM containing fetal bovine serum (FBS), streptomycin/penicillin (S/P) and L-glutamine. Passages 20–35 were utilized and medium was changed every 3–4 days.

Preparation of Test Botanicals

American ginseng berry was obtained from the Wisconsin Ginseng Board in Wausau, WI. The extract was prepared and freeze-dried, as described elsewhere (Attele et al., 2002). Appropriate amounts of the extract were diluted in distilled water to achieve final concentrations of 0.1, 0.5, and 1.0 mg/ml. Ginsenoside Re was obtained from Sigma (St. Louis, MI). A stock solution of Re was dissolved in DMSO and added to cells to achieve final concentrations of 0.05, 0.1, and 0.5 mg/ml.

Treatment and Measurements of Oxidants and ROS

Hydrogen peroxide (H₂O₂; Sigma, St. Louis, MI) was used to induce exogenous oxidative stress. MIN-6 cells were exposed to increasing concentrations of H₂O₂ from
100–500 µM for 60 min. Intracellular ROS was measured by using the probe 2′,7′-dichlorofluorescin diacetate (DCFH-DA) and a fluorescence spectrophotometer at excitation 488 nm/emission 529 nm. DCFH-DA can cross cell membranes and is hydrolyzed enzymatically by intracellular esterases to form nonfluorescent DCFH. DCFH reacts readily in the presence of ROS to form its oxidative state, highly fluorescent dichlorofluorescein (DCF). MIN-6 cells were incubated with DCFH-DA for 20 min prior to addition of H$_2$O$_2$.

**Evaluation of Acute and Chronic Effects**

To evaluate acute effects, MIN-6 cells were incubated with DCFH-DA and baseline measurements were read for 10 min. Ginseng extract and Re was then added to cells and measurements were taken for an additional 10 min. The extract remained in medium and H$_2$O$_2$ was then added to cells. Measurements were taken for 30 min. To evaluate chronic effects, MIN-6 cells were treated with the extract and Re for 48 hours. Cells were washed with modified PBS buffer to remove any traces of ginseng just before measurements. Cells were exposed to DCFH-DA for 20 min and baseline measurements were taken for 10 min. H$_2$O$_2$ was then added and measurements were taken for 50 min.

**Measurement of Insulin Secretion**

Insulin was measured by first incubating MIN-6 cells in Krebs-Ringer bicarbonate buffer (KRBB) containing 2 mM glucose for 2 hours. Cells were then exposed to varying concentrations of H$_2$O$_2$ for 30 min. We then proceeded to stimulate cells with glucose by increasing the glucose concentration of the KRBB to 25 mM. Cells were placed on a waterbath shaker at 37°C for 30 min. After incubation, the medium was centrifuged, and the supernatants were assayed using radioimmunoassay.

**Data Analysis**

Data are expressed as means with standard error of the mean (SEM). All data were analyzed using ANOVA followed by Dunnett’s test with $p < 0.05$ considered statistically significant.

**Results**

**Effects of H$_2$O$_2$ Exposure to MIN-6 Cells**

Oxidant stress was assessed by measuring oxidation of DCFH to DCF. Exposure to H$_2$O$_2$ led to increased oxidation of DCFH, indicating increasing oxidative stress experienced by the cell. The control cell without H$_2$O$_2$ showed a 116 ± 4.0% increase in DCF fluorescence from baseline, while those exposed to 100, 250, and 500 µM H$_2$O$_2$ experienced 1433 ± 15.9%, 1509 ± 51.4%, and 1583 ± 21.4%, respectively.
Acute Effects of American Ginseng

Under acute conditions, ginseng extract appeared to directly scavenge \( \text{H}_2\text{O}_2 \) radicals. Upon addition of extract, a slight increase in DCF fluorescence was observed. However, once \( \text{H}_2\text{O}_2 \) was added, oxidative stress within control cells increased significantly, whereas cells treated with the extract experienced very little additional stress. As shown in Fig. 1, control cells without \( \text{H}_2\text{O}_2 \) exposure underwent a 116 ± 4.0% change over the course of 30 min and cells where only \( \text{H}_2\text{O}_2 \) was added experienced a 517 ± 8.5% change. In cells exposed to \( \text{H}_2\text{O}_2 \) and pretreated with the extract 0.1, 0.5, and 1.0 mg/ml, oxidative stress was significantly reduced to 145 ± 7.5%, 116 ± 6.7%, and 114 ± 4.0%, respectively. The difference between treated groups and the group exposed to \( \text{H}_2\text{O}_2 \) only was statistically significant (all \( p < 0.01 \)).

Ginsenoside Re at 0.05 mg/ml caused roughly the same fluorescence as the group treated only with \( \text{H}_2\text{O}_2 \) at 512 ± 15.5%, but at 0.1 mg/ml, Re significantly reduced fluorescence to 485 ± 13.8% (\( p < 0.05 \)). These results suggest that for pancreatic \( \beta \)-cells, American ginseng and high concentrations of Re improves acute protection against oxidative stress.

Chronic Effects of American Ginseng

As seen in Fig. 2, in cells treated with ginseng extract 0.1, 0.5, and 1.0 mg/ml, oxidative stress decreased from 1509 ± 51.4% in \( \text{H}_2\text{O}_2 \) exposed cells to 1261 ± 16.4% (\( p < 0.05 \)), 824 ± 18.0% (\( p < 0.01 \)), and 323 ± 5.7% (\( p < 0.01 \)), respectively. Cells exposed to \( \text{H}_2\text{O}_2 \) and treated with Re 0.05, 0.1, and 0.5 mg/ml decreased to 1494 ± 12.1%, increased to 1521 ± 14.2%, and decreased to 1362 ± 16.4% (\( p < 0.05 \)), respectively. The data suggests
AMERICAN GINSENG IN PANCREATIC β-CELLS

Figure 2. Chronic effects of American ginseng berry extract (AGBE) and ginsenoside Re (Re) at 250 μM H₂O₂. Increasing concentrations of AGBE lead to reduced levels of DCF fluorescence, indicating reduction of oxidative stress. Higher concentration of Re also appears to produce some antioxidant effects. H₂O₂ = 250 μM H₂O₂ AGBE and Re concentrations are in mg/ml. *p < 0.05, **p < 0.01, compared to H₂O₂ only.

that the ginseng extract and higher concentration of Re may help to improve β-cell protection via chemical mediation and signaling.

**Effects of Ginseng on Insulin Secretion**

Our data showed that under exposure to 1.0 mM H₂O₂, MIN-6 cells secreted 13.2 ± 3.4 μU/ml of insulin. The cells treated with ginseng extract 0.1, 0.5, and 1.0 mg/ml showed increased insulin responses at 21.3 ± 6.5 μU/ml, 25.41 ± 10.0 μU/ml, and 25.3 ± 4.1 μU/ml, respectively, indicating a trend of increased insulin secretion with increasing concentrations of the extract. This result shows promise of improving insulin secretion by enhancing β-cell function under oxidative stress.

**Discussion**

Several clinical trials have shown that ginseng treatment is capable of reducing blood glucose for diabetic and non-diabetic subjects (Vuksan et al., 2000; Sotaniemi et al., 1995). However, the mechanism by which American ginseng acts on pancreatic β-cells has not been evaluated to date. The degree of oxidative stress which β-cells experience in diabetes and their minimal levels of antioxidant defense (West, 2000) make them prone to oxidant injury and point towards a need to explore methods of antioxidant protection.

In this study, our data suggest that the antioxidant properties of American ginseng are related to its ability to improve hyperglycemic conditions in MIN-6 cells. Under various concentrations and incubation periods of ginseng, we have observed that ginseng does retain its antioxidant properties in pancreatic β-cells. Whether via direct scavenging of radicals or
via signaling pathways, the data suggest that American ginseng has the ability to almost reverse the effects of oxidative stress within the cell culture.

We used the berry of American ginseng in our study which is known to have a distinctive profile of ginsenosides, the vital constituents of ginseng (Wang et al., 2006). We also tested the antioxidant properties of a major ginsenoside in the berry, ginsenoside Re. Re did show some significant improvements in oxidative stress protection; however, results from the extract were much stronger. This suggests that other constituents of the extract may be responsible for the antioxidant protection in the MIN-6 model and future experiments may include isolating other ginseng components to determine the effective constituent(s) and potentially develop a new class of antidiabetic agents.

In the current study, we used exogenous H2O2 to emulate the endogenous oxidative stress which ß-cells experience. High levels of glucose, however, have been shown to normally induce endogenous oxidative stress and free radical production in ß-cells (Robertson, 2004; Robertson et al., 2004). Future directions include using a more physiological model for oxidative stress in which MIN-6 cells are chronically exposed to levels of high glucose, a condition associated with Type 2 diabetes.

Previous data suggest that long-term American ginseng use can produce effects on cells by stimulating cells to synthesize chemical mediators such as superoxide dismutase (Yokozawa and Liu, 2000). Further studies evaluating the antioxidant enzymes such as catalase and superoxide dismutase are necessary to provide information regarding ginseng’s long-term mechanism of action. Nonetheless, our results are one of the first steps in shedding light on the mechanism of the anti-hyperglycemic abilities of American ginseng.

Acknowledgments

This work was supported in part by the Howard Hughes Institute Undergraduate Education Initiative Grant at the University of Chicago and by the NIH/NCCAM grant AT003255.

References

AMERICAN GINSENG IN PANCREATIC ß-CELLS


