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EFFECTS OF GINSENG AND ITS FOUR PURIFED GINSENOSIDES (Rb2, Re, Rg1, Rd) ON HUMAN PANCREATIC ISLET β CELL IN VITRO.

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ABSTRACT

Ginseng has attracted interest because of its potential therapeutic role in diabetes therapy. No direct evidence has shown the effects of ginseng and its components, ginsenosides, on human islet β cell. In this study, we evaluated ginseng extract and ginsenosides (Rb2, Re, Rg1, Rd) on human pancreatic β cell function. The results provide direct evidence that ginseng extract promotes human pancreatic β cell function. Ginsenoside Rb2 increased islet β cell insulin release and promoted β cell migration. Ginsenoside Re had some impact on cell migration, but had no effect on islet function by evaluating insulin release. The other ginsenosides had no effect on insulin release and islet migration. To date, this is the first study that examines the impact of ginsenosides on human pancreatic islets in vitro.

KEYWORDS: Human islets, American Ginseng, Ginsenosides, Diabetes, β cells, Insulin.

INTRODUCTION

Ginseng root (Panax) has been used for many centuries as an herbal remedy.[1-5] A few of the believed benefits of ginseng are stress relief, anti-aging, and anti-oxidant.[3, 5-8] The effects vary between different species of ginseng. The active components of ginseng have been identified as a group of saponins called ginsenosides. These are differentiated into subclasses Ro, Ra, Rb, Rc, Rd, Re, Rf, Rg, and Rh based on retention factor values in thin layer chromatography.[3, 9-12]

The different species of ginseng have different ginsenosides. Wan et al analyzed the ginsenoside composition of two popular ginseng species, Asian (Panax *ginseng*) and American ginseng (Panax *quinquefolius*). Asian ginseng contains ginsenoside Rf and Rg2, while American ginseng does not. Concentrations of some ginsenosides such as Rg1 and Re vary drastically.[13, 14]

There are multiple studies looking at the effects of ginseng, ginsenosides, and ginseng preparations.[8] A number of studies looked at their effect on hyperglycemia in rats and in mice,[1, 15-55] in diabetic patients,[2, 56-74] and in patients with insulin resistance.[66, 75-79] Ginseng increased insulin production in rat pancreatic β cells,[8, 68, 80-85] and decreased apoptosis in rat and mice β cells.[18, 86] Ginseng was protective against chemotherapeutic injury in mice pancreatic β cells.[87] However, there are no studies in human pancreatic islet β cells in vitro.[8, 13]

This study investigates the effects of ginseng extract and four purified ginsenosides, Rb2, Re, Rg1, and Rd, on human pancreatic islet β cell insulin release and islet migration. Our data suggested that ginseng extract can promote human islet β cell insulin release. Ginsenosides Rb2 and Re affect pancreatic islet insulin release and migration. Ginsenoside Rg1 and Rd have no effect.

MATERIALS AND METHODS

Ginseng root extracts

Sliced American ginseng (Panax *qinquefolium* L.) purchased from H.S.U. Corp. (WI, USA) was soaked in ddH_2O for 24 h at 4°C on a rotator. Following centrifugation, the supernatant was lyophilized to yield a solid which was weighed and diluted to the final concentration of 5 µg/µL. One lot of ginseng was used to prepare this solution over the course of the study and it was stored at -4°C for less than 1 week. There was no evidence of degradation during the 1 week storage period since ginsenoside is stable in pH 7.0 water solution but unstable in acid solution. The concentration is determined by weight per volume.[68] Since there is no standard positive control for insulin secretion in human pancreatic β cells, ginseng root extract was the positive control and was used at 5 µg/mL.

Ginsenosides
Ethanol/water extracted HPLC purified ginsenosides Rb2, Re, Rg1, Rd (Indofine Chemical, Hillsborough, NJ) were utilized in this study (purity >98%). Purified ginsenosides were administered in liquid form at 0.5 µg/mL during cultures.

Human pancreatic β cell islets
Human pancreatic islet tissue, from normal cadaveric donors, was obtained from Islet Resource Centers (ICRs) in the ICR Basic Science Islet Distribution Program from human islet laboratories, such as University of Pennsylvania (Philadelphia, PA), University of Illinois (Champaign, IL), University of Miami (Coral Gables, FL), and City of Hope National Medical Center (Duarte, CA), et al (http://icr.coh.org/). The total amount of islets, cell viability, and purity were assessed by islet count and by measurement of diameter (single islet diameter ±150 µm), trypan blue, and dithizone staining of islets. Use of these tissues was approved by the institutional review board (IRB) at Roger Williams Hospital (Providence, RI) and the ICRs Committees. Pancreatic islets were received within 48 hour after procurement from donors. Purity assessed by dithizone identification was >70%. Viability was >95%, determined by trypan blue dye exclusion. Islets were divided amongst wells at 50 islets/mL.[88]

Culture conditions
Human pancreatic β cells were cultured in RPMI 1640 (Gibco, Grand Island, NY) medium with 10% heated inactivated fetal bovine serum (HiFBS, Hyclone, Logan, Utah), 5.5 mM glucose, 10 mM HEPES, and 1% pencillin/streptomycin. The medium was changed twice a week.

Evaluation of islet function
Islet function was measured by the level of insulin release. This was performed with or without a high glucose challenge (22 mM glucose concentration). The media were collected on a weekly basis and stored at -80°C. High glucose challenge was performed every four weeks as follows: media was collected, and cells were washed once with RPMI medium. Media was replaced with 20mM glucose RPMI 1640 for 1 hour and then collected and stored at -80°C.

Assay for insulin: ELISA with insulin antibody
Insulin secretion was measured using a human insulin ELISA kit (Linco Research, St. Charles, MO). Assay was performed following manufacturer instructions, and the results were analyzed using the KC Junior® microplate reader software (Bio-Tek Instruments, Winooski, VT).

Observing cell morphology
Pancreatic islets were observed under a Zeiss Axiovert 200M© microscope (Zeiss, Oberkochen, Germany) and morphological photos of the same islets were taken on a weekly basis to observe changes in islet morphology and migration. Representative images are presented in this paper.

Statistical analysis
All data are presented as the mean ± SEM and analyzed by the Analysis of Variance (ANOVA) followed by a t test, unless otherwise indicated. Data is labeled with p value if it is <0.05 or <0.01. Microsoft Excel® (Microsoft Corporation, Seattle, WA) was used to draw graphs. All data represent the results of three independent experiments unless otherwise indicated.

RESULTS

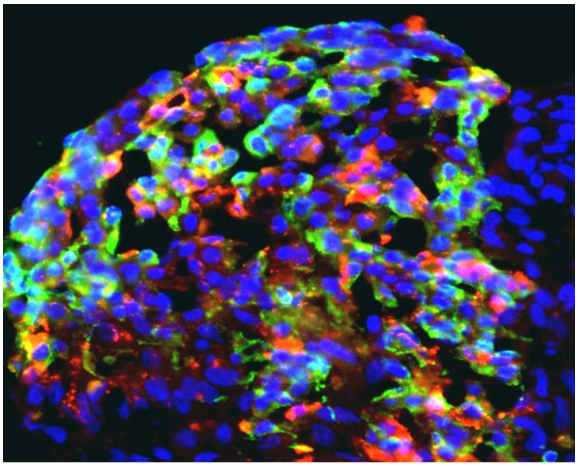

Figure 1: Human pancreatic islet with immunohistochemistry

Fluorescence histology with insulin antibody, glucagon antibody indicated β cells (green), α cells (red) and nuclei staining blue, which show about 70% β cells and 25% α cells in islet.

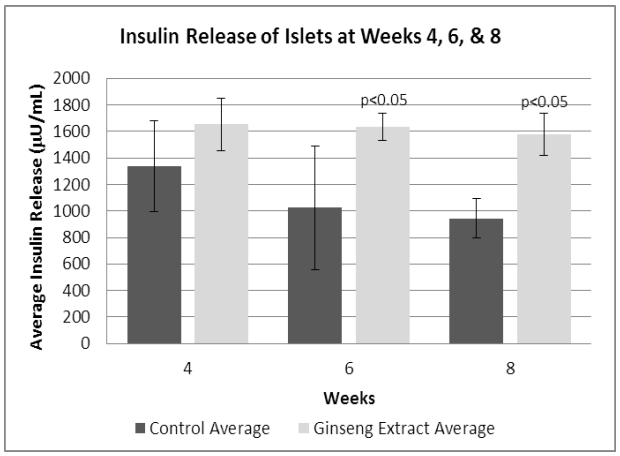

Figure 2: Average insulin releases of pancreatic islets at weeks 4, 6, and 8

In figure 2, human pancreatic islets were exposed to ginseng extract. We selected a culture period of 8 weeks to identify long term insulin release. At weekly intervals, insulin release was measured. The data at week 4, 6, and 8 are shown. The human islets exposed to ginseng extract released more insulin than islets alone. This difference reached statistical significance at week 6 and

8. From week 1 to 4, the insulin release in both ginseng extract and control decreased steady. The steady decline is likely due to the expected apoptosis of human islets in vitro. The insulin release in ginseng extract has no difference between ginseng treatment and control. The ginseng extract had effects on the pancreatic islets until week 4.

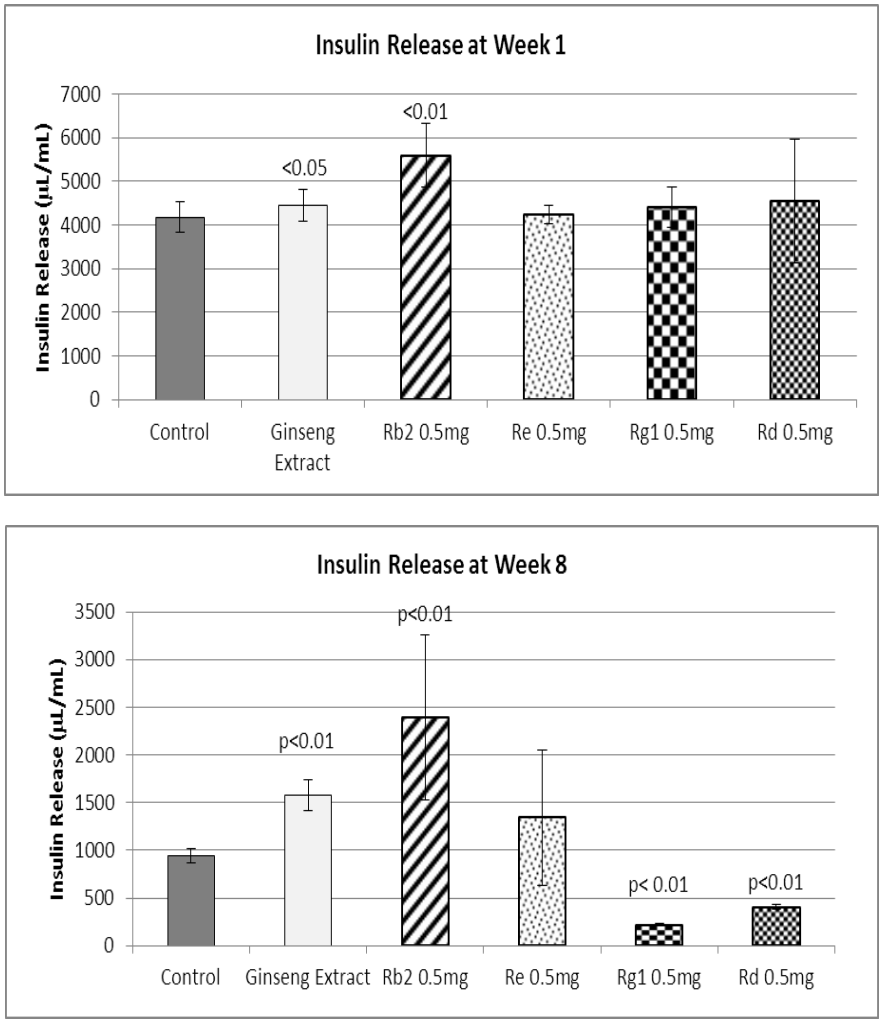

Figure 3: Human pancreatic islet insulin release at week 1 and 8 of culture without exposure to glucose

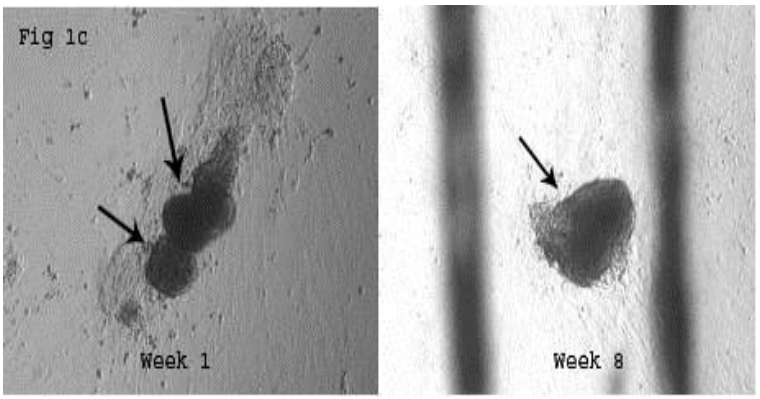

Figure 4: Image of human pancreatic islets exposed to ginsenoside Rb2 at week 1 and 8

Arrows indicated islet in cultures of 1 and 8 weeks and islet migration was traced with a grid under the culture wells (black lines). After 8 weeks culture islets migrated and merged together.

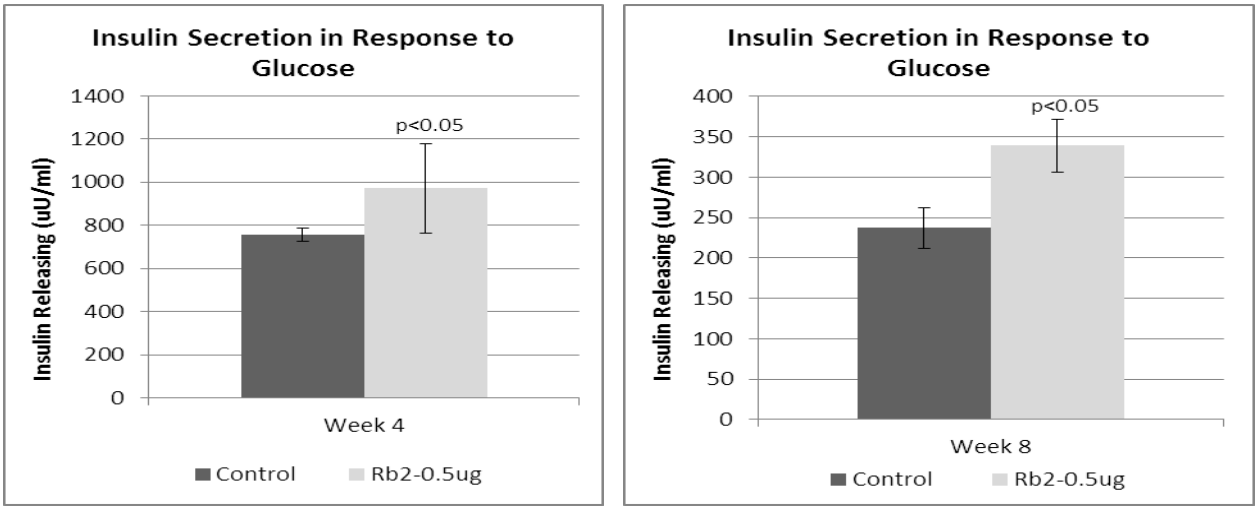

Figure 5: Insulin secretion of human pancreatic islets when exposed to high glucose challenge (22 mM glucose) and exposed to ginsenoside Rb2 at week 4 and 8

Ginsenoside Rb2 at 0.5 μg/mL increased insulin secretion compared to human pancreatic islets alone and ginseng extract at weeks 1 and 8 (figure 3). Rb2 promotes insulin secretion in response to high glucose challenge (figure 5). Both findings were statistically significant. In figure 4, islet migration was observed under microscopy. Pancreatic islets formed into a large size islet, which leads us to believe that Rb2 stimulates islet migration and reconstitution.

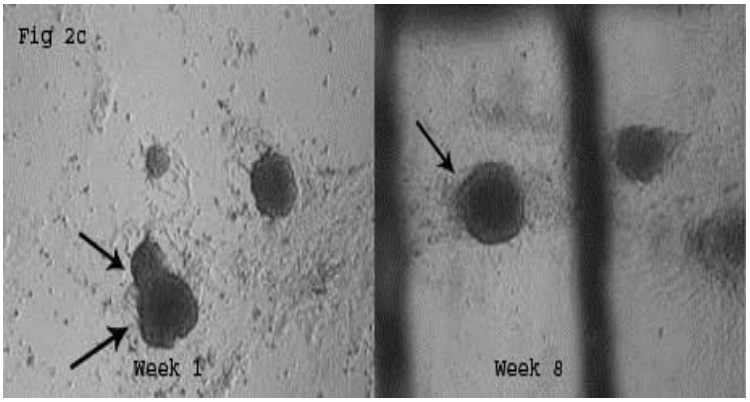

Figure 6: Image of human pancreatic islet exposed to ginsenoside Re at week 1 and 8

No islets significantly migrated and merged occurred under Re treatment culture group.

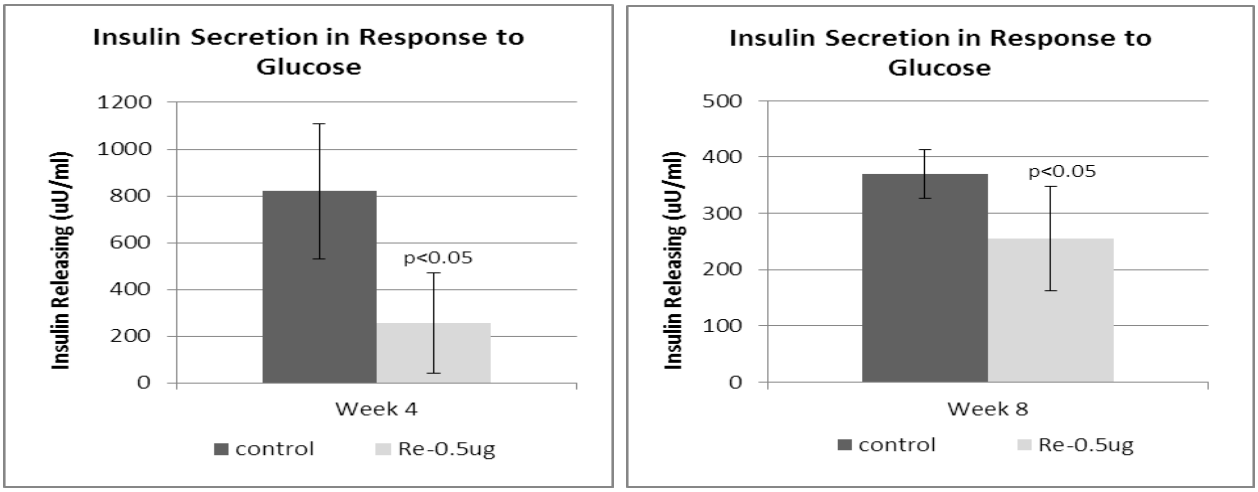

Figure 7: Insulin secretion of human pancreatic islets when exposed to high glucose challenge (22 mM glucose) and exposed to ginsenoside Re at week 4 and 8

In figure 3, the pancreatic β cell response to ginsenoside Re treatment was not significant. At week 4 and 8, 0.5 μg/mL of ginsenoside Re had generally the same effects as the control group (figure 3). Their effects were less than that of the positive control, ginseng extract (figure 3). There was very little difference between islets exposed to Re and the control islets in response to a high glucose challenge (figure 7).

While the efficacy of ginsenoside Re was limited, the same phenomenon where several islets migrated to form a single islet was observed in islets treated with Re in figure 6. Ginsenoside Re may not have an impact on β cell function, but it has a role in islet neogenesis.

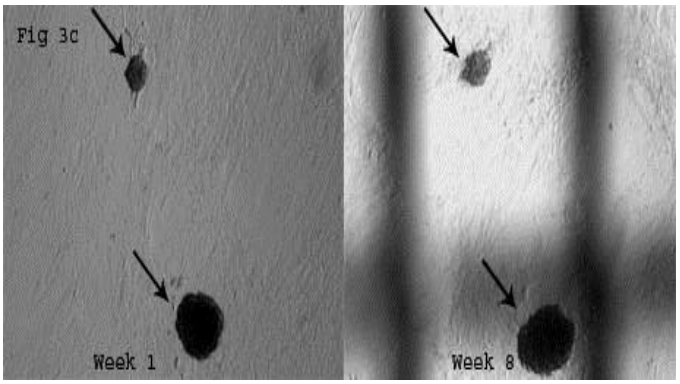

Figure 8: Image of human pancreatic islet exposed to ginsenoside Rg1 at week 1 and 8

No islets significantly migrated and merged occurred under Rg1 treatment culture group.

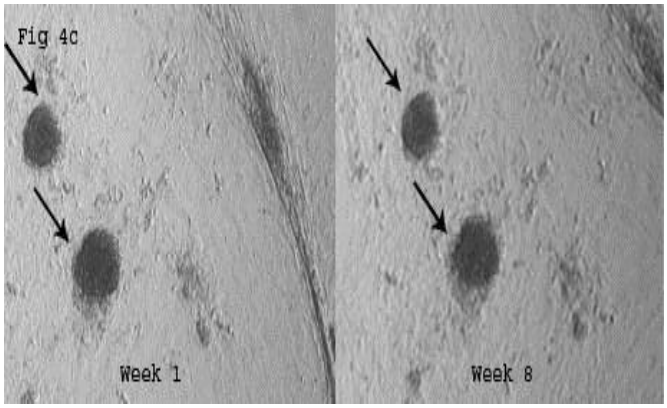

Figure 9: Image of human pancreatic islet exposed to ginsenoside Rd at week 1 and 8

No islets significantly migrated and merged occurred under Rd treatment culture group.

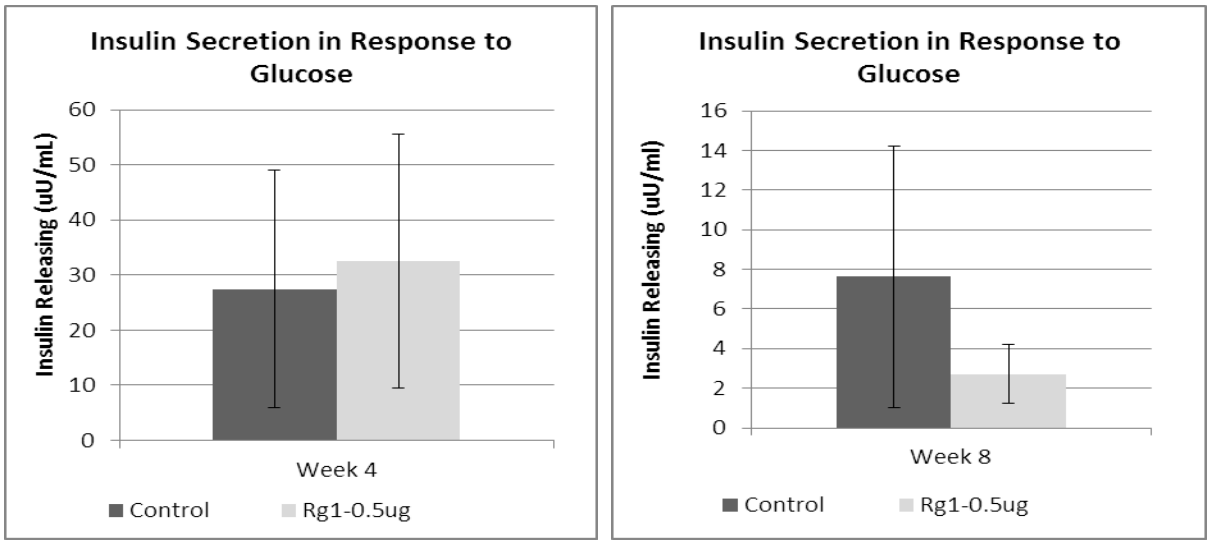

Figure 10: Insulin secretion of human pancreatic islets when exposed to high glucose challenge (22 mM glucose) and exposed to ginsenoside Rg1 at week 4 and 8

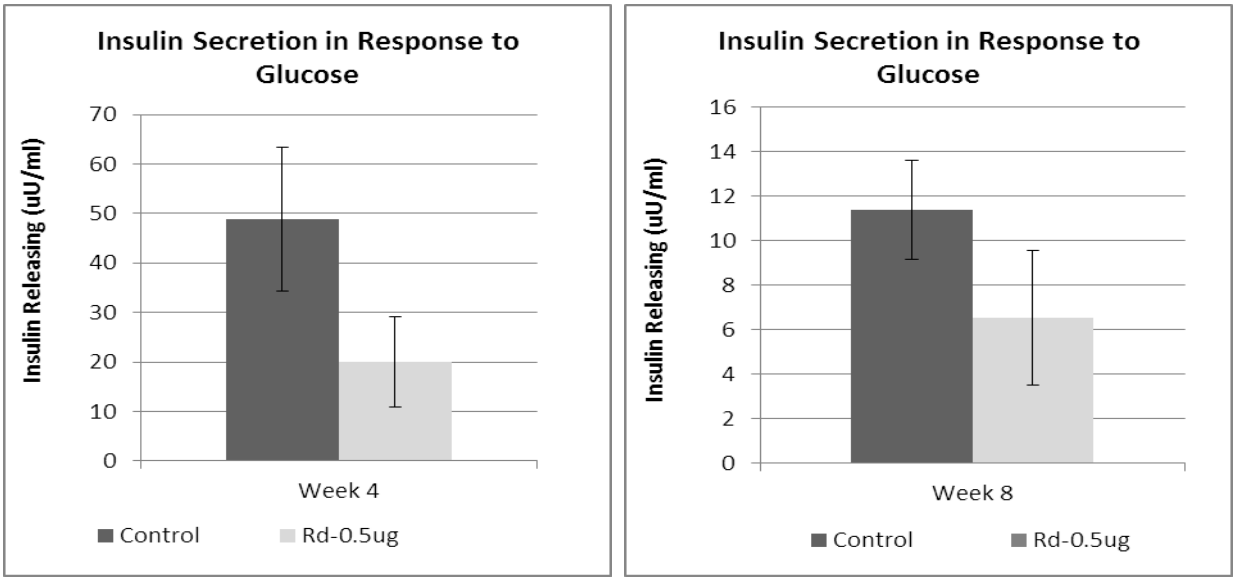

Figure 11: Insulin secretion of human pancreatic islets when exposed to high glucose challenge (22 mM glucose) and exposed to ginsenoside Rd at week 4 and 8

The last two ginsenosides Rg1 and Rd had limited effectiveness. When ginsenoside Rg1 was administered to pancreatic islets, no significant difference in the level of insulin secretion between experimental group and control group was observed. Insulin release levels of the experimental group were lower than those of the positive control (figure 3). A null response from islets exposed to high glucose challenge further confirmed no effect of Rg1 on human islet β cell function (figure 10). Islets under Rg1 treatment did not migrate and did not form larger islets with surrounding cells (figure 8).

The effects of ginsenoside Rd were also not significant. Insulin secretion levels of the experimental group were similar to those of the control islets (figure 3). They were lower than those of the positive control (figure 3). High glucose challenge was not significant. It is possible that Rd suppressed insulin secretion and hindered cell function under high glucose (figure 11). Very little morphological change was observed under microscopy in figure 9.

DISCUSSION

Much research on ginseng has been done in the mouse model, but a very limited number of studies have been performed on human pancreatic islets in vitro.[8] This study marks the first attempt to identify the effects of specific ginsenosides on human islets. Our study showed that not all ginsenosides contribute to an increase in insulin production. Of the four ginsenosides, Rb2 had the most significant effect on increasing insulin production in both regular culture and in response to high glucose challenges (figure 3, 5). Likely, Rb2 has a direct effect on pancreatic islet β cells. Curiously, no previous study in an animal model has looked at the effect of Rb2 on the β cell.[89]

The published data on ginsenoside Re has been conflicting. Several studies in diabetic mice consistently report reduced hyperglycemia in response to Re.[15, 16, 35, 54, 55] However, one clinical study where ginsenoside Re was administered orally did not demonstrate improved β cell function or insulin sensitivity.[67] The results in this study provides a possible explanation for this discrepency. Ginsenoside Re has no effect on *human* pancreatic β cells (figure 3, 7), and may only be effective in *animal* pancreatic β cells.

Ginsenoside Rg1 had little effect in stimulating insulin production (figures 3, 10). This is surprising since a number of animal model studies suggest that Rg1 and Rd support pancreatic islet function. Ginsenoside Rg1 has an anti-apoptotic effect in rat β cells,[86] enhances glucose stimulated insulin secretion in β cells,[84] and has anti-hyperglycemic effects.[90] It is possible that Rg1 may be effective in animal pancreatic islets, but not in human islets.

Even though Re, Rg1 and Rd did not have an effect on insulin secretion, they could still play a role in treating hyperglycemia. They might act synergistically with more active ginsenosides. One study has demonstrated an anti-hyperglycemic effect by a group of saponins.[35]

It is also possible that a ginsenoside in and of itself may not affect β cell function. Rather, the ginsenoside could become metabolites in the digestive system. The metabolites may subsequently be responsible for the anti-hyperglycemic activity.

CONCLUSION

Understanding the effects of individual ginsenosides on human islet β cell function will aid in the standardization of ginseng as a clinical supplement for hyperglycemia. Though this study addresses the effects of ginsenoside on

islet function, further tests should be performed to examine the effect of ginsenosides on other tissues, such as the hepatic or muscular. The molecular mechanism by which Rb2 improves human islet β cell function also needs to be addressed.

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