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The protective effect of berberine on β-cell lipoapoptosis

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ABSTRACT. ***Aims:*** **To evaluate the protective effect of berberine on β-cell lipoapoptosis induced by palmitate and to explore the possible underlying mechanisms.** ***Materials and methods:*** **HIT-T15 pancreatic β-cells were divided into the following treatment groups: untreated controls; 100 μM berberine; 0.5 mM palmitate; 0.5 mM palmitate + 0.1 μM berberine; 0.5 mM palmitate + 1 μM berberine; 0.5 mM palmitate + 10 μM berberine; and 0.5 mM palmitate + 100 μM berberine. After 48 h, cell apoptosis was assessed by flow cytometry and the Hoechst 33258 fluorescent assay. Basal and glucose-stimulated insulin levels in culture medium were measured by radioimmunoassay. Peroxisome proliferator-activated receptor-γ (PPAR-γ) mRNA and protein levels were determined by real-time PCR and immunocytochemistry, respectively.** ***Results:*** **Apoptosis was significantly increased upon treatment with palmitate as compared to the untreated controls ($p<0.0001$). In addition, glucose-stimulated insulin secretion (GSIS), PPAR-γ mRNA and protein expression were significantly reduced in response to palmitate ($p<0.0001$); however, palmitate-induced apoptosis and reduction in PPAR-γ expression were reversed in response to berberine in a dose-dependent manner ($p<0.05$). Furthermore, there was a non-significant increase in GSIS with increasing berberine dose.** ***Conclusion:*** **Palmitate exerted lipotoxic effects on HIT-T15 cells, inducing apoptosis and reducing GSIS. Berberine reduced palmitate-induced lipoapoptosis and tended to increase GSIS in HIT-T15 cells, possibly through increased PPAR-γ expression.**
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INTRODUCTION

Insulin resistance and insufficiency are the hallmarks of diabetes mellitus (1). Although the mechanisms underlying insulin resistance and insufficiency are complex, lipotoxicity appears to play an important role (2). Indeed, the concept of lipotoxicity has led to important developments in diabetes mellitus research, particularly pertaining to Type 2 diabetes mellitus

Lipotoxicity is a pathological condition in which free fatty acid (FFA) levels in the blood are elevated to such an extent that adipose tissue storage, and tissue FFA oxidizing capacities are exceeded. As a consequence, the excess FFA accumulate in non-adipose tissues as triglycerides, inducing tissue damage. It has been demonstrated that elevated FFA levels not only contribute to insulin resistance, but also impair insulin secretion via induction of pancreatic β-cell apoptosis (i.e., lipoapoptosis). Hence, β-cell lipotoxicity caused by increased blood FFA levels would appear to be an important mediator of Type 2 diabetes mellitus (3).

Apoptosis of pancreatic β-cells results in decreased cell number and, as a consequence, insufficient insulin secretion. It has been recently reported that the mechanisms of lipoapoptosis may include oxidative stress, endoplasmic reticulum (ER) stress, and altered regulation of a number of pathways including those involving ceramide, β-cell lymphoma 2 (Bcl-2) protein family, insulin receptor substrate-2 (IRS-2), 12-lipoxygenase, protein kinase B (PKB), G-protein-coupled receptors (GPCR), and peroxisome proliferator-activated receptors (PPAR) (2).

The PPAR family is a group of nuclear receptor proteins, including PPAR-α, PPAR-β, and PPAR-γ, which are involved in fat formation, glucose metabolism, inflammation, and cell multiplication and differentiation. The expression of different PPAR subtypes is variable in different tissues; PPAR-γ is expressed in human pancreatic and INS-1 cells (4, 5). Furthermore, there is evidence suggesting that PPAR-γ expression may ameliorate the deleterious effects of FFA on pancreatic β-cells (6, 7). It has also been demonstrated that pioglitazone, a known stimulator of PPAR-γ expression (8), reduces fatty acid-induced oxidative stress and apoptosis in pancreatic β-cells (9).

At present, there are a number of anti-lipoapoptotic pharmacological options available to protect pancreatic β-cells from FFA-related damage, including thiazolidinediones, a PPAR-α agonist, glucagon like peptide-1, and anti-oxidants (10). Although these medications can protect β-cells, they tend to be very expensive and have a number of unwanted side effects (10). Therefore, such treatments are not available to all patients, particularly those for whom expense is a significant concern (10).

There is an obvious need to find safe, effective, and economic anti-lipoapoptotic medications to prevent FFA-associated pancreatic β-cell damage. Chinese Goldthread is a *Ranunculaceae* plant that grows widely in China and has been used to treat diabetes mellitus in China for many years. It contains many alkaloids, of which the content of berberine, an isoquinoline alkaloid, is the highest. Recently, it has been reported that berberine reduced plasma glucose concentrations and prevented diabetic complications in rats (11, 12). In addition, the anti-diabetes effect of berberine has been demonstrated in a clinical study (13). Hypolipidemic effects of berberine have also been demonstrated and shown to be mechanistically different to those of statins (14).

Key-words: Berberine, β-cell, lipoapoptosis, palmitate, peroxisome proliferator-activated receptor-γ.

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In the present study, we tested the hypothesis that berberine has the capacity to regulate the expression of PPAR-γ in β-cells and thus prevent lipoapoptosis. Specifically, we examined palmitate-mediated β-cell lipoapoptosis and PPAR-γ expression in the presence or absence of differing concentrations of berberine.

MATERIALS AND METHODS

Cell culture and reagents

HIT-T15 cells (SV 40-transformed pancreatic β-cells from Syrian hamsters) were grown in culture Dulbecco's modified Eagle medium (Gibco, New York, USA) containing 20% BSA, 5.6 mM glucose, 0.3 g/l glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin and maintained at 37 C, 5% carbon dioxide (CO_2), and 95% humidity for 24 to 48 h until cells had grown to confluence. Cells were divided into the following 7 treatment groups: untreated controls (no palmitate nor berberine); 100 µM berberine; 0.5 mM palmitate; 0.5 mM palmitate + 0.1 µM berberine; 0.5 mM palmitate + 1 µM berberine; 0.5 mM palmitate + 10 µM berberine; and 0.5 mM palmitate + 100 µM berberine. A 2.68 mM berberine hydrochloride (concentration: 99.5%; Sichuan Yabao Guangtai Pharmaceutical Co Ltd, Pengzhou, Sichuan, China) stock solution in distilled water was initially prepared and diluted to obtain the appropriate concentrations in culture medium. Specifically, to make a 10 mmol/l palmitate solution, 51.284 mg (0.2 mmol) palmitate and 8 mg sodium bicarbonate (0.2 mmol) were dissolved in 2 ml distilled water, shaking the solution in a 70 C water bath. In addition, 1.8 mg BSA was dissolved in 18 ml distilled water, shaking the solution in a 55 C water bath. Both solutions were combined (10 mmol/l palmitate) and diluted in 180 ml culture DMEM (1 mmol/l palmitate). Finally, a final 0.5 mmol/l palmitate solution was prepared by diluting the 1 mmol/l palmitate solution in DMEM, after which it was filtered and stored at 4 C. The effects of berberine were assessed in triplicate for each group.

Assessment of apoptosis

Flow cytometry

After 48-h treatment, cells were harvested by trypsinization and washed three times with phosphate buffered saline (PBS). Cell pellets were then resuspended in 500 µl binding buffer with 5 µl of Annexin V-FITC (Beyotime, JiangSu, China) at final 1:100 dilution concentration and propidium iodide (Beyotime, JiangSu, China) and incubated for 5 to 15 min in the dark. The cell suspensions were then analyzed by flow cytometry (CoulterFlow, Beckman Coulter, Brea, CA). The rate of apoptosis (given as the percentage of the total number of cells undergoing apoptosis) was automatically calculated according to the intensity of fluorescence.

Hoechst 33258 fluorescence assay

After 48-h treatment, cells were trypsinized and washed twice with PBS. Cell were then fixed with 4% paraformaldehyde and stained with Hoechst 33258 (Sigma, St Louis, MO). The cells were then observed using fluorescent microscopy, and three separate fields were counted. These findings are presented as the integrated optical density calculated by multiplying the average optical density (the average intensity of all positive cells) by the area of examination.

Insulin secretion

Cell culture media (0.5 ml) was removed from each well after 48-h treatment and centrifuged; supernatant was stored at –20 C to assess baseline insulin concentrations. Glucose was added to the culture medium remaining in the wells at a final concentration of 22 mM. Cells were cultured for a further 60 min after which 0.5 ml of supernatant were obtained and stored at –20 C for assessment of glucose-stimulated insulin secretion (GSIS). GSIS was determined by subtracting the baseline insulin concentration from the insulin concentration obtained following the glucose stimulation.

Insulin concentrations were measured using a commercially available radioimmunoassay kit (Beijing North Biotechnology Research Institute, Beijing, China) following the instructions provided. Briefly, ^{125}I-insulin and samples were mixed with insulin antibodies provided, and incubated at 4 C for 24 h. A precipitative reagent was added, and the samples were incubated at room temperature for 60 min, centrifuged, and the supernatant isolated. Insulin concentrations were measured using the SN695B type intelligent radioimmunoassay apparatus (Shanghai Hesuorihuanguandian Apparatus Co. Ltd, China) and are presented in µIU/ml.

Real-time PCR

Total RNA was extracted from the treated HIT-T15 cells using Trizol reagent (MRC, Cincinnati, OH) and stored at –80 C. RNA was reverse transcribed to obtain cDNA. Real-time PCR (FTC-2000A Real-Time PCR system, Canada) was then performed using SYBR green (TOYOBO, Japan) and the following PPAR-γ primer sequences obtained from the National Center for Biotechnology Information (NCBI) GenBank: forward 5′-tcatgcttgcgaaggattgaag-3′ and reverse 5′-ccaaacctgatggcattgtgagac-3′. Samples were normalized for actin expression using the following primers: forward 5′ctggcaccacaccttctacaatg-3′ and reverse 5′cctcgtagatgggcacagtgtg-3′.

The PCR parameters were as follows: 95 C for 30 sec, 95 C for 5 sec, 60 C for 5 sec and 72 C for 20 sec for 50 cycles, followed by 60 C for 10 sec and 98 C for 1 sec. PPAR-γ mRNA expression was determined using the ΔΔCt method, where expression in each experimental group was relative to that of the control group.

PPAR-γ protein immunocytochemistry

Following 48 h of treatment, the cells were washed 3 times with PBS and fixed with 4% paraformaldehyde for 10 to 30 sec. After 3 PBS washes (5 min each), the cells were incubated with 0.03% hydrogen peroxide (H_2O_2) for 5 to 10 sec at room temperature to inactivate endogenous peroxidase activity. The cells were then washed 3 times with distilled water and permeabilized with 0.1% triton X-100 in PBS at room temperature. Several drops of 5% BSA were added, and the cells were incubated for 10 min at room termperature. The primary PPAR-γ antibody (Boster, Wuhan, China) was then added (10 µg/ml final concentration), followed by an overnight incubation at 4 C. The secondary antibody, biotinylated horseradish peroxidase labeled goat anti-rabbit IgG [IgG (H+L)] (Boster; 1:200 dilution), was then added, followed by the addition of an avidin-biotin horseradish peroxidase complex (Boster; 1:200 dilution) for 40 min at 37 C. Slides were stained at room temperature using a diaminobenzidine (DAB) kit and then lightly co-stained with hematoxylin for 2 min. Images were captured and analyzed using image analysis software (Image Pro-Plus, Media Cybernetics Co, USA).

Statistical analysis

Statistical analysis was performed using SPSS 11.5 statistical software (SPSS, Chicago, IL). Data were expressed as mean±SD, and comparisons were performed using one-way analysis of variance (ANOVA) followed with two by two comparisons adjusted using the Bonferroni approach. A *p*-value <0.05 was considered statistically significant.

RESULTS

Effect of berberine on apoptosis

Apoptosis of HIT-T15 cells in response to palmitate combined with different concentrations of berberine were detected by flow cytometry (Fig. 1A-G). These findings are summarized in Figure 2. The Hoechst 33258 fluorescent assay was also performed to examine the apoptosis (Fig. 2B). A representative fluorescent image obtained from each treatment group using the Hoechst 33258 fluorescent assay is shown in Figure 3. The DNA observed in dead cells was condensed and prominently stained (arrows). Similar responses were observed using either of the two methods of assessment. Whereas 100 μM berberine did not influence apoptosis as compared to the control group, apoptosis was significantly increased in cells exposed to 0.5 mM palmitate ($p<0.0001$). Palmitate-induced apoptosis decreased in a dose-dependent manner with increasing concentrations of berberine. In fact, apoptosis was significantly reduced in all palmitate-berberine treatment groups as compared to the 0.5 mM palmitate group ($p<0.05$). Furthermore, although there was no significant difference in apoptosis between the 0.5 mM palmitate + 0.1 μM berberine and the 0.5 mM palmitate + 1 μM berberine groups, apoptosis levels were significantly reduced in the groups treated with 10 and 100 μM berberine, indicating a dose-responsive effect of berberine in these cells ($p<0.05$).

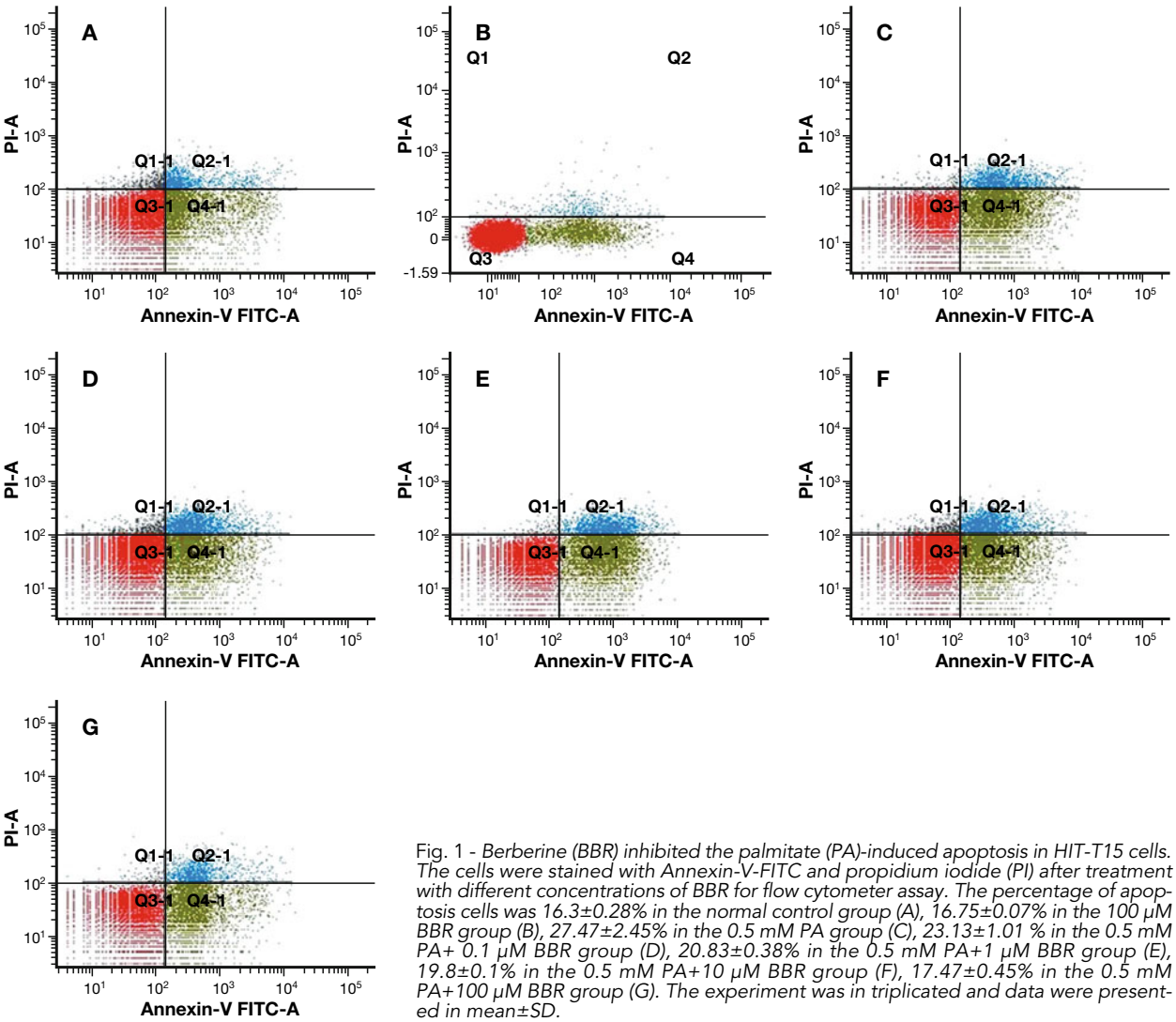

Fig. 1 - *Berberine (BBR) inhibited the palmitate (PA)-induced apoptosis in HIT-T15 cells. The cells were stained with Annexin-V-FITC and propidium iodide (PI) after treatment with different concentrations of BBR for flow cytometer assay. The percentage of apoptosis cells was 16.3±0.28% in the normal control group (A), 16.75±0.07% in the 100 μM BBR group (B), 27.47±2.45% in the 0.5 mM PA group (C), 23.13±1.01 % in the 0.5 mM PA+ 0.1 μM BBR group (D), 20.83±0.38% in the 0.5 mM PA+1 μM BBR group (E), 19.8±0.1% in the 0.5 mM PA+10 μM BBR group (F), 17.47±0.45% in the 0.5 mM PA+100 μM BBR group (G). The experiment was in triplicated and data were presented in mean±SD.*

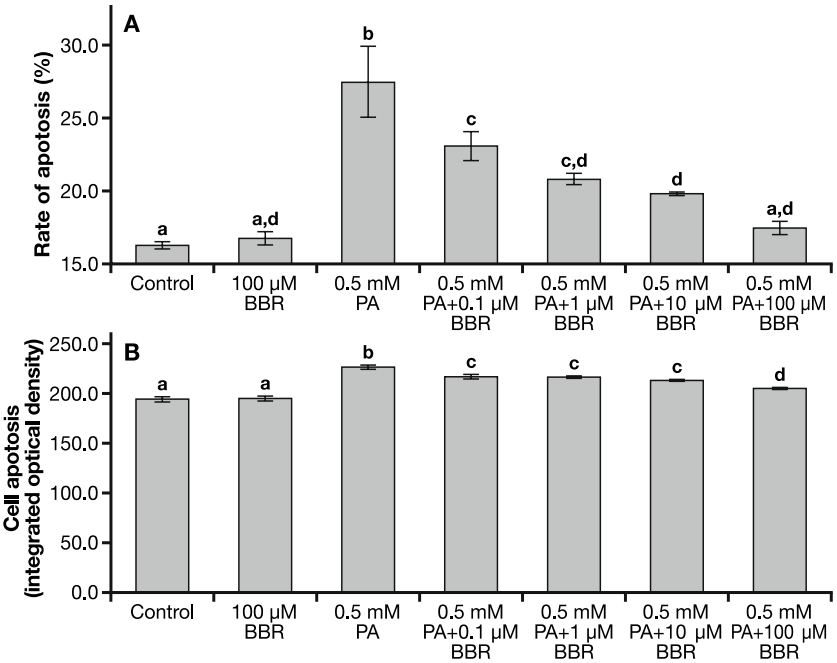

Fig. 2 - *The effects of palmitate (PA) and different concentrations of berberine (BBR) on HIT-T15 cell apoptosis as determined by flow cytometry (A) and Hoechst 33258 fluorescent assay (B). Data are presented as mean±SD (no.=3 per group). Different letters indicate significant difference among groups at* $p<0.05$ *after adjustment.*

Effect of berberine on insulin secretion

As shown in Figure 4A, there were no significant differences in baseline insulin concentrations or GSIS between the control and 100 μM berberine groups. Baseline insulin levels were significantly increased, whereas GSIS levels were significantly decreased in response to 0.5 mM palmitate ($p<0.0001$). However, no differences in baseline insulin concentrations or GSIS were observed between cells treated with palmitate and those treated with palmitate and berberine.

Effect of berberine on PPAR-γ mRNA expression

To determine whether the anti-lipoapoptotic action of berberine was mediated by PPAR-γ expression, the effects of berberine on PPAR-γ mRNA expression were analyzed. As shown in Figure 4B, significantly decreased

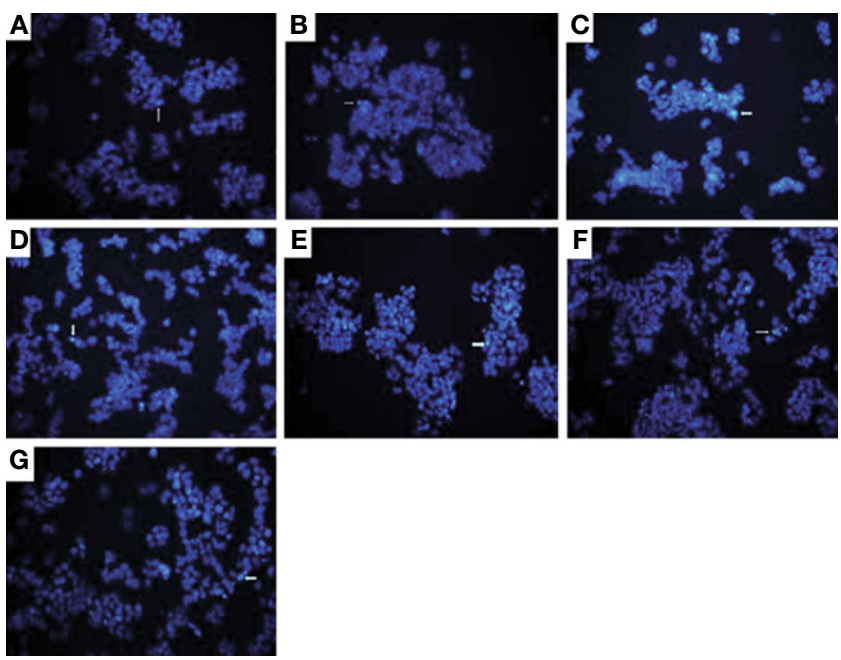

Fig. 3 - *Hoechst 33258 fluorescence analysis showing the prominent DNA staining of dead cells indicated by arrows in the untreated control (A), 100 μM BBR (B), 0.5 mM palmitate (C), 0.5 mM palmitate + 0.1 μM BBR (D), 0.5 mM palmitate + 1 μM BBR (E), 0.5 mM palmitate + 10 μM BBR (F), or 0.5 mM palmitate + 100 μM BBR (G) groups (200× magnification)*

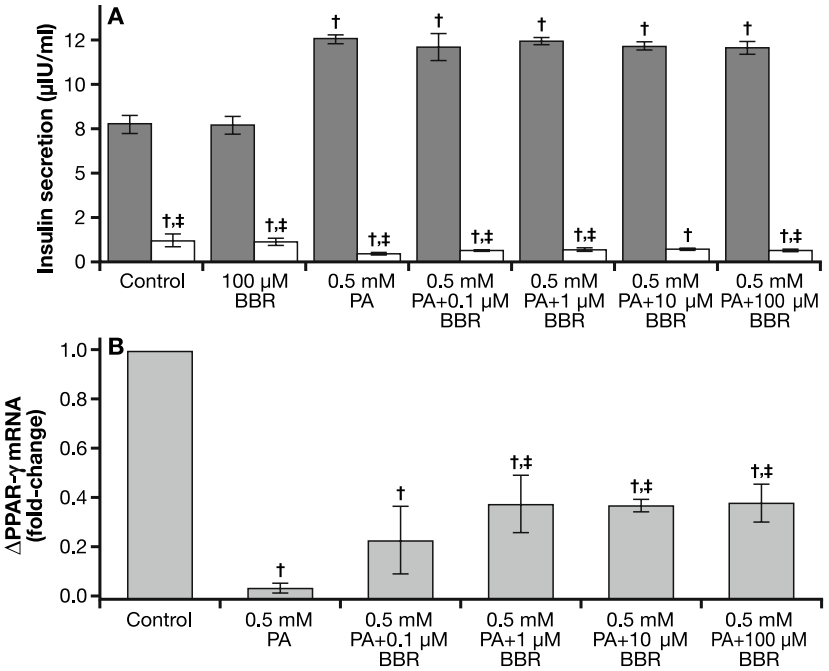

Fig. 4 - *A) The effects of palmitate (PA) and various concentrations of berberine (BBR) on baseline (gray bars) and glucose-stimulated (white bars) insulin concentrations in HIT-T15 cells. Data were presented as mean±SD (no.=3 per group). †Adjusted p<0.05 vs untreated control; ‡adjusted p<0.05 vs 100 μM BBR. B) The effects of PA and various concentrations of BBR on PPAR-γ mRNA expression in HIT-T15 cells. Data are presented as mean±SD (no.=3 per group). †Adjusted p<0.05 vs untreated control; ‡adjusted p<0.05 vs 0.5 mM PA.*

PPAR-γ mRNA expression was observed in cells treated with 0.5 mM palmitate ($p<0.0001$). In addition, PPAR-γ mRNA expression levels were significantly higher in all cells treated with palmitate + berberine as compared to those treated with palmitate alone ($p<0.05$). Finally, PPAR-γ mRNA levels were significantly reduced in the 0.5 mM palmitate + 0.1 μM berberine group as compared to all other palmitate + berberine groups ($p<0.05$).

Effect of berberine on PPAR-γ protein expression

To verify the effects of berberine on PPAR-γ expression at the level of protein expression, immunocytochemical analysis was performed. Representative images demonstrating PPAR-γ expression in HIT-T15 cells from each treatment group are shown in Figure 5. As shown in Figure 6, PPAR-γ protein levels were significantly decreased in the palmitate group as compared to the control group ($p<0.0001$). PPAR-γ protein levels were significantly higher in all palmitate + berberine groups as compared to the palmitate group ($p<0.05$). In addition, PPAR-γ protein levels increased in a dose-dependent manner with increasing berberine concentrations. Specifically, PPAR-γ protein levels were significantly higher in the 0.5 mM palmitate +100 μM berberine group as compared to both the 0.5 mM palmitate + 0.1 μM berberine and 0.5 mM palmitate + 1 μM berberine groups ($p<0.05$).

DISCUSSION

Lipotoxicity is an important mediator of Type 2 diabetes mellitus. Long-term elevation of FFA levels not only induces fat deposition in peripheral tissues thereby con-

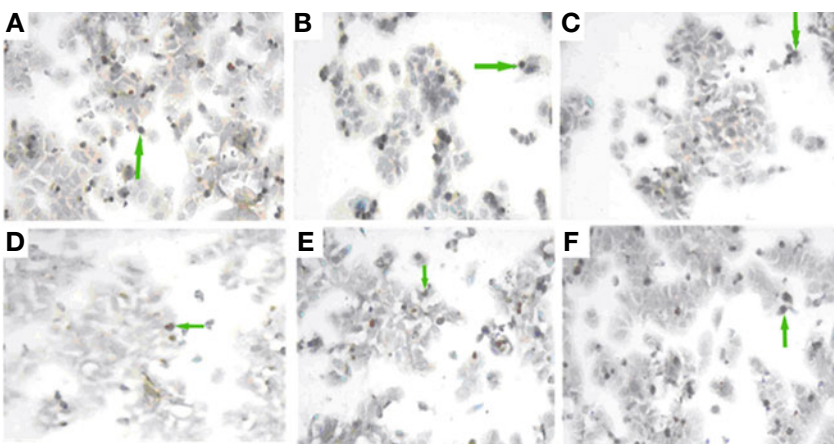

Fig. 5 - *The effects of palmitate (PA) and various concentrations of berberine (BBR) on PPAR-γ protein expression in HIT-T15 cells. PPAR-γ expression (brown dots indicated by arrows) as determined by immunocytochemistry was analyzed in the untreated control (A), 0.5 mM palmitate (B), 0.5 mM palmitate + 0.1 μM BBR (C), 0.5 mM palmitate + 1 μM BBR (D), 0.5 mM palmitate + 10 μM BBR (E), and 0.5 mM palmitate + 100 μM BBR (F) groups (400× magnification).*

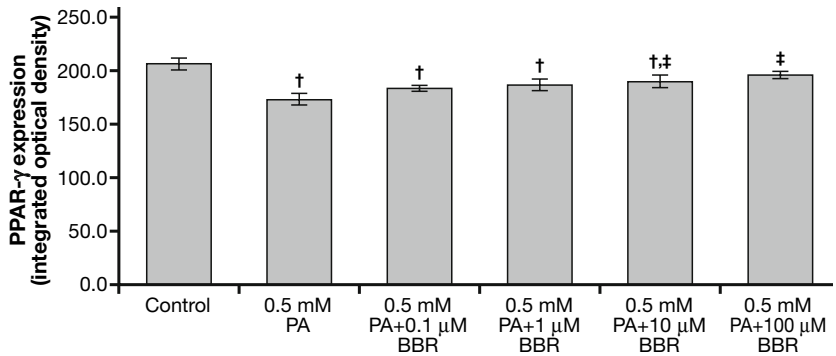

Fig. 6 - *The effects of palmitate (PA) and various concentrations of berberine (BBR) on PPARμM protein levels in HIT-T15 cells. Data are presented as mean±SD (no.=3 per group). †Adjusted p<0.05 vs untreated control; ‡adjusted p<0.05 vs 0.5 mM PA.*

tributing to insulin resistance, but also leads to apoptosis of pancreatic β-cells. In the present study, we found that palmitate-mediated apoptosis of HIT-T15 pancreatic β-cells could be reduced by concurrent treatment with berberine, an extract from an herb commonly used in Chinese medicine. This effect appears to be mediated at least in part by increased PPAR-γ expression.

Findings from an *in vitro* study have revealed increased β-cell GSIS following short-term (<24 h) exposure to FFA; however, upon long-term exposure (>24 h), decreased GSIS was observed (15). In addition, Dixon et al. reported that basal insulin secretion from BRIN-BDII tumor cells, a β-cell line, was increased by nearly two-fold following exposure to 100 μM palmitate for 24 h (16). In the present study, we found that baseline insulin secretion was significantly increased in HIT-T15 cells in response to 0.5 mM palmitate for 48 h; however, GSIS was significantly decreased with palmitate treatment. The reason for this decrease following glucose exposure is presumably due to a stronger effect of glucose (as compared to palmitate) on insulin secretion. Furthermore, decreased GSIS in our study coincided with increased HIT-T15 cell apoptosis; the palmitate-mediated apoptosis presumably reduced the number of cells, thereby decreasing insulin secretion. Additional study is needed to confirm this.

We found that there was a non-significant tendency towards increased GSIS following HIT-T15 cell exposure to berberine and palmitate as compared to palmitate alone. The trend for increased GSIS in cells treated with berberine may be attributed to reduced apoptosis in these cells. In addition, the lack of significance may be due to insufficient culture duration. Further experiments are warranted to assess GSIS at a later time after glucose exposure.

Findings from previous *in vitro* studies have demonstrated that berberine induces apoptosis in a number of different tumor cell lines such as PG (human prostate cancer), HSC-3 (human oral squamous carcinoma), MCF-7, and MDA-MB-231 (human breast cancer) cells (17-21). However, other studies have found that berberine inhibits apoptosis, particularly that induced by ischemia (22). In addition, an isoquinoline alkaloid, protopine, which has a similar structure to berberine, has the capacity to reduce cerebral cell apoptosis caused by ischemic injury (23). Our study also shows that berberine can significantly reduce palmitate-mediated apoptosis in HIT-T15 cells in a dose-dependent manner.

Coinciding with the decreased rates of apoptosis in cells treated with berberine and palmitate, we observed dose-dependent increases in PPAR-γ mRNA and protein expression levels. We did not assess the effects of berberine alone on PPAR-γ expression. This should be ascertained in future studies. Nevertheless, our findings suggest that the berberine-associated protection against palmitate-induced apoptosis in HIT-T15 cells is at least in part due to increased PPAR-γ expression. Other studies have demonstrated that β-cells are less susceptible to FFA-associated damage when PPAR-γ expression is increased. For instance, Du et al. found pancreatic β TC3 cells were protected from FFA-induced cell damage when PPAR-γ1 expression was elevated (6). In addition, Lin et al. reported that treatment of pancreatic β-cells with the PPAR-γ activator, rosiglitazone, inhibited islet amyloid polypeptide-induced apoptosis (24). Mechanistically, findings from a number of studies suggest that PPAR-γ has the capacity to interfere with the natural factor-κB (NF-κB) signalling pathway, thus inhibiting inducible nitric oxide synthase expression and the activation of proteinases involved in apoptosis (25, 26). Further studies using PPAR-γ antagonists are warranted to confirm the importance of PPAR-γ in mediating the berberine-associated protection described in this report.

We found that HIT-T15 cells exposed to palmitate alone exhibited decreased expression of PPAR-γ as compared to control cells. It is unclear precisely how this effect is mediated. Findings from a previous study indicate that palmitate can reduce expression of PPAR-γ coactivator 1α in skeletal muscle cells (27), and that this is mediated by altered mitogen-activated protein kinase-extracellular signal-related kinase and NF-κB activation (27). Additional research is needed to determine whether similar mechanisms of action mediate the changes observed in this study.

While apoptosis was clearly detected using the Hoechst 33258 fluorescence assay, a dose-dependent effect (seen with flow cytometry) was not apparent. The different apoptosis pattens obtained by flow cytometry and Hoechst 33258 fluorescence assay may be methodology-related. Annexin-V FITC (used in flow cytometry) allows for the detection of apoptotic cells at both the early and late stages of apoptosis, while Hoechst 33258 does not allow for such differentiation and is semi-quantitative.

The apoptosis of pancreatic β-cells caused by hyperlipemia is an important underlying mediator of Type 2

diabetes mellitus. In this study, we report that berberine, a Chinese medicinal herbal extract, can reduce the level of palmitate-induced apoptosis in β-cells and that this may be due to associated increases in PPAR-γ expression. Our findings suggest that berberine may be of use in the clinical setting to protect pancreatic β-cells from lipoapoptosis. Further *in vitro* and *in vivo* animal studies are required to delineate the underlying mechanisms and confirm the efficacy of berberine treatment to this end.

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REFERENCES

1. Ward WK, Beard JC, Halter JB, Pfeifer MA, Porte D Jr. Pathophysiology of insulin secretion in non-insulin-dependent diabetes mellitus. Diabetes Care 1984, 7: 491-502.
2. Lou DJ, Yang YW. The lipotoxicity and β cell apoptosis. Foreign Medical Sciences (Section of Internal Medicine) 2006, 33: 401-4.
3. Girard J. Fatty acids and beta cells. Diabetes Metab 2000, 26 (Suppl 3): 6-9.
4. Lupi R, Del Guerra S, Marselli L, et al. Rosiglitazone prevents the impairment of human islet function induced by fatty acids: evidence for a role of PPARgamma2 in the modulation of insulin secretion. Am J Physiol Endocrinol Metab 2004, 286: E560-7.
5. Kawai T, Hirose H, Seto Y, et al. Troglitazone ameliorates lipotoxicity in the beta cell line INS-1 expressing PPAR gamma. Diabetes Res Clin Pract 2002, 56: 83-92.
6. Du YF, Yang LY. The protective effect of high expression of mouse PPAR-γ1 on β-cell damage mediated by free fatty acid. Zhong Hua Diabetes Journal 2005, 12: 296-301.
7. Misra A, Kumar S, Kishore VN, Kumar A. The role of lipids in the development of diabetic microvascular complications: implications for therapy. Am J Cardiovasc Drugs 2003, 3: 325-38.
8. Heikkinen S, Auwerx J and Argmann CA. PPARgamma in human and mouse physiology. Biochim Biophys Acta 2007, 1771: 999-1013.
9. Saitoh Y, Chun-ping C, Noma K, Ueno H, Mizuta M, Nakazato M. Pioglitazone attenuates fatty acid-induced oxidative stress and apoptosis in pancreatic beta-cells. Diabetes Obes Metab 2008, 10: 564-73.
10. Wyne KL. Free fatty acids and type 2 diabetes mellitus. Am J Med 115 (Suppl 8A): 29S-36S, 2003.
11. Leng SH, Lu FE, Xu LJ. Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion. Acta Pharmacol Sin 2004, 25: 496-502.
12. Tang LQ, Wei W, Chen LM, Liu S. Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. J Ethnopharmacol 2006, 108: 109-15.
13. Zhang Y, Li X, Zou D, et al. Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. J Clin Endocrinol Metab 2008, 93: 2559-65.
14. Kong W, Wei J, Abidi P, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 2004, 10: 1344-51.
15. Ravnskjaer K, Boergesen M, Rubi B, et al. Peroxisome proliferator-activated receptor alpha (PPARalpha) potentiates, whereas PPARgamma attenuates, glucose-stimulated insulin secretion in pancreatic beta-cells. Endocrinology 2005, 146: 3266-76.
16. Dixon G, Nolan J, McClenaghan NH, Flatt PR, Newsholme P. Arachidonic acid, palmitic acid and glucose are important for the modulation of clonal pancreatic beta-cell insulin secretion, growth and functional integrity. Clin Sci (Lond) 2004, 106: 191-9.
17. Hao Y, Wang P, Wu J,Qiu QY. Effects of ginsenoside and berberine on secretion of immunosuppressive cytokines in lung carcinoma cell line PG. Zhong Xi Yi Jie He Xue Bao 2008, 6: 278-82.
18. Meeran SM, Katiyar S, Katiyar SK. Berberine-induced apoptosis in human prostate cancer cells is initiated by reactive oxygen species generation. Toxicol Appl Pharmacol 2008, 229: 33-43.
19. Kim JB, Lee KM, Ko E, et al. Berberine inhibits growth of the breast cancer cell lines MCF-7 and MDA-MB-231. Planta Med 2008, 74: 39-42.
20. Lin JP, Yang JS, Chang NW, et al. GADD153 mediates berberine-induced apoptosis in human cervical cancer Ca ski cells. Anticancer Res 2007, 27: 3379-86.
21. Lin CC, Yang JS, Chen JT, et al. Berberine induces apoptosis in human HSC-3 oral cancer cells via simultaneous activation of the death receptor-mediated and mitochondrial pathway. Anticancer Res 2007, 27: 3371-8.
22. Zheng L, Zhou Z, Tao D, Lan T. Protective effect of berberine on cardiac myocyte injured by ischemia-reperfusion. Sichuan Da Xue Xue Bao Yi Xue Ban 2003, 34: 452-4.
23. Xiao X, Liu J, Hu J, Li T, Zhang Y. Protective effect of protopine on the focal cerebral ischaemic injury in rats. Basic Clin Pharmacol Toxicol 2007, 101: 85-9.
24. Lin CY, Gurlo T, Haataja L, Hsueh WA, Butler PC. Activation of peroxisome proliferator-activated receptor-gamma by rosiglitazone protects human islet cells against human islet amyloid polypeptide toxicity by a phosphatidylinositol 3'-kinase-dependent pathway. J Clin Endocrinol Metab 2005, 90: 6678-86.
25. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 1998, 391: 79-82.
26. Kharroubi I, Ladriere L, Cardozo AK, Dogusan Z, Cnop M, Eizirik DL. Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress. Endocrinology 2004, 145: 5087-96.
27. Coll T, Jove M, Rodriguez-Calvo R, et al. Palmitate-mediated downregulation of peroxisome proliferator-activated receptor-gamma coactivator 1alpha in skeletal muscle cells involves MEK1/2 and nuclear factor-kappaB activation. Diabetes 2006, 55: 2779-87.