JPP
Journal of
Pharmacy and Pharmacology

JPP 2009, 61: 789–794
© 2009 The Authors
Received August 26, 2008
Accepted March 18, 2009
DOI 10.1211/jpp/61.06.0012
ISSN 0022-3573

Research Paper

Study on the antidiabetic mechanism of a shark liver peptide, S-8300, in alloxan-induced diabetes

Fengjie Huang and Wutong Wu

School of Life Science and Technology, China Pharmaceutical University, Nanjing, China

Abstract

Objectives The aim was to evaluate the antidiabetic mechanism of S-8300 in alloxan-diabetic mice.

Methods Diabetes was induced by a single intravenous injection of alloxan (60 mg/kg). The effects of S-8300 on diabetic mice were investigated by observing the change in fasting plasma glucose, detecting Fas mRNA by reverse transcriptase–polymerase chain reaction, Fas protein expression in the pancreas by immunohistochemistry and Western blot, and the DNA fragmentation pattern forming a ladder by electrophoresis.

Key findings A significant decrease in fasting plasma glucose was observed, and Fas mRNA and Fas protein expression in the pancreas were attenuated in diabetic mice treated with S-8300. Treatment with S-8300 also attenuated DNA ladder formation.

Conclusions The results suggest that S-8300 inhibits Fas protein-mediated apoptosis of pancreas cells.

Keywords alloxan-diabetes; apoptosis; reverse transcriptase–polymerase chain reaction; Fas; immunohistochemistry

Introduction

The compound S-8300, a small (8200.901 Da) antidiabetic peptide, was purified and characterized from shark livers.[1] It was isolated by extraction of the water-soluble fraction, dialysis, ultrafiltration and DEAE-Sepharose, Bio-Gel P-10, FPLC monoQ and C18 RPHPLC column chromatography. The peptide (S-8300) contained 17 amino acids and the 15 N-terminal amino acid residues of S-8300 were NH_2-Met-Leu-Val-Gly-Pro-Ile-Gly-Ala-Ala-Lys-Val-Val-Tyr-Glu-Gln-. The peptide was stable at 95°C for 30 min and at pH 3.0–9.0. We previously reported that S-8300 had significant protective effects against carbon tetrachloride induced liver injury in mice[2] and an immunoregulatory effect on the immunosuppression caused by cyclophosphamide in mice.[3] We also reported that S-8300 produced a 60.5%, 53.5% and 36.6% decrease in blood glucose at doses of 1, 3 and 10 mg/kg in alloxan-diabetic mice after 4 weeks treatment.[4] In streptozotocin-diabetic mice treated with S-8300 at 3 and 10 mg/kg for 28 days, a significant reduction in blood glucose was observed (14.8% and 29.4%, respectively).[5]

The Fas protein is a well known apoptosis-associated cell surface molecule, which is a receptor protein mediating cell death signals intercellularly. Fas belongs to a family of nerve growth factor receptors/tumour necrosis factor receptors and induces the apoptotic signal when cross-linked by either agonistic anti-Fas antibody or its natural ligand. Originally, Fas was detected in fibroblasts, some tumour cell lines including gliomas, and some cells of lymphocyte lineage. Subsequently, a variety of normal cells have been found to express this molecule. In mice, the mRNA coding for Fas has been detected in the thymus, heart, liver, and ovaries at high levels.[6]

S-8300 could protect the structural integrity and recover the damage of NIT-1 cells after exposure to the toxin streptozotocin.[1] Treatment with S-8300 also attenuated the degree of injury of β cells in pancreatic islets.[4,5] However, the effects of S-8300 in the prevention of damage to diabetic mouse pancreas have not been carefully investigated. The present study was undertaken to systematically demonstrate the preventive effect of S-8300 on pancreatic cell damage in alloxan-diabetic mice. To explore the mechanism of the antidiabetic effect of this active substance, we measured the change of fasting plasma glucose, and Fas mRNA and Fas protein expression levels in the pancreas after administration of S-8300 to alloxan-diabetic mice.

Correspondence: Wutong Wu, School of Life Science and Technology, China Pharmaceutical University, Tongjia-Xiang 24#, Nanjing 210009, China.
E-mail: wuwutongmailbox@163.com

Materials and Methods

Reagents

The reagents used in this study were: alloxan (Sigma Chemicals Ltd Shanghai, China), glucometer (Beijing Yicheng Bio-electron Technology Co., Ltd, Beijing, China), TRIzol reagent (Sangon Gene Ltd, Shanghai, China), reverse transcription polymerase chain reaction (RT-PCR) reagent (Promega, Beijing, China), Fas ABC kit and DAB substrate kit (Boshide, Wuhan, China). All the other chemicals used were of analytical grade.

Animals

Healthy female ICR mice, 22–26 g, were obtained from the Experimental Animal Center of China Pharmaceutical University (Nanjing, China). They were housed in plastic cages and maintained under standard conditions (12-h light–dark cycle; 22–25°C; 35–60% humidity). Before and during the experiment, mice were fed a normal laboratory pellet diet and water *ad libitum*. After randomization into various groups, the mice were acclimatized for a period of 2 days in the new environment before the start of the experiment. The experiments were overseen and approved by the Science and Technological Committee and the Animal Use and Care Committee of China Pharmaceutical University, Nanjing, China.

Preparation of S-8300

S-8300 is a peptide extracted and purified from healthy *Squalus mitsukurii*.[2] For purification of S-8300,[1] fresh, healthy shark livers were first washed in distilled water. After freezing and melting several times, the material was heated to 95°C for 5 min to obtain the heat-stable peptides. After centrifugation, the supernatant containing the heat-stable proteins was subjected to ultrafiltration and the extract was loaded onto a DEAE-Sepharose column, which was equilibrated with 10 mmol/l Na-phosphate buffer at pH 8.0. The high active fractions were collected, dialysed and lyophilized. The lyophilized powder was dissolved in distilled water and applied to a Bio-Gel P-10 column, which was then eluted with distilled water. The high active fractions were collected and dialysed with buffer A (20 mmol/l Tris-HCl buffer at pH 8.0). The dialysate was loaded onto a FPLC monoQ column, which was eluted with a linear gradient of 0–25% buffer B (buffer A containing 1 mol/l NaCl). The active peak was collected, dialysed and lyophilized. The lyophilized powder was dissolved in 0.1% (v/v) trifluoroacetic acid in HPLC-grade water and loaded onto a C18 RPHPLC column equilibrated with 0.1% trifluoroacetic acid. The peptides were eluted with a linear gradient of acetonitrile (10–95%) containing 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. The peptide S-8300 was collected and lyophilized.

Induction of experimental diabetes

Diabetes was induced in mice by a single intravenous injection of alloxan dissolved in sterile normal saline at a dose of 60 mg/kg.[7] Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, mice were treated with a 5% glucose solution (1 ml) intraperitoneally after 6 h. After 5 days, the diabetic mice (glucose levels over 15 mmol/l) were used in the experiment.

Experimental design

A total of 70 mice (56 diabetic surviving mice, 14 normal mice) were used. The mice were divided into four groups of 14 mice each after the induction of alloxan-diabetes. Group 1: normal control mice were given 0.4 ml saline intraperitoneally daily for 4 weeks. Group 2: diabetic mice were given 0.4 ml saline intraperitoneally daily for 4 weeks. Group 3: diabetic mice were given S-8300 (3 mg/kg) intraperitoneally daily for 4 weeks. Group 4: diabetic mice were given S-8300 (10 mg/kg) intraperitoneally daily for 4 weeks.

The antidiabetic mechanism of S-8300 in alloxan-diabetic mice was determined by measuring NO and NOS levels, Fas mRNA and Fas protein expression level in the pancreas. The extracted DNA in the pancreas was analysed by agarose gel electrophoresis. Day 5 of induction was designated as Day 1 for S-8300 administration to diabetic mice. Fasting plasma glucose was estimated on Day 1, 8, 15, 22 and 29 of S-8300 administration. All other biochemical parameters were determined on Day 29 when the animals were fasted for 12 h and then killed by decapitation.

Sample collection

At the end of 4 weeks the animals were fasted overnight and killed by decapitation. Plasma was separated for the estimation of NO and NOS. The pancreas was dissected out. One part was used for DNA, RNA and protein extraction. The other specimens were preserved in 10% neutral formalin and were processed for paraffin embedding.

Biochemical measurements

Fasting plasma glucose levels were determined by the glucose oxidase method using a reflective glucometer.

RNA extraction and RT-PCR analysis

Total RNA was extracted from mouse pancreas tissues with TRIzol reagent, a monophasic solution of phenol and guanidine isothiocyanate. The amount of RNA was estimated by spectrophotometry at 260 nm. Single-stranded cDNA was obtained by reverse transcription of 2 μg of total RNA using oligo d(T)18 as primers (0.5 μg), incubation at 70°C for 5 min and then chilling on ice for 5 min. Then, 2.5 μl dNTP (20 mmol/l), 40 units RNase inhibitor (Promega), and 10 units M-MLV reverse transcriptase (Promega) were added to the tube and the final volume was made up to 25 μl with DEPC-water. The reaction was carried out at 42°C for 1 h and then inactivated at 94°C for 5 min. The cDNA was obtained and stored at –20°C. Each PCR run was conducted using the same RT mixture, with GAPDH primer or Fas primer. The PCR reactions (25 μl) were prepared on ice and contained 2 units Taq polymerase (Promega), 1.5 mM $MgCl_2$, 100 mM dNTPs, 2 μM of each primer and 1 μl of cDNA. After denaturing at 94°C for 3 min, PCR amplification was performed under the following conditions: 94°C for 45 s, 58°C for 45 s, and 72°C for 45 s, for a total of 30 cycles. The reaction was terminated after a 10-min elongation step at 72°C. After amplification, agarose gel electrophoresis of the DNA was performed through a 1.8%

agarose gel containing 0.5 μg/ml ethidium bromide using a Mupid electrophoretic apparatus. PCR products were subcloned and sequenced to confirm sequence identities. As an internal standard, GAPDH was co-amplified in the same tube to allow semiquantitative comparisons among the cDNA. The PCR amplification was performed under the following conditions: 94°C for 30 s, 56°C for 30 s, and 72°C for 45 s, for a total of 28 cycles. The reaction was terminated after a 10-min elongation step at 72°C, and the PCR products were separated by electrophoresis on 8% polyacrylamide gels. Band intensities were estimated by densitometric scanning using a GDS 8000 White/Ultraviolet Transilluminator. Photographs were taken with Laboratory film (Chicago, USA). Data were expressed as Fas/GAPDH mRNA ratios. RT-PCR primers were designed inside separate exons to avoid any bias due to residual genomic contamination. Moreover, for all primers, no amplification was observed when PCR was performed on RNA preparations. The following primers were used: Fas (209 bp) upper 5′-CTG AGG AGG CGG GTT CAT-3′; lower 5′-GAG TGT GCA TCT TCT GCA-3′; GAPDH (584 bp) as housekeeping gene, upper, 5′-ATC ACC ATC TTC CAG GAG CG -3′; lower, 5′-CCT GCT TCA CCA CCT TCT TG-3′.

Immunohistochemistry assay

Tissue fixation and immunostaining procedures were performed essentially according to the method described previously.[8] Briefly, the pancreas was immersed in 4% paraformaldehyde/phosphate-buffered saline (PBS) solution, irradiated twice in a microwave oven for 20 s and fixed in 4% paraformaldehyde/PBS solution for 4 h on ice. It was then immersed in cold PBS overnight, dehydrated with 100% ethanol, embedded in polyester wax and then sectioned at 5 mm. After dewaxing and inactivating the endogenous peroxidase, the sections were blocked by applying PBS containing 5% fetal calf serum, 5% skim milk and 20 mg/ml mouse and goat IgG for 30 min. The blocking solution was then removed and the sections were incubated overnight with either 20 mg/ml of anti-Fas monoclonal antibody (mAb; rat IgG2a) or control rat IgG2a in a fresh blocking solution. Thereafter, the sections were washed in cold PBS containing 0.02% Tween 20 and incubated with peroxidase-conjugated anti-rat IgG antibody and 20 mg/ml goat IgG for 1 h. After washing in cold PBS, the sections were stained with a metal enhanced DAB substrate kit. The sections were dehydrated and mounted with coverslips for photomicrography.

Western blot analysis

Equal amounts of the extracted pancreas tissue protein were separated by 10% SDS-PAGE. The Western blot was done as described elsewhere.[9] In brief, total cellular proteins were prepared and then quantified by the Bradford method. Membranes were blocked with 10% fat-free milk powder at room temperature for 2 h and incubated with the primary antibody against Fas (1 : 1000 dilution) or anti-β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight. After three washes for 15 min in TBST, the membrane was incubated with the HRP-conjugated goat anti-mouse IgG antibody (Wuhan, Hubei, China) for 2 h at room temperature. The membrane was washed again in TBST; enhanced chemiluminescence (Amersham Life Science, Piscataway, NJ, USA) was added and monitored for the development of colour. All results were normalized to β-actin levels in the same sample.

Apoptosis assay

DNA was prepared from pancreas as follows. Tissues were washed twice in PBS and then lysed in cold 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA and 0.5% Triton X-100. After tissue lysis, debris was removed by centrifugation at 15 000*g* for 20 min. DNase-free RNase was added to the lysates to a final concentration of 40 μg/ml and the lysates were then incubated for 1 h at 37°C with gentle shaking. Proteinase K was added to the RNase-treated lysates at a final concentration of 40 μg/ml. The lysates were further incubated for 1 h at 37°C with gentle shaking. DNA in the supernatant was precipitated with 2-propanol and sodium chloride overnight at –20°C. After centrifugation and drying, DNA was dissolved in TE-buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Agarose gel electrophoresis of the DNA was performed through a 2.0% agarose gel containing 0.5 μg/ml ethidium bromide using a Mupid electrophoretic apparatus. Molecular weight standards were run on the same gels. For visualization of apoptotic alterations to DNA integrity, DNA bands were observed on a UV transilluminator. Photographs were taken with laboratory film.

Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was evaluated by one-way analysis of variance followed by the Student–Newman–Keuls test for multiple comparisons to evaluate the difference between two groups. $P < 0.05$ was considered significant.

Results

Fasting plasma glucose

Table 1 shows the levels of plasma glucose in the normal, diabetic control and experimental groups. The diabetic mice showed a significant increase in blood glucose. The administration of S-8300 to diabetic mice restored the level of blood glucose in a dose-dependent manner ($P < 0.01$). It produced a 28.2 and 48.6% decrease in blood glucose at a dose of 3 and 10 mg/kg, respectively, in alloxan-diabetic mice.

Expression of Fas mRNA

We analysed the mRNA levels of Fas genes that are specifically expressed in pancreas tissues. Fas expression decreased in a dose-dependent manner after S-8300 treatment. Indeed, a significant ($P < 0.01$) decrease in Fas mRNA levels was detected after S-8300 treatment. We examined the level of Fas mRNA in the pancreas by RT-PCR. As shown in Figure 1, RT-PCR products for Fas mRNA were detected in all experimental groups. We also examined the expression of Fas/GAPDH mRNA. The changes in the ratio of Fas/GAPDH in pancreas of normal, diabetic control and experimental groups are shown in Table 2. A marked increase in the ratio of Fas/GAPDH was observed in diabetic mice. S-8300 treatment significantly reversed the changes in

Table 1 Effect of S-8300 on fasting plasma glucose in alloxan-diabetic mice

Group	Dose	Fasting plasma glucose (mmol/l)				
		Week 0	Week 1	Week 2	Week 3	Week 4
Normal	–	6.51 ± 0.42**	6.61 ± 0.55**	6.56 ± 0.38**	6.30 ± 0.52**	6.43 ± 0.43**
Diabetic control	–	20.14 ± 1.55	20.42 ± 2.35	20.20 ± 1.58	19.76 ± 2.03	19.02 ± 1.55
S-8300	3 mg/kg	20.22 ± 1.72	18.87 ± 2.05*	18.07 ± 2.50*	16.81 ± 2.30**	14.52 ± 2.09**
S-8300	10 mg/kg	20.01 ± 1.42	17.84 ± 2.15*	15.04 ± 1.63**	13.91 ± 2.40**	10.28 ± 2.38**

Data are mean ± SD, $n = 14$. *$P < 0.05$ and **$P < 0.01$, significantly different compared with the respective diabetic control (analysis of variance followed by the Student–Newman–Keuls test).

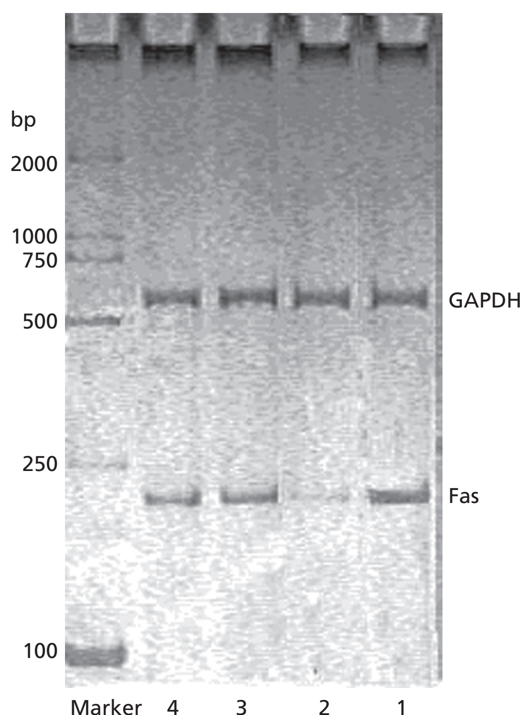

Figure 1 Expression of Fas and GAPDH mRNA in the pancreas. Agarose gel electrophoresis: lane 1: model group (60 mg/kg alloxan); lane 2: normal group; lane 3: low dose group (3 mg/kg S-8300); lane 4: high dose group (10 mg/kg S-8300); marker: standard DNA marker. GAPDH: 584 bp; Fas: 209 bp.

Table 2 Effects S-8300 on the expression of the Fas gene in alloxan-diabetic mice

Group	Dose	Fas/GAPDH
Normal	–	0.212597 ± 0.014478**
Diabetic control	–	0.54022 ± 0.076913
S-8300	3 mg/kg	0.310502 ± 0.015666**
S-8300	10 mg/kg	0.248378 ± 0.018545**

Data are mean ± SD, $n = 14$. **$P < 0.01$, significantly different compared with the diabetic control (analysis of variance followed by the Student–Newman–Keuls test).

the ratio of Fas/GAPDH in diabetic mice in a dose-dependent manner.

Expressions of Fas protein in the pancreas

To detect Fas expression at the protein level, we examined the section of pancreas by a immunohistochemical method with anti-Fas mAb. A control antibody of the same subclass (IgG2a) did not stain any cells in either group. Representative results of Fas-positive immunostaining are shown in Figure 2. While immunoreactivity was found to be very weak or absent in the normal group (Figure 2a), the diabetic group displayed strong Fas-positive immunoreactivity (Figure 2b). Moreover, as shown in Figure 2c, a significant decrease of Fas-positive immunoreactivity was, when compared with the diabetic group, barely observed in pancreas from mice treated with S-8300. The changes in distribution of Fas immunoreactive cells in the pancreas of normal, diabetic control and experimental groups are shown in Table 3. A marked increase in the number of immunoreactive cells was observed in the diabetic mice. As shown in Table 3, the expression of Fas declined significantly ($P < 0.01$) in a dose-dependent manner after S-8300 treatment. The decrease of Fas expression in the pancreas of diabetic mice treated with S-8300 was confirmed by Western blot (Figure 3).

Apoptosis in pancreas

The extracted DNA was analysed by agarose gel electrophoresis. In diabetic animals, a DNA fragmentation pattern forming a ladder of multiple bands was observed (Figure 4). The normal control group did not show any apoptotic features (Figure 4). S-8300 treatment in diabetic mice significantly reversed these changes as shown in Figure 4.

Discussion

We examined the effect of S-8300 on Fas protein-mediated apoptosis in pancreas cells. The results demonstrate that treatment of diabetic mice with S-8300 decreased Fas expression, indicating that S-8300 ameliorates the diabetic state by preventing the apoptosis of pancreatic cells. Normal pancreatic beta cells do not express Fas,[10] but diabetic beta cells do. Our findings are consistent with this: in this study, Fas immunoreactivity was found to be very weak or absent in the normal group (Figure 2a), whereas the diabetic group displayed strong Fas-positive immunoreactivity (Figure 2b).

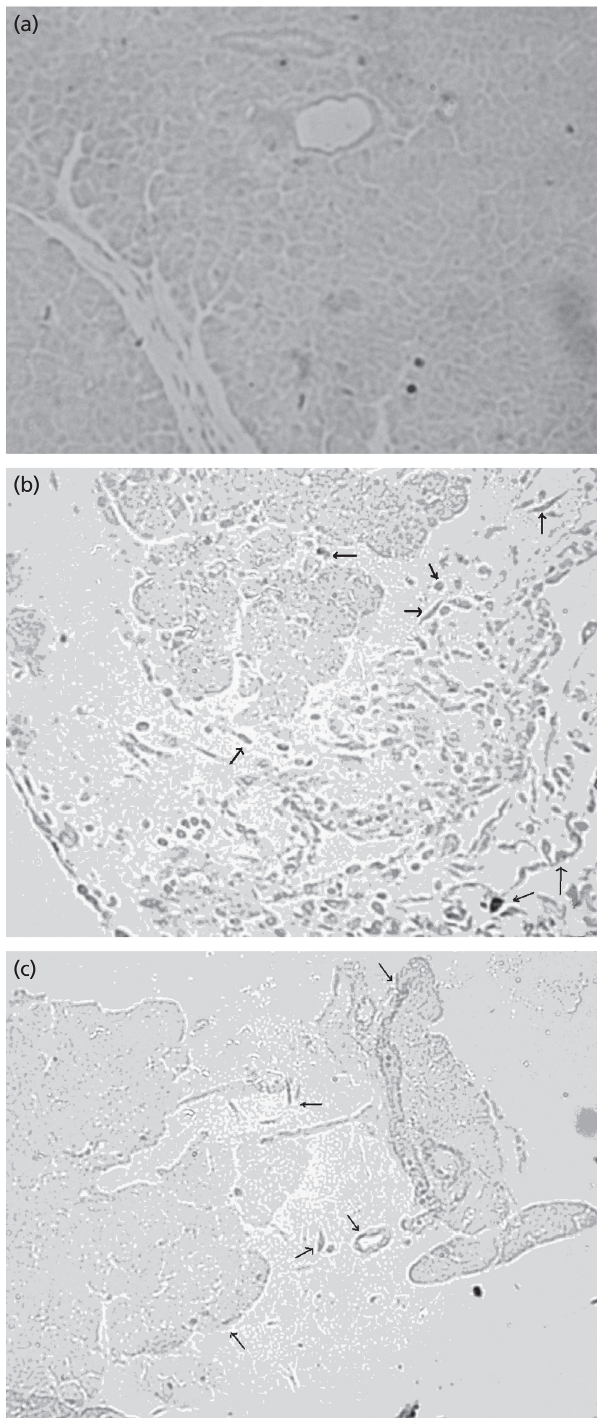

Figure 2 Fas immunoreactive cells detected by immunohistochemistry in the pancreas. (a) Normal group, pancreas stained with anti-Fas antibody (×100). (b) Alloxan alone, pancreas stained with anti-Fas antibody (×100). (c) Alloxan + S-8300 (10 mg/kg), pancreas stained with anti-Fas antibody (×100); Arrows indicate Fas immunoreactive cells.

Table 3 Distribution of Fas immunoreactive cells in the pancreas of alloxan-diabetic mice

Group	Dose	Fas (cell number/mm)
Normal	–	–**
Diabetic control	–	28.18 ± 1.06
S-8300	3 mg/kg	14.38 ± 0.70**
S-8300	10 mg/kg	3.80 ± 0.66**

Data are mean ± SD, n = 3. **P < 0.01, significantly different compared with the diabetic control (analysis of variance followed by the Student–Newman–Keuls test).

Figure 3 Western blot analysis of Fas expression in isolated islets. The expression of Fas declined significantly (P < 0.01) in a dose-dependent manner after S-8300 treatment. Lane 1: normal group; lane 2: high dose group (10 mg/kg S-8300); lane 3: low dose group (3 mg/kg S-8300); lane 4: model group (60 mg/kg alloxan).

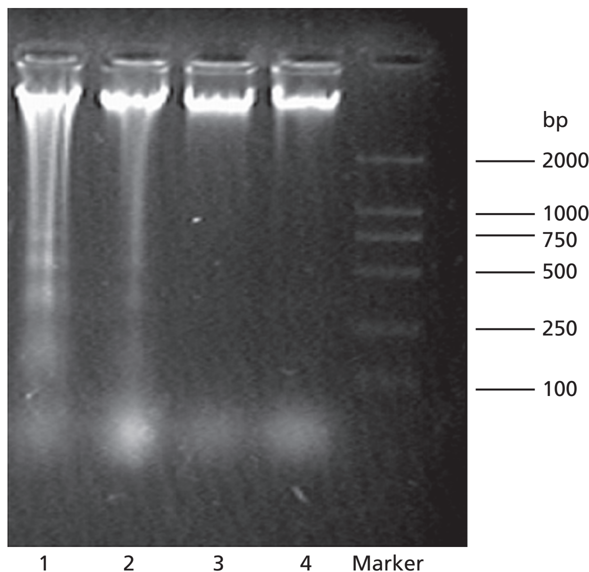

Figure 4 DNA ladder formation in the pancreas. Extracted DNA was electrophoresed through an agarose gel and stained with ethidium bromide. Lane 1: model group (60 mg kg^{-1} alloxan); lane 2: low dose group (3 mg/kg S-8300); lane 3: high dose group (10 mg/kg S-8300); lane 4: normal group; marker: standard DNA marker.

The Fas expression in pancreas was confirmed by Western blot (Figure 3). Fas mRNA expression determined by RT-PCR was decreased by S-8300 treatment. The DNA fragmentation pattern forming a ladder of multiple bands was observed in diabetic mice by agarose gel electrophoresis, and S-8300 treatment of diabetic mice significantly reversed these changes, as shown in Figure 4. These results confirmed that Fas was involved in the apoptosis of pancreas cells

induced by alloxan. Quantitative analysis showed that S-8300 decreased the apoptosis of pancreatic cells in a dose-dependent manner. The results indicate that a pronounced destruction of active pancreatic cells could be induced by alloxan in mice with blood glucose levels of 20 mmol/l, leading to pancreas cell apoptosis. Based on these results, we can outline a link between the decrease in apoptosis and the decrease in blood glucose, which suggests that apoptosis may be involved in the early stages of diabetes. The expression of mRNA and Fas protein in pancreatic cells coincided with the occurrence of apoptosis in pancreatic cells. In the pancreas under the influence of S-8300, the signal of the Fas–Fas ligand may be down-regulated so that injured pancreatic cells are recovered to maintain homeostasis of fasting plasma glucose. Together, these results suggest an important role of S-8300 in the regulation of Fas gene expression. Thus, the significant antidiabetic activity of S-8300 in our study may be attributed to the protection of pancreatic cells from apoptosis.

Our study provides substantial evidence of the hypoglycemic effect of S-8300 as well as its role as an anti-apoptotic agent and thus reveals the mechanism of its cytoprotective action. We previously reported the influence of S-8300 on the immune system of mice with experimentally induced immunosuppression, whereby administration of S-8300 not only decreased the carbon tetrachloride induced elevated levels of aspartate transaminase, alanine transaminase, lactate dehydrogenase and maleic dialdehyde, but also increased levels of superoxide dismutase and glutathione.[11] This suggested the maintenance of structural integrity of the hepatocytic cell membrane or regeneration of damaged liver cells by inhibiting lipid peroxidation activity of S-8300. Thus, S-8300 could serve a beneficial role as an antioxidant, capable of removing free radicals from a system either by prolonging the initiation phase or by inhibiting the propagation phase of autooxidation. It could also scavenge free radicals in humans, and with its immunostimulatory effect it could prevent foreign pathogenic invasion. Our studies strongly support the notion that supplementation of S-8300 to diabetic patients would help in achieving good glycaemic and metabolic control as a result of the protection offered by its anti-apoptotic action, probably preserving the residual β-cell mass without further loss. This is the first report of the anti-apoptotic role of S-8300, suggesting its potential use in the control of diabetes and thus improving quality of life free of secondary diabetic complications due to sustained residual pancreas cell mass. It was a limitation that there was no positive control group in the present study.

Conclusions

This study demonstrates that S-8300 has beneficial effects in an experimental model of alloxan-induced diabetes in mice by preventing apoptosis of pancreas cells. The effects of S-8300 are exerted through its hypoglycaemic, anti-apoptotic and cytoprotective action. The reduction in pancreas cell apoptosis and attenuation of diabetes seen in our model may be as a result of the effects of S-8300 on the regulation of Fas gene expression.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported by Hi-Tech Research and Development Program of China (863 Project), the National Natural Science Foundation of China (no. 30701036) and the 211 Project of China Pharmaceutical University.

References

1. Huang F, Wu W. Purification and characterization of a new peptide (S-8300) from shark liver. *J Food Biochem* (in press).
2. Huang F *et al*. Protective effect of peptide S-8300 from shark liver on acute liver injury in mice. *Zhongguo Haiyang Yaowu* 2004; 97: 17–20.
3. Huang F, Wu W. Study on immunoregulation effect of peptide S-8300 from shark liver. *Zhongguo Tianran Yaowu* 2005; 3: 121–123.
4. Huang F, Wu W. Antidiabetic effect of a new peptide from *Squalus mitsukurii* liver (S-8300) in alloxan-induced diabetes. *Clin Exp Pharmacol Physiol* 2005; 32: 521–525.
5. Huang F, Wu W. Antidiabetic effect of a new peptide from *Squalus mitsukurii* liver (S-8300) in streptozotocin-induced diabetes. *J Pharm Pharmacol* 2005; 57: 1575–1580.
6. Cheho P *et al*. Expression of Fas protein in the normal mouse brain. *Biochem Biophys Res Commun* 1998; 52: 623–628.
7. Hong G *et al*. Protective effects of shark hepatic stimulator substance on alloxan-induced diabetes in mice. *J China Pharm Univ* 2003; 34: 155–159.
8. Fang ZP *et al*. Immunocytochemical localization of nine teleosts using monoclonal antibody of grass carp growth hormone. *Shuisheng Shengwu Xuebao* 1998; 22: 355–360.
9. Pan Y *et al*. Expression of seven main Rho family members in gastric carcinoma. *Biochem Biophys Res Commun* 2004; 315: 686–691.
10. Stassi G *et al*. Nitric oxide primes pancreatic beta cells for Fas-mediated destruction in insulin-dependent diabetes mellitus. *J Exp Med* 1997; 186: 1193–1200.
11. Huang F *et al*. Study on hepatoprotective effect of peptide S-8300 from shark liver. *World J Gastroenterol* 2005; 11: 1809–1812.