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Hypoglycemic and hypolipidemic activities of MT-α-glucan and its effect on immune function of diabetic mice

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ABSTRACT

The hypoglycemic and hypolipidemic effect of an α-glucan (designed here as MT-α-glucan) from fruit body of maitake (*Grifola frondosa*) on diabetic mouse model induced by streptozotocin and high-fat-diet were evaluated, and its effect on immune function of diabetic mice was investigated. Treatment with MT-α-glucan (300 or 100 mg kg^{-1}) could decrease the levels of fasting plasma glucose, triglycerides, cholesterol, free fatty acid, the proliferative response of macrophages and IL-1, NO production by macrophages significantly. Treatment with MT-α-glucan could increase the serum insulin, the proliferative response and IL-2 production of splenocytes induced by ConA significantly. Ultrastructural changes of pancreatic β-cells were ameliorated in the treatment group. These data suggest that MT-α-glucan has hypoglycemic and hypolipidemic effect on the diabetic mice model, which might be related to its benefit effect on immune reactions involved in pathogenesis of diabetes mellitus, leading to attenuate the degree of injured β-cells of the pancreatic islets.

1. Introduction

Type 2 diabetes mellitus (T2DM) affects approximately 5% of population world wide and its incidence is increasing every year (Xie & Zhou, 2002), characterized by abnormalities in carbohydrate and lipid metabolism, which lead to postprandial and fasting hyperglycemia, dyslipidemia, and relative insulin secretion shortage (Defronzo, Bondonna, & Ferranini, 1992). Deterioration of pancreatic β cell secreting function is considered to be the significant pathogenic factor in T2DM (Olefsky & Nolan, 1995).

Some natural products possess the ability to lower blood glucose and utilization of natural products over a prolonged period should be safer than chemical drugs. Maitake mushrooms (*Grifola frondosa*) belonging to Basidiomycetes in fungi represent a natural alternative. Because of its enticing taste, maitake has been praised and consumed by Chinese people for hundreds of years. Moreover, the medicinal properties of maitake have been claimed for years and some of them have been demonstrated scientifically and experimentally. For instance, maitake has been shown to have anti-tumor effect (Liu, Chen, & Wu, 2005), immune regulatory activity (Inoue, Kodoma, & Nanba, 2002), anti-hyperliposis (Kubo, 1997), anti-common and specific infections effects such as hepatitis (Kubo & Nanba, 1998; Ooi, 1996) and AIDS/HIV (Nanba, Kodama, Schar, & Turner, 2000). Extracts from maitake are already in practice use in the clinic as BRMs (biological response modifiers). The polysaccharide extracted from maitake was found to have a favorable effect in autoimmune hepatitis though its effect on the immune reaction (Kubo & Nanba, 1998). The immune system dysfunction has been recognized to be closely related to the onset of T2DM (Hirotada, Noriko, & Hiroaki, 2000). We extended our study to see if similar effect of MT-α-glucan could be found for T2DM.

Previous studies have shown that ingesting maitake mushrooms, or some of its extracts, influences glucose/lipid metabolism and has anti-diabetic effect (Kubo, 1994, 1997). But studies on its active part and mechanism of action have not been carried out. Based on previous studies, a new kind of α-glucan extracted and purified from the fruit body of maitake, designed here as MT-α-glucan, was prepared in our laboratory. Previous studies in our laboratory showed that MT-α-glucan has hypoglycemic activity in KK-Ay mice, a model of type 2 diabetes, by ameliorating peripheral insulin resistance and enhancing insulin sensitivity (Lei, Ma, & Wu, 2007). Compared to our previous work, the differences of this work were that we used the different animal model of type 2 diabetes and we elucidated the different mechanism of action of MT-α-glucan concerning immune reaction. The present study was therefore designed to determine the hypoglycemic effect of MT-α-glucan in an another animal model of type 2 diabetes, exploiting high-fat diet and low dose Streptozotocin (STZ). Moreover, its mechanisms of action concerning protection of pancreatic β cells from destruction by immunity-adjusting mechanism were also investigated.

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2. Materials and methods

2.1. Preparation of MT-α-glucan

MT-α-glucan was extracted and purified from fruit body of maitake (*G. frondosa*) as previously described (Lei et al., 2007), which is a basically homogeneous polysaccharide fraction and its molecular weight is about 400,000–450,000 Da. MT-α-glucan was dissolved in 1% sodium carboxymethylcellulose (CMC-Na) and diluted to the concentration needed.

2.2. Animals

Healthy male C_{57}BL/6J mice (three weeks of age, 6–9 g) were obtained from Experimental Animal Institute of Nanjing University (Nanjing, China). They were housed in plastic cages and maintained under standard conditions (12 h light/dark cycle; 23–25 °C; 35–60% humidity). Before and during the experiment, mice were fed with a high fat or normal laboratory pellet diet and water was freely available. After randomization into various groups, the mice were acclimatized in the new environment for two days before initiation of the experiment. The study complied with the current ethical regulations for the care and use of laboratory animals of Hefei University of Technology (Anhui, China), and all mice used in the experiment received human care.

2.3. Main reagents

Streptozotocin (STZ) was from Sigma. The glucometer was from Beijing Yicheng Bio-electron Technology Co., Ltd. (Beijing, China). Various measuring kits were used during the study. These were as follows: triglyceride measurement kit (Zhejiang Dongou Bioengineering Co., Ltd., Hangzhou, China), cholesterol measurement kit (Shanghai Rongsheng Biotech Co., Ltd., Shanghai, China), free fatty acid measurement kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), insulin analysis kit (Shanghai Yuanxiang Medical Instrument Co., Ltd., Shanghai, China). Concanavalin A (ConA), lipopolysaccharides (LPS) were obtained from Sigma; RPMI 1640 medium was obtained from Gibco which was supplemented with HEPES buffer 25 mmol L^{-1}, sodium pyruvate 1 mmol L^{-1}, L-glutamine 2 mmol L^{-1}, penicillin 100 kU L^{-1}, streptomycin 100 mg L^{-1} and 10% new born bovine serum and were adjusted to pH 7.2. All the other biochemicals and chemicals used in the experiment were of analytical grade.

2.4. Induction of experimental type 2 diabetes (T2DM)

C_{57}BL/6J mice at three weeks of age were fed on diets enriched in fat (25%, wt/wt). After exposure to the high-fat diets for three weeks, mice were injected intraperitoneally with a single dose of STZ 100 mg kg^{-1} body weight and kept on the same diet for the next five weeks (Luo et al., 1998). Normal control mice were fed on conventional chow and were injected intraperitoneally with vehicle (0.1 mol/L citric acid, pH 4.4).

2.5. Experimental design

In the experiment a total of 40 C_{57}BL/6J mice were used and were divided into four groups, each containing 10 animals as follows: normal control; T2DM mice model control; two treatment groups (given MT-α-glucan 300, 100 mg kg^{-1}). Mice were given drugs or dissolvent 0.1 ml 10 g^{-1} orally by gavage once a day for eight weeks from three weeks of age.

The effects of administration of MT-α-glucan on T2DM mice were determined by measuring the levels of fasting blood glucose, serum triglycerides, cholesterol, free fatty acid and insulin levels. Moreover, the immune function as the proliferative response and IL-2 production of splenocytes, the proliferative response and IL-1, NO production of macrophages were observed. Moreover, ultrastructural changes of pancreatic β-cells were also observed by electro-microscope.

2.6. Biochemical measurements

Blood samples were obtained from the distal part of the tails of animals after a 4-h fast. Fasting plasma glucose was estimated on 1 d pre-STZ and 1, 2, 3, 4 and 5 weeks post-STZ, which were determined by the glucose oxidase method using a reflective glucometer. At the end of the eight-week treatment mice were deprived of food overnight and killed by decapitation. Serum was separated for the estimation of insulin, triglyceride, cholesterol and free fatty acid. The above biochemical parameters were determined using commercial kits according to the guidelines indicated.

2.7. Proliferative response of splenocytes

The splenocytes suspension (1×10^{10} L^{-1}) was prepared in a general way. Splenocytes suspension 100 μl was seeded on 96-well microtiter plate in the presence of ConA (final concentration 3 mg L^{-1}). The splenocytes were incubated at 37 °C in 5% CO_2 atmosphere for 48 h. The supernatant 150 μl was collected and stored at −20 °C until tested for IL-2 activity. Then RPMI-1640 medium containing 10% fetal calf serum (FCS) 150 μl and MTT (5 mg/ml) 20 μl were added into each well and splenocytes were cultured for another 6 h. Then the supernatant was discarded and DMSO 150 μl were added into each well. After shaking the plate gently, the optical density (OD) values were measured at 570 nm (Song et al., 2007). The results were expressed as means of OD value of triplicate wells.

2.8. IL-2 assay

The supernatant containing IL-2 was diluted by forty times. Activated splenocytes suspension (2×10^9 L^{-1}) 100 μl was seeded on 96-well microtiter plate in the presence of the dilution 100 μl. The splenocytes were incubated at 37 °C in 5% CO_2 atmosphere for 24 h. MTT assay was done according to the methods stated above (Song et al., 2007). The results were expressed as means of OD value of triplicate wells.

2.9. Proliferative response of macrophages and NO, NOS and iNOS assay

Mouse peritoneal macrophages were collected by lavage with cold D-Hank's solution (pH 7.4) and suspended in RPMI-1640 medium containing 10% FCS. Cells suspension (2×10^9 L^{-1}) 1 ml was seeded in a 24-well microtiter plate and were cultured at 37 °C in 5% CO_2 atmosphere for 2 h. After removing the culture supernatant, the adherent cells were washed with 37 °C PBS and were cultured in RPMI-1640 medium containing 10% FCS in the presence of LPS (final concentration 6 mg L^{-1}) at 37 °C in 5% CO_2 atmosphere for 6 h. Then the cells were washed again with PBS and adherent cells were cultured in RPMI-1640 medium containing 10% FCS at 37 °C in 5% CO_2 atmosphere for 42 h. The supernatant in each well was collected and was used for NO, NOS and iNOS assay and IL-1 assay. NO, NOS and iNOS assay were determined by using commercial kits according to the guidelines indicated. Cell activity was assayed by MTT method according to the methods stated above (Andras, Gyongyi, Laszlo, Tamas, & Gyorgy, 2006). The results were expressed as means of OD value of triplicate wells.

Table 1
Effect of MT-α-glucan on serum insulin level and NO production by macrophage in T2DM mice.

Group	Dose (mg kg^{-1})	Insulin (μIU/ml)	NO (μmol/L)	NOS (U/ml)	iNOS (U/ml)
Normal control	–	21.55 ± 5.60**	217.86 ± 64.84**	6.04 ± 0.27**	3.31 ± 0.32**
T2DM model	–	12.74 ± 3.51	501.49 ± 52.65	8.19 ± 0.52	4.07 ± 0.22
MT-α-glucan	300	17.92 ± 5.13*	344.05 ± 69.11**	6.70 ± 0.36**	3.25 ± 0.37**
	100	16.27 ± 3.29*	323.04 ± 71.49**	6.63 ± 0.49**	3.40 ± 0.21**

Data are the mean ± s.d. (n = 10). Analysis of variance followed by the Student–Newman–Keuls test. In each vertical column, *. P < 0.05 and **P < 0.01 compared with the model group.

2.10. IL-1 assay

The supernatant containing IL-1 was diluted by thirty times. Mouse thymocytes were prepared in a general way which was used for measuring IL-1 activity. Thymocytes suspension (2×10^{10} L^{-1}) 50 μl was seeded in a 96-well microtiter plate in the presence of the dilution 100 μl and ConA (final concentration 3 mg L^{-1}). The thymocytes were incubated at 37 °C in a 5% CO_2 incubator for 48 h. Cell activity was assayed by MTT method according to the methods stated above (Song et al., 2007). The results were expressed as means of OD value of triplicate wells.

2.11. Ultrastructural assay

The pancreas specimens were preserved in 4% glutaric dialdehyde for consolidation overnight. Following dehydration by series of alcohol, the specimens for ultrastructural observation were prepared in a routine way and were stained by osmic acid. Then the specimens were observed by transmission electron microscopy (Type H-600-4, HITACHI, Japan) and photographs of pancreatic β-cells were taken (15,000×). The changes of ultrastructural of pancreatic β-cells were assayed.

2.12. Statistical analysis

Data were expressed as means ± s.d. Statistical analysis was evaluated by one-way analysis of variance, followed by the Student–Newman–Keuls test for multiple comparisons, which was used to evaluate the difference between two groups. P < 0.05 was considered significant.

3. Results

3.1. Effect of MT-α-glucan on blood glucose of T2DM mice

Fig. 1 shows the level of fasting plasma glucose in normal, T2DM and experimental groups. The blood glucose of T2DM mice increased significantly. Three weeks after administration of STZ, treatment with MT-α-glucan (300 mg kg^{-1}) decreased the level of blood glucose significantly. Four weeks after administration of STZ, treatment with MT-α-glucan (100 mg kg^{-1}) decreased the level of blood glucose significantly. This suggested that MT-α-glucan has hypoglycemic effect on T2DM mice.

3.2. Effect of MT-α-glucan on serum lipid levels of T2DM mice

The levels of serum cholesterol, triglycerides and free fatty acid are shown in Fig. 2. The levels of serum cholesterol, triglycerides and free fatty acid were significantly higher in T2DM mice as compared with normal control (P < 0.01). Treatment with MT-α-glucan (300, 100 mg kg^{-1}) lowered the serum lipid levels markedly as compared with untreated T2DM mice. This suggested that MT-α-glucan has hypolipocemia effect.

3.3. Effect of MT-α-glucan on serum insulin level of T2DM mice

Table 1 shows the level of serum insulin of normal, T2DM and experimental groups. The level of serum insulin of T2DM mice decreased significantly. Administration of MT-α-glucan (300, 100 mg kg^{-1}) to T2DM mice raised the level of insulin markedly. This suggested that MT-α-glucan has promoting-insulin-secretion effect on T2DM mice.

3.4. Effect of MT-α-glucan on splenocyte proliferative response and IL-2 production of T2DM mice

The proliferative response and IL-2 production of splenocytes induced by ConA are shown in Fig. 3. The levels of splenocyte proliferative response and IL-2 production were significantly lower in T2DM mice as compared with normal control (P < 0.01 or 0.05). Treatment with MT-α-glucan (300, 100 mg kg^{-1}) increased the proliferative response and IL-2 production markedly. This suggested that MT-α-glucan caused proliferation and activation of B cells, as detected by MTT analysis of whole spleen cell lymphocytes. Cytokines secretion from whole spleen cells was investigated, which showed that IL-2 production increased. MT-α-glucan promoted activation and proliferation of splenocytes and consequently to accelerate the generation of IL-2, which is the β-cell protecting

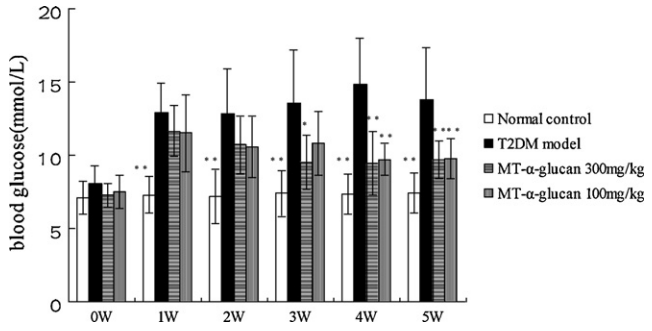

Fig. 1. Effect of MT-α-glucan on fasting plasma glucose in T2DM mice. Data are the mean ± s.d. (n = 10). Analysis of variance followed by the Student–Newman–Keuls test. *P < 0.05 and **P < 0.01 compared with the T2DM model group.

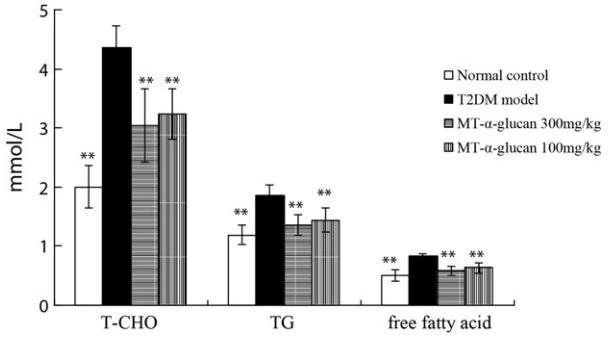

Fig. 2. Effect of MT-α-glucan on serum lipids in T2DM mice. Data are the mean ± s.d. (n = 10). Analysis of variance followed by the Student–Newman–Keuls test. *P < 0.05 and **P < 0.01 compared with the T2DM group.

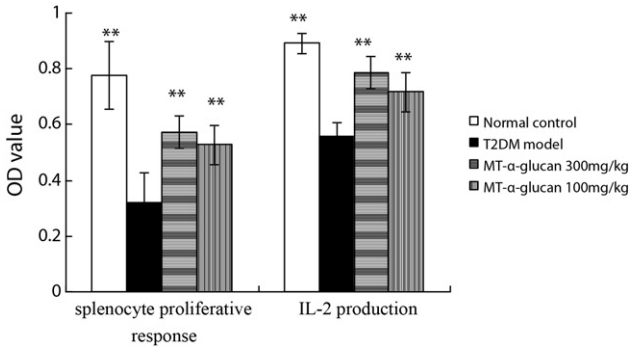

Fig. 3. Effect of MT-α-glucan on splenocyte proliferative response and IL-2 production in T2DM mice. Data are the mean ± s.d. ($n = 10$). Analysis of variance followed by the Student–Newman–Keuls test. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the T2DM group.

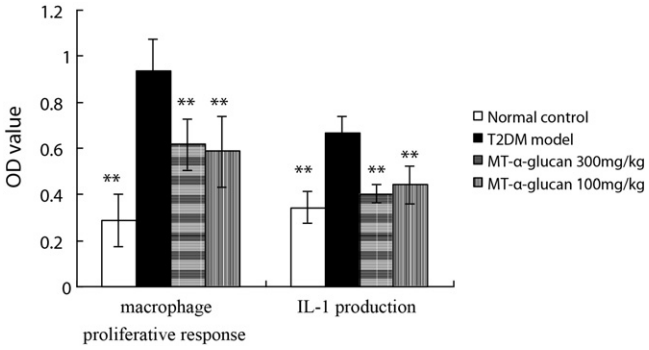

Fig. 4. Effect of MT-α-glucan on macrophage proliferative response and IL-1 production in T2DM mice. Data are the mean ± s.d. ($n = 10$). Analysis of variance followed by the Student–Newman–Keuls test. In each vertical column, $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the T2DM group.

factor. This is accordance with the references previously reported (Dimitry, Natalia, & Pavel, 2008; Thorvaldson, Holstad, & Sandler, 2003).

3.5. Effect of MT-α-glucan on macrophage proliferative response and IL-1 production of T2DM mice

The levels of macrophage proliferative response and IL-1 production are shown in Fig. 4. The levels of macrophage proliferative response and IL-1 production were significantly higher in T2DM mice as compared with normal control ($P < 0.01$). Treatment with MT-α-glucan (300, 100 mg kg^{-1}) decreased the macrophage proliferative response and IL-1 production markedly. This suggested that MT-α-glucan suppressed activation and proliferation of macrophages and consequently to inhibit the generation of IL-1, which is a kind of β-cell destructive factors. This is accordance with the references previously reported (Hirotada et al., 2000; Rosenwasser, 1998).

3.6. Effect of MT-α-glucan on NO production by macrophage in T2DM mice

The levels of NO production by macrophage are shown in Table 1. The levels of NO production and the content of NOS and iNOS were significantly higher in T2DM mice as compared with normal control ($P < 0.01$). Treatment with MT-α-glucan (300, 100 mg kg^{-1}) decreased the NO, NOS and iNOS markedly. This suggested that MT-α-glucan has the effect of suppressing the generation of NO, which is an another β-cell destructive factor. This is accordance with the references previously reported (Hirotada et al., 2000; Philippe, Ulrich, Nenad, Dominik, & Beat, 2003).

3.7. Ultrastructural changes of pancreatic β-cells

Healthy pancreatic β-cells were observed in normal mice. The shape and size of pancreatic β-cells were regular and arranged tightly. The shape of nuclei were oval and located in the middle of the cells (Fig. 5A). As compared with normal control, the shape of pancreatic β-cells in T2DM model mice was markedly destroyed, whose nuclei were distorted and the membrane of secretive granule confused in a large scale, leading to aggregation or disappearance of the secretive granule, which dispersed in the cells leading to leave a large blank area in the cell (Fig. 5B). Treatment with MT-α-glucan markedly attenuate the degree of destruction of pancreatic β-cells. The shape of some pancreatic β-cells restored normal and the shape of nuclei were oval, in which the size of secretive granule were uniform and the membranes of secretive granule were integrate and unconfused (Fig. 5C and D). This suggested that obvious pancreatic β-cell-destroying phenomenon was seen in T2DM model mice, while MT-α-glucan could ameliorate pancreatic β-cell-destruction significantly and had β-cell-protecting effect.

4. Discussion

The purity of the compound MT-α-glucan estimated by HPGPC demonstrated that the molecular was basically homogeneous, which had the molecular weight about 400,000–450,000 Da.

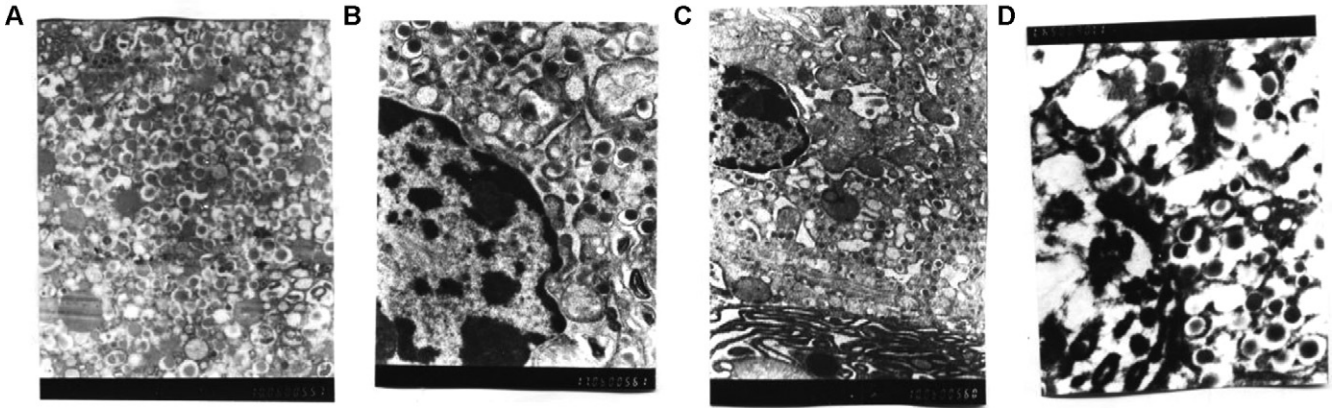

Fig. 5. Effect of MT-α-glucan on ultrastructure of pancreatic β-cells in T2DM mice. (A) Normal control group (original magnification 15,000×); (B) T2DM model group (original magnification 15,000×); (C) MT-α-glucan 300 mg kg^{-1} group (original magnification 15,000×); (D) MT-α-glucan 150 mg kg^{-1} group (original magnification 15,000×).

Results of structure analyses (IR, ^{1}H NMR and ^{13}C NMR) and monosaccharide analysis (TLC and GC) demonstrated that the molecule is an α-glucan (Lei et al., 2007), rather than a β-glucan, molecules hitherto reported to be most commonly produced by this mushroom strain. The β-glucan has been demonstrated that its anti-diabetic activity was not related to the inhibition of glucose absorption at the enteron, but with the process of metabolism of absorbed glucose (Kubo, 1994, 1997).

The metabolic characteristics of fat-fed STZ-injected mice bear a striking resemblance to those of patients with T2DM, providing an easily accessible and inexpensive experimental models used to study T2DM. Hyperglycemia was induced by feeding these mice with diets enriched in fat and injecting with a relatively low-dose of STZ (Luo et al., 1998). In this study, we replicated this kind of T2DM animal model to investigate the hypoglycemic effect of MT-α-glucan.

Results of the present study showed that the mice model showed obvious characteristics of T2DM such as hyperglycemia, hyperlipidemia and relative low serum insulin level. Treatment with MT-α-glucan lowered circulating glucose in a dose-dependent manner significantly in T2DM mice. The most common lipid abnormalities in diabetes are free fatty acids, hypertriglyceridemia and hypercholesterolemia. Treatment with MT-α-glucan significantly decreased these plasma lipid parameters.

Recently, there have been many reports about the deterioration of pancreatic β cell functions in type 2 diabetes (Malek & Mohammad, 2006). In this study, ultrastructural results showed that the shape and nuclei of pancreatic β-cells in T2DM model mice markedly destroyed as compared with normal control. Treatment with MT-α-glucan could restore the shape and nuclei of some of the pancreatic β-cells of T2DM mice markedly. Moreover, administration of MT-α-glucan to T2DM mice restored the decreased level of serum insulin significantly. This suggested that MT-α-glucan has pancreatic-β-cell-protecting effect.

Proliferation and activation of whole spleen cells and of macrophages, combined with immunocytokines secreted from these immunocytes were investigated in this study. Spleen cells are important immune cells and play a pivotal role in immune responses, which are able to produce many kinds of immunomodulatory cytokines, such as IL-2, TNFα, IFNγ, IL-4, IL-5, IL-6 IL-10 and IL-13 (Thorvaldson et al., 2003). Of all these immunocytokines, the quantity of IL-2 is more than other cytokines, and IL-2 is secreted at first stage and then other cytokines are secreted at later stage. IL-2 has critical actions on the immune system and has extensive immuno-enhancing activity. IL-2 remains the most effective cytokine for T lymphocyte cell expansion and regulation of many critical functions in T cell biology. Moreover, IL-2 exerts beneficial effects on other cell types. For example, it can augment B-cell proliferation and increases immunoglobulin synthesis, boost the cytolytic activity of natural killer (NK) cells, and exert actions on neutrophils and monocytes (Ruth, Carlos, & Gershwin, 2008). Accordingly, major IL-2 dysfunctions in both humans and mice are associated with immunodeficiencies. Hence, IL-2 is a kind of the major and well known protective immunocytokines secreted from splenocytes, which is also the pancreatic β-cell protecting factor (Dimitry et al., 2008; Thorvaldson et al., 2003). The results showed that the proliferative response and IL-2 production of splenocytes induced by ConA of T2DM mice were all lower than that of the normal control mice. Treatment with MT-α-glucan could enhance the cellular immunity of T2DM mice, and thus exerting pancreatic-β-cell-protecting effect.

The development of T2DM involves pancreatic β-cells destruction introduced by disrupted immunity (Malek & Mohammad, 2006). Macrophages are considered to be one of the effectors that cause the immunity disruption. IL-1 is primarily produced by cells of the mononuclear phagocytic lineage and has remained one of the most studied inflammatory cytokines to date. The biologic effects and function of IL-1 involve systemic and local effects that have influence on immunologic properties, including T-cell activation, lymphocyte activating factor, increasing antibody production, inducing the synthesis of other cytokines. IL-1 also has profound effects on endothelial, smooth muscle, vascular, and myocardial cells. Because of its many, varied, and multiple biologic effects, IL-1 may play a significant role in the mediation of a number of inflammatory diseases. Hypotension, myocardial suppression, atherosclerotic vascular disease, septic shock, and death all can be physiologic responses to macrophage activation and overwhelming expression of IL-1. Moreover, IL-1 has also been associated with specific cytotoxicity mediated against pancreatic β-islet cells (Hirotada et al., 2000; Rosenwasser, 1998). NO, synthesized and secreted by macrophages, is a β-cell-destroying factor which damages pancreatic β-cells directly. IL-1 is a putative effector molecular in immune cell-mediated β-cell damage which damages pancreatic β-cells indirectly by accelerating NO production (Hirotada et al., 2000). Nitric oxide (NO) is a message for a wide variety of physiological functions which is synthesized through the L-arginine pathway by the enzyme NO synthase (NOS) (Ji, Ji, & Won, 2006). NOS has three different isoforms: endothelial constitutive NOS (ecNOS), neural NOS, and inducible NOS (iNOS). However, NO is produced in a large amount by iNOS. Increased NO generation damages DNA, resulting in destruction of pancreatic β-cells and endothelial cells in diabetic blood vessels, which contributes to the development of diabetes and macrovascular complications. There is some evidence to support the role of nitric oxide (NO) in diabetes development. It has been reported that patients with T2DM had significantly higher circulating NO levels (Philippe et al., 2003). In the present study, proliferation and activation of intraperitoneal macrophages were found to be significantly inhibited by MT-α-glucan, which resulted in the inhibition of NO and IL-1 production from the macrophages. NOS and iNOS were also found to be inhibited. So we assumed that MT-α-glucan could inhibit the β-cell-damaging factors in T2DM mice, and thus exerting pancreatic-β-cell-protecting effect.

5. Conclusion

Our study showed that MT-α-glucan has hypoglycemic and hypolipidemic activity in the mouse model of type 2 diabetes. MT-α-glucan could adjust the immunity which have been disrupted in T2DM, which manifests its benefit effect on protective immune reactions and its effect of decreasing levels of β-cell-destroying immune factors, leading to attenuate the degree of injured β-cells in the pancreatic islets, and consequently exerting pancreatic β-cells protecting effect.

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