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Anti-diabetic effect of an α-glucan from fruit body of maitake (*Grifola frondosa*) on KK-Ay mice

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Abstract

We have evaluated the anti-diabetic effect of a α-glucan (MT-α-glucan) from the fruit body of maitake mushrooms (*Grifola frondosa*) on KK-Ay mice (a kind of genetical type 2 diabetes animal model). The effects of MT-α-glucan (450 or 150 mg kg^{-1}) on diabetic mice were investigated by observing the changes in body weight, the level of fasting plasma glucose, glycosylated serum protein (GSP), hepatic glycogen, serum insulin, triglycerides, cholesterol, free fatty acid, liver superoxide dismutase (SOD), glutathione peroxidase (GSHpx), reduced glutathione (GSH) and malondialdehyde (MDA). Moreover, the binding capacity of insulin receptors on liver crude plasma membranes was assayed and histopathological changes in the pancreas were observed. Treatment with MT-α-glucan significantly decreased the body weight, level of fasting plasma glucose, GSP, serum insulin, triglycerides, cholesterol, free fatty acid and MDA content in livers. Treatment with MT-α-glucan significantly increased the content of hepatic glycogen, GSH and the activity of SOD and GSHpx. Moreover, the insulin binding capacity to liver crude plasma membranes increased and histopathological changes in the pancreas were ameliorated in the treatment group. These data suggest that MT-α-glucan has an anti-diabetic effect on KK-Ay mice, which might be related to its effect on insulin receptors (i.e., increasing insulin sensitivity and ameliorating insulin resistance of peripheral target tissues).

Introduction

Diabetes is classified into two types: type 1 (insulin-dependent diabetes mellitus, IDDM) and type 2 (non-insulin-dependent diabetes mellitus, NIDDM). Type 2 diabetes represents 90% of all cases of diabetes, affecting approximately 3% of the population worldwide and its incidence is increasing every year (Xie & Zhou 2002). Type 2 diabetes is a chronic metabolic disorder characterized by abnormalities in carbohydrate and lipid metabolism, which lead to postprandial and fasting hyperglycaemia, dyslipidaemia, and hyperinsulinaemia (Defronzo et al 1992). Insulin resistance is considered to be the significant pathogenic factor in type 2 diabetes and an obvious target for anti-diabetic medication (Olefsky & Nolan 1995). Because of insulin resistance, current oral therapy using sulfonylurea derivatives, which primarily stimulate insulin secretion from pancreatic β-cells, often fails to achieve the expected level of efficacy. Thus, more effective treatment of type 2 diabetes relies mainly on how to overcome insulin resistance. Pharmaceuticals such as the thiazolidinedione group of drugs have been used to enhance peripheral insulin sensitivity and ameliorate insulin resistance. Although effective in glycaemic control, there may be potential adverse effects, such as hepatotoxicity, cardiomegaly and haemotoxicity (Chakrabarti & Reeba 2002). Thus, safer natural agents available to overcome insulin resistance could be a better treatment for type 2 diabetes.

Some natural products possess the ability to prevent/treat type 2 diabetes and utilization of natural products over a prolonged period should be safer than chemical drugs. Maitake mushrooms (*Grifola frondosa*) belonging to Basidiomycetes in fungi represents 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 an anti-tumour effect (Liu et al 2005), immune stimulatory activity (Inoue et al 2002), an anti-hyperliposis (Kubo & Nanba 1997)

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effect and activity against common and specific infections such as hepatitis (Ooi 1996; Kubo & Nanba 1998) and AIDS/HIV (Nanba et al 2000). Previous studies have shown that ingesting maitake mushrooms, or some of its extracts, influences glucose/lipid metabolism and has anti-diabetic effect (Kubo 1994; Kubo & Nanba 1997). However, 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, designated here as MT-α-glucan, was prepared in our laboratory. This study was therefore designed to determine the anti-diabetic effect of MT-α-glucan in an insulin-resistance animal model of type 2 diabetes (KK-Ay mouse). Moreover, its mechanisms of action were also investigated.

Materials and Methods

Preparation, purity and structure of MT-α-glucan

MT-α-glucan was extracted and purified from the fruit body of maitake (*Grifola frondosa*), manufactured in Zhejiang province and identified by China Pharmaceutical University. Dried powdered fruit bodies of maitake were refluxed with diethylether and ethylalcohol mixture at 70°C for 6 h, centrifuged and the residue collected and extracted by distilled water at 121°C for 30 min. The extract was centrifuged and the supernatant was collected and precipitated in 95% ethyl alcohol at 4°C for 12 h. This was followed by centrifugation and the precipitate was collected and re-dissolved in distilled water, then fractionated by DEAE Sepharose Fast Flow chromatography. The fraction was collected, concentrated and freeze dried, and was designated as MT-α-glucan.

The homogeneity of the compound MT-α-glucan was estimated by high-performance gel-permeation chromatography (HPGPC). HPGPC was performed using an Agilent 1100 series HPLC pump equipped with a Shodex SUGAR KS804 column, using distilled water as a mobile phase (column temperature, 30°C; flow rate, 0.4 mL min^{-1}). The molecular weight of MT-α-glucan was estimated based on a calibration curve made by HPGPC using Dextran T series glucan as standards (Wei & Fang 1989). The structure of the compound was examined using infrared (IR), ^{1}H nuclear magnetic resonance (NMR) spectroscopy and ^{13}C NMR spectroscopy. Sugar composition was determined by thin-layer chromatography (TLC) analysis of the acid hydrolysed product and gas chromatography (GC) analysis of the acetylized product. MT-α-glucan was dissolved in 1% sodium carboxymethylcellulose (CMC-Na) and diluted to the concentration needed.

Animals

Healthy female spontaneously diabetic mice (KK-Ay), 40–45 g, were obtained from Shanghai Experimental Animal Center of Academia Sinica (Shanghai, China). Healthy C_{57}BL/6J mice, 20–25 g, were obtained from the 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% r.h.). Before and during the experiment, mice were fed with a 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 China Pharmaceutical University (Nanjing, China), and all mice used in the experiment received humane care.

Main reagents

Rosiglitazone was from Zhejiang Wanma Pharmaceutical Co., Ltd (Hangzhou, China). Bovine monocomponent insulin and bovine serum albumin (BSA) were from Sigma. ^{125}I-Labelled insulin (5000 GBq g^{-1}) was from China atom energy institute (Beijing, China). The glucometer was from Beijing Yicheng Bio-electron Technology Co., Ltd (Beijing, China). Various measuring kits were used during the study: 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, glycosylated serum protein (GSP) measurement kit, glycogen measurement kit, superoxide dismutase (SOD) measurement kit, glutathione peroxidase (GSHpx), reduced glutathione (GSH) and malondialdehyde (MDA) measurement kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Insulin measuring kit was from China Atom Energy Institute (Beijing, China). All the other biochemicals and chemicals used in the experiment were of analytical grade.

Experimental design

In the experiment a total of 40 mice (32 KK-Ay mice, 8 C_{57}BL/6J mice) were used and were divided into five groups, each containing 8 mice as follows: C_{57} BL/6J mice as normal control; KK-Ay mice as model control; three treatment groups (given MT-α-glucan 450, 150 mg kg^{-1} or rosiglitazone 1 mg kg^{-1}). Mice were given drugs or solvent 0.1 mL/10 g orally by gavage twice a day (every 12 h) for two weeks. The effects of administration of MT-α-glucan on KK-Ay mice were determined by measuring the levels of fasting blood glucose, GSP, liver glycogen and serum lipid levels. Initial and final changes in body weight and feed consumption were recorded also. In the liver tissues the levels of anti-oxidative capacity were measured. The insulin receptor on the membrane of liver cells was also investigated. Histopathological changes in the pancreas were observed under a light microscope.

Biochemical measurements

Blood samples were obtained from the tails of mice after a 4-h fast. Fasting plasma glucose was estimated on days 0, 4, 8 and 14 of MT-α-glucan administration, and was determined by the glucose oxidase method using a reflective glucometer. At the end of the two-week treatment mice were deprived of food overnight and killed by decapitation. Serum was separated for the estimation of GSP, insulin, triglyceride,

cholesterol and free fatty acid. The liver homogenate was used for estimation of the content of hepatic glycogen and levels of SOD, GSHpx, GSH and MDA. The above biochemical parameters were determined using commercial kits according to the guidelines indicated.

Insulin binding to liver crude plasma membranes

Crude plasma membranes, as the target of insulin binding sites, were prepared for binding assay from the liver according to the method previously described (Ray 1970; Nishimura et al 1988). Membranes were suspended in 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM $MgCl_2$ and 2% bovine serum albumin and incubated with 0.25 ng mL^{-1} ^{125}I-labelled, and varying concentrations of unlabelled, insulin (0–100000 ng mL^{-1}) in a total volume of 400 μL. The amount of protein in each tube was 100 μg. The incubation was performed for 22 h at 4°C. After incubation, 1 mL ice-cold buffer containing 15.8% PEG was added, and the tube immediately centrifuged at 3500 rev min^{-1} for 10 min. The supernatant was discarded, and the pellet was washed with 1 mL of the same buffer. The radioactivity in each tube was determined by a gamma counter. Competitive binding to insulin receptors of ^{125}I-insulin and unlabelled insulin was assayed. Protein content was determined using commercial kits according to the guidelines indicated. Data were analysed by Scatchard plot analysis (Bonen & Hood 1984; Nishimura et al 1989). The affinity constants (KD_1, KD_2) and binding sites (RT_1, RT_2) were calculated.

Histopathological assay

The pancreas specimens were preserved in 10% neutral formalin and were processed for paraffin embedding. Following standard micro-techniques, 5-μm sections of pancreas were stained with alum haematoxylin and eosin for microscopic observation of histopathological changes. The size of islet in each examined section was estimated in a blinded manner using a microscope attached to a camera and ImageJ 1.37 software (http://rsb.info.nih.gov/ij/). Islet area was determined in 10 consecutive sections of the pancreas from each mouse and 80 islets were measured per group. The value each area (mm^2) was obtained directly from the photographs (×200).

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.

Results

Purity and structure of MT-α-glucan

Figure 1 shows a relatively symmetric peak on HPGPC, indicating a basically homogeneous fraction. The retention time

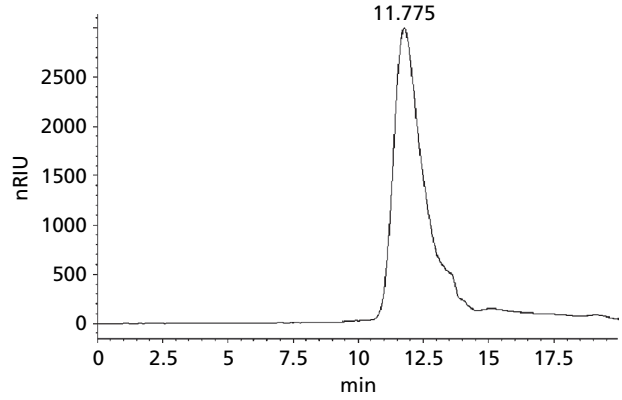

Figure 1 HPGPC chromatography of MT-α-glucan.

was 11.775 min. The molecular weight of MT-α-glucan was about 400000–450000 Da estimated by HPGPC. The anomeric configuration was revealed by the absorption at 920 and 858 cm^{-1} in the IR spectrum. The chemical shifts of the H-1 signals in the ^{1}H NMR spectra was > δ 5.0 and the chemical shifts of the C-1 signals in the ^{13}C NMR spectra was δ 97–101. So the spectra obtained by IR, ^{1}H NMR and ^{13}C NMR analyses of the compound revealed that it contained an α-glucosidic bond. Results of TLC analysis of the acid hydrolysed product and GC analysis of the acetylized product demonstrated that the polysaccharide was composed of D-glucose, as the only carbohydrate present in the polysaccharide chain. Thus, the main compound in the extract was demonstrated to be an α-glucan.

Effect of MT-α-glucan on the body weight and feed consumption of KK-Ay mice

Table 1 shows the effect of MT-α-glucan on body weight and feed consumption of KK-Ay mice. The body weight of KK-Ay mice was significantly higher than that of the normal control ($P < 0.01$). MT-α-glucan (450 mg kg^{-1}) markedly decreased the body weight of KK-Ay mice ($P < 0.01$). Rosiglitazone (1 mg kg^{-1}) had no such effect. The amount of food intake did not change obviously after one week of treatment with MT-α-glucan, while after 2 weeks of treatment, feed consumption decreased markedly.

Effect of MT-α-glucan on blood glucose, GSP, liver glycogen and serum insulin level in KK-Ay mice

Tables 2 and 3 show the level of fasting plasma glucose, GSP, liver glycogen and serum insulin in normal, KK-Ay and experimental groups. KK-Ay mice showed a significant increase in blood glucose and in the level of GSP and serum insulin. A significant decrease in liver glycogen was observed. Administration of MT-α-glucan to KK-Ay mice restored the level of blood glucose, GSP, liver glycogen and serum insulin significantly. Rosiglitazone (1 mg kg^{-1}) had the same effect. This suggested that MT-α-glucan has a hypoglycaemic effect, promoting glycogen synthesis and hypoinsulinaemia, in KK-Ay mice.

Table 1 Effect of MT-α-glucan on body weight of KK-Ay mice

Group	Dose (mg kg^{-1})	Body weight (g)		
		Week 0	Week 1	Week 2
C_{57} BL/6J	—	21.18 ± 0.96** (2.96)	21.54 ± 0.75** (3.20)	22.15 ± 0.97** (3.28)
KK-Ay	—	41.40 ± 2.13 (3.89)	43.16 ± 2.05 (4.11)	44.68 ± 1.32 (4.20)
MT-α-glucan	450	40.64 ± 2.85 (4.05)	40.41 ± 2.96 (3.94)	39.56 ± 2.81** (3.38)
	150	41.39 ± 2.52 (3.92)	42.14 ± 2.64 (3.87)	42.24 ± 2.91 (3.35)
Rosiglitazone	1	41.28 ± 2.89 (4.21)	43.03 ± 2.54 (3.90)	43.36 ± 2.37 (3.39)

Data are the mean ± s.d., n = 8. In each vertical column, *$P < 0.05$ and **$P < 0.01$, compared with the KK-Ay group (analysis of variance followed by the Student–Newman–Keuls test). Data in brackets represent feed consumption (g/day/mouse).

Table 2 Effect of MT-α-glucan on fasting plasma glucose of KK-Ay mice

Group	Dose (mg kg^{-1})	Fasting plasma glucose (mmol L^{-1})			
		Day 0	Day 4	Day 8	Day 14
C_{57} BL/6J	—	7.05 ± 0.87**	7.11 ± 0.82**	7.03 ± 0.96**	7.18 ± 0.66**
KK-Ay	—	10.29 ± 1.15	10.04 ± 1.13	10.18 ± 1.27	10.60 ± 0.97
MT-α-glucan	450	10.23 ± 1.07	7.70 ± 1.73*	7.93 ± 1.55*	7.90 ± 1.16**
	150	10.48 ± 1.52	8.85 ± 1.48	8.88 ± 1.47	9.25 ± 1.28*
Rosiglitazone	1	10.20 ± 0.96	8.35 ± 1.32*	8.21 ± 1.17*	8.06 ± 0.93**

Data are the mean ± s.d., n = 8. In each vertical column, *$P < 0.05$ and **$P < 0.01$, compared with the KK-Ay group (analysis of variance followed by the Student–Newman–Keuls test).

Table 3 Effect of MT-α-glucan on GSP, liver glycogen and serum insulin level in KK-Ay mice

Group	Dose (mg kg^{-1})	GSP (mmol L^{-1})	Liver glycogen (mg g^{-1})	Serum insulin (μU mL^{-1})
C_{57} BL/6J	—	2.86 ± 0.32**	14.32 ± 1.11**	20.95 ± 4.94**
KK-Ay	—	5.32 ± 0.49	6.33 ± 1.29	104.26 ± 25.38
MT-α-glucan	450	4.08 ± 0.43**	13.89 ± 1.96**	56.75 ± 10.61**
	150	4.50 ± 0.33**	10.48 ± 1.55**	66.74 ± 7.96**
Rosiglitazone	1	4.34 ± 0.41**	12.31 ± 2.19**	57.16 ± 14.40**

Data are the mean ± s.d., n = 8. In each vertical column, *$P < 0.05$ and **$P < 0.01$, compared with the KK-Ay group (analysis of variance followed by the Student–Newman–Keuls test).

Effect of MT-α-glucan on serum lipid levels in KK-Ay mice

The levels of serum cholesterol, triglycerides and free fatty acid are shown in Table 4. The levels of serum cholesterol, triglycerides and free fatty acid were significantly higher in KK-Ay mice as compared with normal control ($P < 0.01$). Treatment with MT-α-glucan (450, 150 mg kg^{-1}) lowered the serum lipid levels markedly as compared with untreated KK-Ay mice. Rosiglitazone (1 mg kg^{-1}) did not have any significant effect on lipid parameters in this model. This suggested that MT-α-glucan has a hypolipidaemic effect.

Effect of MT-α-glucan on levels of liver SOD, GSHpx, GSH and MDA in KK-Ay mice

The changes in the levels of SOD, GSHpx, GSH and MDA in the liver of normal mice, KK-Ay mice and experimental groups are shown in Table 5. Results showed that the activity of SOD, GSHpx and the content of GSH in the livers of KK-Ay mice markedly decreased compared with the normal control ($P < 0.01$). Treatment with MT-α-glucan (450, 150 mg kg^{-1}) and rosiglitazone (1 mg kg^{-1}) markedly increased SOD, GSHpx activity and GSH content. MDA content was markedly increased in KK-Ay mice compared with the normal control ($P < 0.01$). MT-α-glucan (450, 150 mg kg^{-1}) and

Table 4 Effect of MT-α-glucan on serum lipids in KK-Ay mice

Group	Dose (mg kg^{-1})	Cholesterol (mmol L^{-1})	Triglycerides (mmol L^{-1})	Free fatty acid (μmol L^{-1})
C_{57} BL/6J	—	2.27 ± 0.65**	1.67 ± 0.19**	530.00 ± 88.24**
KK-Ay	—	5.66 ± 1.01	2.40 ± 0.21	986.36 ± 109.05
MT-α-glucan	450	3.79 ± 1.05**	1.75 ± 0.27**	794.50 ± 69.27**
	150	4.34 ± 0.44*	1.90 ± 0.40*	805.45 ± 40.77**
Rosiglitazone	1	5.12 ± 0.61	2.31 ± 0.22	934.09 ± 87.44

Data are the mean ± s.d., n = 8. In each vertical column, *$P < 0.05$ and **$P < 0.01$, compared with the KK-Ay group (analysis of variance followed by the Student–Newman–Keuls test).

Table 5 Effect of MT-α-glucan on liver SOD, GSHpx, GSH and MDA in KK-Ay mice

Group	Dose (mg kg^{-1})	SOD (U (mg protein)$^{-1}$)	GSHpx (U (mg protein)$^{-1}$))	GSH (mg (g protein)$^{-1}$)	MDA (nmol (mg protein)$^{-1}$)
C_{57} BL/6J	—	82.47 ± 3.29**	199.82 ± 12.99**	59.53 ± 8.07**	1.066 ± 0.074**
KK-Ay	—	63.68 ± 6.46	168.90 ± 16.05	43.26 ± 5.43	1.526 ± 0.115
MT-α-glucan	450	76.89 ± 3.45**	196.18 ± 11.07**	55.61 ± 6.10**	1.225 ± 0.098**
	150	70.56 ± 3.91*	182.34 ± 11.98	51.84 ± 5.62*	1.240 ± 0.134**
Rosiglitazone	1	70.55 ± 4.35*	181.04 ± 9.27	51.82 ± 6.06*	1.161 ± 0.103**

Data are the mean ± s.d., n = 8. In each vertical column, *$P < 0.05$ and **$P < 0.01$, compared with the KK-Ay group (analysis of variance followed by the Student–Newman–Keuls test).

rosiglitazone (1 mg kg^{-1}) markedly decreased MDA. The effect of MT-α-glucan was more prominent than that of rosiglitazone. This suggested that MT-α-glucan has antioxidant effect.

Effect of MT-α-glucan on insulin receptors on the membrane of liver in KK-Ay mice

We analysed the data by Scatchard plots. The binding curves of ^{125}I-insulin to crude plasma membranes of livers resembled a rectangle hyperbola. This suggests there are two kinds of types of receptor on the membranes, with different specific affinities, which can be referred as high- and low-affinity receptors. The values of affinity constants (KD_1, KD_2) and binding sites (RT_1, RT_2) were also calculated. KD_1 and KD_2 are, respectively, the high- and low-affinity dissociation constants. RT_1 and RT_2 are binding sites that are indicative of the numbers of high- and low-affinity receptor sites, respectively. Table 6 shows the changes in the KD_1, KD_2, RT_1 and RT_2 values in normal mice, KK-Ay mice and experimental groups. The values of RT_1 and RT_2 were lower in KK-Ay mice than in normal control mice ($P < 0.01$), while the values of KD_1 and KD_2 did not change significantly. Treatment with MT-α-glucan (450, 150 mg kg^{-1}) markedly increased the value of RT_2. Rosiglitazone (1 mg kg^{-1}) had the same effect. This suggested that MT-α-glucan could increase the number of low affinity receptors, but has no effect on the affinity of the two kinds of receptors and the number of high affinity receptors. Therefore, the increase in binding was mainly due to an increase in the receptor number, especially low-affinity receptor number, rather than affinity.

Histopathological changes

Healthy acinar cells and ducts in exocrine pancreas and pancreatic islets in endocrine pancreas were observed in normal

Table 6 Effect of MT-α-glucan on insulin binding to liver crude plasma membranes in KK-Ay mice

Group	Dose (mg kg^{-1})	KD_1 (nM^{-1})	KD_2 (nM^{-1})	RT_1 (fmol (mg protein)$^{-1}$)	RT_2 (fmol (mg protein)$^{-1}$)
C_{57} BL/6J	—	0.09380 ± 0.04139	6.0853 ± 1.8331	43.43 ± 17.04*	1238.63 ± 168.15**
KK-Ay	—	0.09584 ± 0.07748	7.2127 ± 1.9782	18.95 ± 15.33	646.99 ± 81.80
MT-α-glucan	450	0.1022 ± 0.07118	7.6991 ± 2.2849	39.99 ± 26.11	988.90 ± 96.61**
	150	0.08325 ± 0.06406	9.1974 ± 4.4133	27.24 ± 17.57	795.17 ± 106.20*
Rosiglitazone	1	0.06026 ± 0.03876	7.1698 ± 0.7758	19.71 ± 10.38	1030.32 ± 70.70**

Data are the mean ± s.d., n = 8. In each vertical column, *$P < 0.05$ and **$P < 0.01$, compared with the KK-Ay group (analysis of variance followed by the Student–Newman–Keuls test).

mice (Figure 2A). The size of pancreatic islets in KK-Ay mice markedly increased as compared with normal control (5.94 ± 1.80 vs $17.11 \pm 2.26\,mm^2$, for C_{57}BL/6J and KK-Ay mice, respectively; $P < 0.01$; Figure 2B). Treatment with MT-α-glucan and rosiglitazone markedly reduced the size of islets as compared with the islet size in untreated KK-Ay mice (12.68 ± 2.55 vs $14.51 \pm 2.52\,mm^2$, for MT-α-glucan and rosiglitazone treatment groups, respectively; $P < 0.01$; Figure 2C, D). This suggested that obvious insulin resistance was seen in KK-Ay mice, while MT-α-glucan could ameliorate insulin resistance significantly.

Discussion

The procedure of preparation of MT-α-glucan was studied in our laboratory. The purity of the compound estimated by HPGPC demonstrated that the molecule was basically homogeneous, and had a molecular weight of about 400 000–450 000 Da. Results of structure analyses (IR, ^{1}H NMR, ^{13}C NMR) and monosaccharide analysis (TLC, GC) demonstrated that the molecule is an α-glucan rather than a β-glucan, hitherto reported to be most commonly produced by this mushroom strain (Kubo 1994; Kubo & Nanba 1997). So this molecule is unique to maitake among mushrooms according to references we have searched.

Previous studies on the anti-diabetic effects of maitake and its extract have been reported. It has been demonstrated that anti-diabetic activity was present in a fraction of peptidoglycan (sugar–protein, 65:35) in the extract of maitake. The sugar had a β-main chain, and its anti-diabetic activity was not related to the inhibition of glucose absorption in the enteron, but with the process of metabolism of absorbed glucose (Kubo 1994; Kubo & Nanba 1997). Other pharmacological effects of polysaccharide from maitake have been reported and most of them focused on its anti-tumour effect (Liu et al 2005). Another five groups of polysaccharides having diverse molecular mass (470–1650 kDa) were prepared from mycelium extract and submerged culture of *Grifola frondosa*, which had antioxidant and free radical scavenging activity (Lee et al 2003).

Some other mushroom polysaccharides have been shown to regulate glucose metabolism. It was reported that extractable material in mannentake (*Ganoderma lucidum*), one of the Basidiomycetes, has blood glucose-lowering activity when administrated intraperitoneally. However, this mushroom did not show stronger activity than maitake and its active ingredients and mechanism was not elucidated (Kubo 1994). The hypoglycaemic activity of orally ingested fruiting bodies, submerged culture biomass or the acidic polysaccharide glucuronoxylomannan of *Tremella mesenterica*, an edible jelly mushroom, has also been reported (Lo et al 2006).

The KK-Ay mouse is spontaneously diabetic by means of transplantation of the diabetic gene into the C_{57}BL/6J mouse, which exhibit profound obesity, hyperglycaemia and hyperinsulinaemia. It is an ideal animal model for studying the aetiopathogenesis of insulin resistance and type 2 diabetes (Ojamaa et al 1998). In this study, we employed KK-Ay mice

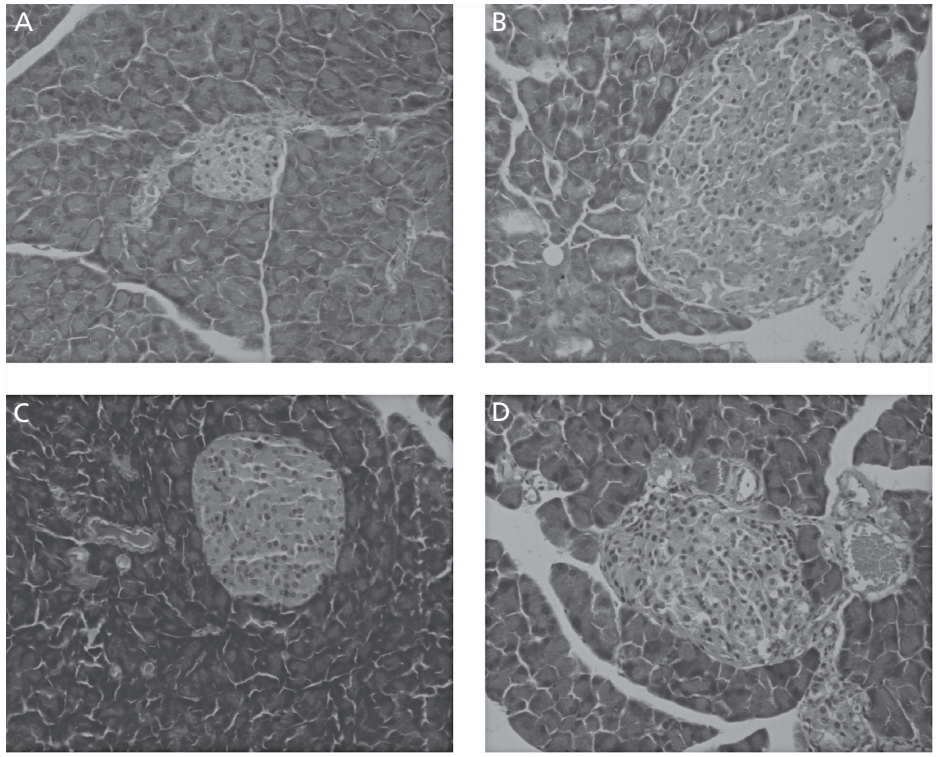

Figure 2 Pancreatic histological findings of MT-α-glucan against KK-Ay diabetic mice. A. Normal group (haematoxylin and eosin (H-E) stain; original magnification × 200). B. KK-Ay group (H-E stain; original magnification × 200). C. MT-α-glucan 450 mg kg^{-1} group (H-E stain; original magnification × 200). D. Rosiglitazone 1 mg kg^{-1} group (H-E stain; original magnification × 200).

as a type 2 diabetes animal model to investigate the anti-diabetic effect of MT-α-glucan. We also used the C_{57}BL/6J mouse as the normal control for it serves as the recipient of the diabetic gene.

The weight of the KK-Ay mouse increases very rapidly as the animal grows because it has hereditary obesity. Treatment with MT-α-glucan significantly reduced body weight in KK-Ay mice. After 2 weeks of treatment with MT-α-glucan, feed consumption decreased markedly, while after 4 days' treatment there was a significant decrease in glucose level. This indicates that the changes in blood glucose occurred before the changes in feed consumption. Therefore, it is considered that the glucose-lowering effect of the compound is not dependent on feed consumption change, but is related to other anti-diabetic mechanisms, which are discussed below.

Treatment with MT-α-glucan significantly lowered circulating glucose and insulin concentrations in a dose-dependent manner in KK-Ay mice. The combination of decreased glucose and insulin level is highly suggestive that the anti-diabetic effect of MT-α-glucan is closely associated with its increasing peripheral insulin sensitivity and its effect on insulin receptors. GSP was found to be increased in patients with diabetes mellitus and the amount of increase was found to be directly proportional to the fasting plasma glucose level (Jackson et al 1979). Administration of MT-α-glucan for two weeks decreased GSP content in KK-Ay mice. This could have been due to the improved glycaemic control produced by MT-α-glucan.

One of the factors for elevating blood glucose may be the reduction of glycogen synthesis and the acceleration of glycogen disassimilation in the liver (Huang & Wu 2005). Results showed that treatment with MT-α-glucan could increase liver glycogen synthesis significantly. This suggested that MT-α-glucan is involved in promoting the synthesis of glycogen and, thus, inhibits elevation of blood glucose. Further investigations on the activity of the hepatic glycogen synthetase will be done.

The most common lipid abnormalities in diabetes are free fatty acids, hypertriglyceridaemia and hypercholesterolaemia. Treatment with MT-α-glucan significantly decreased these plasma lipid parameters. Interestingly, our study showed that rosiglitazone, one of the thiazolidinediones, which are synthetic sensitizers, did not show any significant effect on lipid parameters in this model. Therefore, MT-α-glucan had body-weight-lowering and hypolipidaemic activity, which may in some respects be better than the standard synthetic marketed insulin sensitizing, thiazolidinediones.

In addition, the antioxidant activity of MT-α-glucan could be elucidated by formal study on redox markers, such as SOD, GSHpx, GSH and MDA. Oxidative stress has been shown to play a role in the causation of type 2 diabetes and, as such, antioxidants may have a role in the alleviation of diabetes (Baynes 1991). Treatment with MT-α-glucan for two weeks could lower the content of MDA and restore the activity of SOD and GSHpx and the GSH content of the livers in KK-Ay mice. This suggested that MT-α-glucan has anti-oxidative activity, which may be partly responsible for its anti-diabetic effects.

The above data suggests that MT-α-glucan's anti-diabetic action could be associated with insulin receptors and, thus, functional tests of insulin receptors were conducted. Insulin receptors are divided into two types: high-affinity and low-affinity receptors. The number of low-affinity receptors is greater than that of high-affinity receptors and also, the insulin-binding capacity is stronger and action time is longer for the former than the latter. Therefore, the number and affinity of low-affinity receptors are better indicators of the potency and store of insulin receptors (He et al 1997). A reverse relationship between insulin concentration and number of receptors often is observed to show adaptability (i.e., the receptor number decreases under high insulin concentrations while the number increases under insulin shortage) (Kahn & Crettaz 1980). The value of KD is in inverse proportion to affinity. The less the KD value, the higher the capacity of insulin binding to receptor. The value of RT is indicative of the number of insulin receptors. The more the RT value, the higher the insulin receptor number. Results of this study showed decreased insulin binding to the liver membrane in KK-Ay mice. This could be explained by down-regulation of the insulin receptor under the hyperinsulinaemic state. MT-α-glucan could remarkably raise the number of low-affinity insulin receptors but had no effect on the affinity of the insulin receptor. These suggested that the anti-diabetic activity of MT-α-glucan was mainly due to its increasing the number of low-affinity insulin receptors and insulin-binding activity and, thus, enhancing peripheral insulin sensitivity and reducing the insulin resistance.

Histopathological studies showed that the size of pancreatic islets in KK-Ay mice are larger than the normal control, which is the result of the compensation for peripheral insulin resistance. This finding is in accordance with previous reports (Xu et al 1991). Treatment with MT-α-glucan could reduce the size of pancreatic islets markedly. This confirmed the fact that MT-α-glucan could ameliorate peripheral insulin resistance and increase insulin sensitivity.

Further detailed studies on the chemical nature of MT-α-glucan and its effects on insulin receptors are in progress. Moreover, pharmacokinetic research on this molecule, such as absorption, distribution, metabolism and excretion, will be further elucidated in future studies.

Conclusion

Our study showed that MT-α-glucan has anti-diabetic activity, which is effective in KK-Ay mice, a model of type 2 diabetes. These actions may be exerted by amelioration of peripheral insulin resistance and enhancement of insulin sensitivity. We conclude that MT-α-glucan is a potential anti-diabetic agent that is effective and safe.

References

Baynes, J. W. (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes* **40**: 405–412

Bonen, A., Hood, D. A. (1984) Insulin binding in human skeletal muscle. *Biochim. Biophys. Acta* **801**: 171–176

Chakrabarti, R., Reeba K. (2002) Antidiabetic and hypolipidemic activity of Helicteres isora in animal models. *J. Ethnopharmacol*. **81**: 343–349

Defronzo, R. A., Bondonna, R. C., Ferranini, E. (1992) Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* **15**: 318–337

He, R. H., Gui, Y. G., Zhu, X. S. (1997) Insulin receptors activity research in diabetes mellitus. *Chin. J. Intern Med*. **36**: 607–609

Huang, F.-J., Wu, W.-T. (2005) Antidiabetic effect of a new peptide from *Squalus mitsukurii* liver (S-8300) in streptozocine-induced diabetic mice. *J. Pharm. Pharmacol*. **57**: 1575–1580

Inoue, A., Kodoma, N., Nanba, N. (2002) Effect of Maitake(*Grifola frondosa*) D-fraction on the control of the T lymph node Th-1/Th-2 proportion. *Chem. Pharm. Bull*. **25**: 536–540

Jackson, R. L., Hess, R. L., England, J. D. (1979) Haemoglobin A1c values in children with overt diabetes maintained in varying degree of control. *Diabetes Care* **2**: 391–395

Kahn, C. R., Crettaz, M. (1980) Role of insulin receptors in insulin-resistance states. *Metabolism* 29: 455–457

Kubo, K. (1994) Anti-diabetic activity present in the fruit body of *Grifola frondosa* (maitake). *Biol. Pharm. Bull*. **17**: 1106–1110

Kubo, K., Nanba, H. (1997) Anti-hyperliposis effect of maitake fruit body *(Grifola frondosa). Biol. Pharm. Bull*. **20**: 781–785

Kubo, K., Nanba, H. (1998) Modification of cellular immune responses in experimental autoimmune hepatitis in mice by Maitake *(Grifola frondosa). Mycoscience* **39**: 351–360

Lee, B.-C., Bae, J.-T., Pyo, H.-B., Choe, T.-B., Kim, S.-W., Hwang, H.-J., Yun, J.-W. (2003) Biological activities of the polysaccharides produced from submerged culture of the edible Basidiomycete *Grifola frondosa. Enzyme Microb. Technol*. **32**: 574–581

Liu, X.-W., Chen, X.-D, Wu, W.-T. (2005) Separation, purification and characterization of polysaccharide from *Grifola frondosa. Pharm. Biotechnol*. **3**: 175–178

Lo, H.-C., Tsai, F.-A., Wasser, S.-P., Yang, J.-G., Huang B.-M. (2006) Effects of ingested fruiting bodies, submerged culture biomass, and acidic polysaccharide glucuronoxylomannan of *Tremella mesenterica* Retz.: Fr. on glycemic responses in normal and diabetic rats. *Life Sci*. **78**: 1957–1966

Nanba, H., Kodama, N., Schar, D., Turner D. (2000) Effects of Maitake (*Grifola frondosa*) glucan in HIV-infected patients. *Mycoscience* **41**: 293–295

Nishimura, H., Kuzuya, H., Okamoto, M. (1988) Change of insulin action with aging in conscious rats determined by euglycemic clamp. *Am. J. Physiol*. **254**: E92–E98

Nishimura, H., Kuzuya, H., Okamoto, M. (1989) Postreceptor defect in insulin action in streptozocine-induced diabetic rats. *Am. J. Physiol*. **256**: E624–E630

Ojamaa, K., Suzuki, Y., Hatanaka, Y. (1998) Insulin resistance associated with decreased levels of insulin-receptor mRNA. *J. Clin. Endocrinol. Metabol*. **80**: 1214–1220

Olefsky, J. M., Nolan, J. (1995) Insulin resistance and non-insulin dependent diabetes mellitus: cellular and molecular mechanisms. *Am. J. Clin. Nutr*. **61**: 980–-986S

Ooi, V. (1996) Hepatoprotective effect of some edible mushroom. *Phytother. Res*. **6**: 536–538

Ray, T. K. (1970) A modified method for the isolation of the plasma membrane from rat liver. *Biochem. Biophys. Acta* **196**: 1–9

Wei, Y.-A., Fang, J.-N. (1989) Determination of purity and molecular weight of polysaccharides by high performance gel permeation chromatography. *Acta Pharm. Sin*. **24**: 532–536

Xie, J.-T., Zhou, Y.-P. (2002) Ginseng berry reduces blood glucose and body weight in *db/db* mice. *Phytomedicine* **9**: 254–258

Xu, S.-Y., Bian, R.-L., Chen, X. (1991) *Pharmacology experimental methodology*. People's Hygiene Press, Beijing, pp 1278–1279