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Original Articles

Effects of *Palmijihwangtang* (PMT) and Exercise on Glucose Metabolism in Myocardium Cell Membrane and Pancreas β-Cell of Zucker Diabetic Fatty Rats

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Objective: Non-insulin Dependent Diabetes Mellitus (NIDDM) is characterized by insulin resistance, which affects the glucose transportation inside the cell. The purpose of this study was to find out how *Palmijihwangtang* (PMT) and exercise influence the glucose transport metabolism in the organ muscles of ZDF (zucker diabetic fatty) rat with insulin resistance.

Methods: Using three male normal zucker rats and twelve male obese rats, they were divided into a normal lean group (N=3), obese control group (N=3), obese exercises group (N=3), obese medication group (N=3), obese exercise and medication group (N=3). Treadmill exercise were repeated with 27m/min speed for an hour a day, five days a week, for 8 weeks. And 20ßȼ of PMT was orally administered twice a day for 8 weeks, after that a period blood sample was exsanguinated by heart perforation and was analyzed.

Results: The body weight of the OM and OEM group showed a significant decrease among all the obese groups. The blood insulin level increased significantly of all groups in comparison with the N group. All of the OE, OM and the OEM groups showed a significant decrease of insulin level compared with the OC group; especially the OEM group demonstrated the most among obese groups.

Regarding GLUT-4 level, OEM was the unique group showed a significant increase among all the obese groups. The VAMP-2 level in myocardium cell membrane was increased significantly at OC group in comparison with the N group, whereas the OEM group only showed significant decrease of it. In addition, the VAMP-2 level in pancreas β-cell was significantly decreased at all the obese groups in comparison with the N group. Only the OEM group showed significant increase among all the obese groups.

Conclusion: *Palmijihwangtang* (PMT) and exercise could effectively promote the insulin metabolism in pancreas β-cells and activate the glucose transport process in myocardium cell membrane by lowering the insulin resistance of ZDF rats.

Key Words: Non-insulin Dependent Diabetes Mellitus (NIDDM), insulin resistance, GLUT-4, VAMP-2, *Palmijihwangtang* (PMT)

Introduction

Diabetes mellitus (DM) is a metabolic disorder in which the ability to oxidize carbohydrates is more or less completely lost, due to faulty pancreatic activity, especially of the islets of Langerhans, and consequent disturbance of normal insulin mechanism[1]. This

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produces hyperglycemia and is classified into insulin-dependent type and insulin-independent type[2].

There are VAMP-2 (vesicle associated membrane protein), syntaxin-1 which are involved to secrete insulin in pancreas b-cells. And also there are GLUT-4(glucose transporter-4), VAMP-2, syntaxin-4 which are involved in glucose metabolism in myocardium cell membrane. They increase the glucose transportation into the cell and proliferate the intracellular process of glucose metabolism by insulin activity. GLUT-4 proteins, the glucose transporter affected by insulin or muscle contractive stimulation such as exercise, are usually existed as vesicle in the cell. Once affected by insulin or other stimulation they move to cell membrane[3-4]. There are some reports about vSNARE(VAMP-2, cellubrevin), tSNARE and SNAPs which are related to the affection described above, and they all are involved on glucose transportation[5-9].

Currently Palmijihwangtang (PMT)[10] often used in the management of DM has been one of the most frequently studied medications regarding the renal function[11,12], immune reaction[13], hyperglycemic rat[14] or type I DM model[15]. The studies, however, did not focus on insulin secretion in obese insulin-independent type DM and glucose transportation on cell membrane.

Therefore, using ZDF (zucker diabetic fatty) rats those are animal model of obese insulin-independent type DM, PMT and exercise were tested to check the function of pancreas β-cell and the glucose metabolism in myocardium cell membrane, which is one of the target organs of insulin.

Materials and Methods

1. Materials

Palmijihwangtang (PMT) materials were purchased from the Kangnam Oriental Hospital of Dongguk University (Seoul, Korea) and the traditional methods for the clinical preparation of herbal treatment were employed. The composition of PMT formation is described in Table 1.

4-week-old male ZDF rats were used for this study after one week of acclimation. Totally 15 rats were divided into 5 groups. They are Normal lean group (N, n=3), Obese(fa/fa) control group (OC, n=3), Obese(fa/fa) exercise group (OE, n=3), Obese (fa/fa) medication group (OM, n=3) and Obese(fa/fa) exercise and medication group (OEM, n=3).

OM, and OEM group were administered 20㎖ of PMT b.i.d.(09:00 & 18:00) for 8 weeks. OE and OEM group were exercised on treadmill at the speed of 27m/min[16] one hour everyday for 8 weeks.

2. Removal of pancreas and myocardium

After PMT medication and exercise for 8 weeks hearts and pancreases were removed from the rats under pentobarbital(50㎎/㎏) anesthesia. 2.5g volume of each sample and lysing buffer(0.1M K phosphate, 0.5M EDTA, pH 7.2) were homogenized (homogenizer, PYREX Corning, USA) in the ices for western blot analysis. Each were centrifuged(microcentrifuge, VS-15000CF, Vision Scientific Co. Ltd) at 15,000 rpm for 20 min, and then the supernatant was separated. Total protein concentration was quantified using BSA(bovine serum albumin, 570nm) by Bradford's[17] method.

3. SDS-PAGE

10% separating gel(30% acrylamide: 1.5M Tris, pH 8.8, 10% SDS, TEMED, 10% ammonium persulfate) and 5% stacking gel(30% acrylamide: bisacrylamide, 1M Tris, pH 6.8, 10% SDS, TEMED, 10% ammonium persulfate) were used for gel electrophoresis. The supernatant made above with SDS loading buffer were mixed completely and then boiled at 100℃ for 10 min. Each samples were subjected to gel electrophoresis with prepared standard marker(SDS-PAGE, sodium dodecyl

sulfate-polyacrylamide gel) at 80V for about 2 hr until they were reached to the bottom.

4. Western blot and image analysis

Electrophoresed proteins were transferred onto PVDF membrane (Millipore, Japan) using a blotting apparatus(Mini trans-blot module, BioRad). The membrane was blocked with 2% skim milk(in TNT: 10mM Tris-base pH 8.0, 150mM Nacl, 0.05% Tween-20) on rocker platform for 1 hr and then rinsed 3 times with TNT solution for 10 min each followed membrane transfer.

Molecular weights of the protein were determined prestained molecular weight standards. The lanes were scanned by densitometer(Sharp jx-330) and the intensity of the protein bands were analyzed using image software (ImageMaster ver. 3.0, Biotech pharmacia).

5. Hematological examination

5㎖ of sample blood was collected from each heart by heparin treated disposable syringe. 3㎖ blood was analyzed to check glucose level by standard method using UV-spectrophotometer 2600 (Gilford, USA) and the rest of blood was for insulin level by HPLC.

6. Statistical analysis

The results of the study were analyzed with SPSS/PC+Version 8.0 program. Data from several groups were examined using analysis of variance(one-way ANOVA) and applied Tukey. Significance levels to accept the hypothesis were set at $p<.05$.

Results

1. Changes of body weight

The average body weight(BW) of 5 groups of 4-week-old zucker rats those are N, OC, OE, OM and OEM group was about 186g not much different. However the average BW of 12-week-old rats showed some variation. BW of the OC, OE, OM and OEM groups was very significantly increased ($p<.01$) than that of N group. And also OM and OEM groups showed the significant decrease ($p<.05$) compared with OC group (Fig. 1).

2. Variation of blood insulin

As shown in Fig. 2 blood insulin in OC group was very significantly increased

($p<.01$) than N's blood insulin. And all of 3 other trial groups showed the significant decrease ($p<.05$) compared with OC group. OEM group demonstrated the most remarkable change as far as insulin level was concerned.

3. Changes of GLUT-4 protein concentration in myocardium membrane

GLUT-4 protein which is involved in glucose transport at cell membrane was checked using a densitometer. Each DU (densitometry unit) of the 5 groups were observed and calculated to the % of normal lean value. All the trial groups of OE, OM and OEM group showed the significant increase ($p<.05$), and OEM was the only group which was significantly increased ($p<.05$) compared with the OC group (Fig. 3 and 4).

4. Changes of VAMP-2 contents in myocardium and pancreas

VAMP-2 which affects the GLUT-4 transport to cell membrane was observed in myocardium cell membrane to be increased significantly ($p<.05$) in OC group, however, OE, OM and OEM groups were not. And compared with OC group, OEM showed the significant

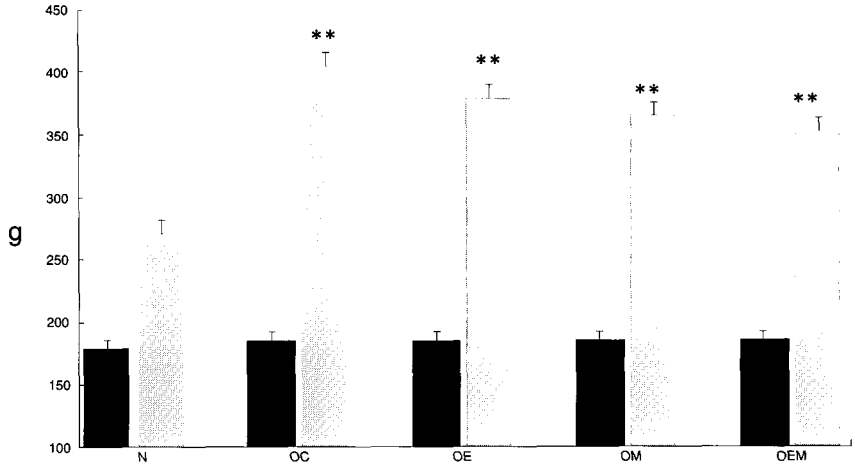

Fig. 1. Changes of Body weight (black; 4weeks, line; 12weeks)
* : statistical significance as compared with N group (** : $p<.01$)
† : statistical significance as compared with OC group († : $p<.05$)
N : normal lean, OC : obese control, OE : obese exercise
OM : obese medication, OEM : obese exercise & medication

decrease ($p<.05$) whereas OE and OM groups did not.

In addition, VAMP-2 in pancreas β-cell was increased significantly ($p<.05$) in all the OC, OE, OM and OEM groups. Compared with OC group, OEM only showed the significant decrease ($p<.05$) (Fig. 5, 6 and 7).

Discussion

Oriental medicines, which have been developed over some 3,000 years are known to have low toxicity and may offer advantages over the longer term over synthetic agent medication[18]. Palmijihwangtang (PMT), often used in the management of DM, is one of those oriental medications. Numerous oriental medical treatises reported that PMT was effective in treating Sogal[10,19] which is characterized by polydipsia, polyuria and polyphagia (All of them are a triad of DM presently). Some clinical studies for PMT were undertaken regarding renal function[11,12], immune reaction[13], hyperglycemic rat[14] or type I DM model[15]. However, there is no scientific evidence of its usefulness for insulin secretion in obese insulin-independent type DM(NIDDM) and glucose transportation on cell membrane.

I have selected the zucker diabetic fatty rats (ZDF rat) as the experimental animals, because they are known as model rats of NIDDM. ZDF rats tend to eat food too much and become severely obese. Once they get to the 4-week-old level, they show not just accumulation of spare fatty tissue in subcutaneous and peritoneal area, but mild hyperglycemia and hyperinsulinemia are also found[2]. Obesity is often accompanied with the carbohydrate metabolic disorder or NIDDM, and the more the body weight increases the worse the diabetic symptoms become. Many articles have reported about the study between obesity and NIDDM[20,21]. It was also

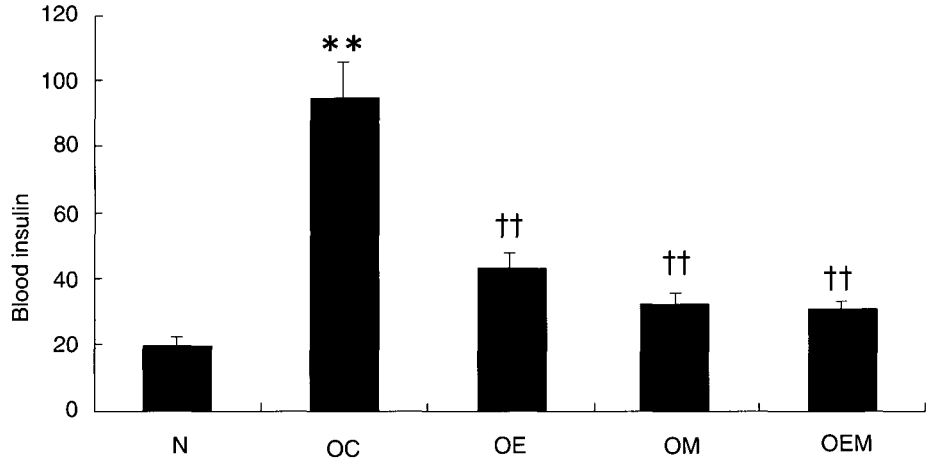

Fig. 2. Blood insulin concentration among groups
* : statistical significance as compared with N group (** : $p<.01$)
† : statistical significance as compared with OC group († : $p<.05$)
N : normal lean, OC : obese control, OE : obese exercise
OM : obese medication, OEM : obese exercise & medication

demonstrated that obesity is the most frequent correlating factor following the wide scope of epidemiologic study[22]. That is, a person who had genetic NIDDM dispositions with 5-7kg gaining of body weight he/she would get DM. On the other hand, a NIDDM patient who already got symptoms lost body weight by 5-7kg, that definitely improved his/her blood glucose level[1,23].

In this study eight weeks after treatment of PMT or exercise, the OM and OEM groups showed a significant decrease ($p<.05$) compared with the OC group whereas the OE group did not. That suggests that PMT reduced the anxiety of polydipsia and polyphagia and resulted in some decreasing body weight. It is well known that one of the reasons for obesity comes from DM. In this case, PMT could improve diabetic obese patients.

Exercise helps glucose enter into the cell with minimum rate of insulin secretion. After the treatment in this study, blood insulin in 3 trial groups (OM, OE and OEM) were decreased significantly ($p<.05$) compared with OC group. This strongly suggests the reduction of insulin resistance which follows that only little amount of insulin works enough to activate the glucose metabolism in the cell. We can easily find the insulin resistance in obesity or NIDDM, in which insulin target cells such as myocardium, skeletal muscle or fatty cell are defective in insulin activity. Exercise and/or PMT helps even little amount of insulin could work enough to activate the blood glucose. In addition, the OEM group showed a greater decrease than the OE/OM group, which means that PMT and exercise affected mutually and powerfully.

Insulin resistance is also decreased when the GLUT-4 protein, which is involved in glucose transport at cell membrane, increased to make the glucose metabolism active. Obese people are often related to higher insulin levels than average; then, they increase the insulin

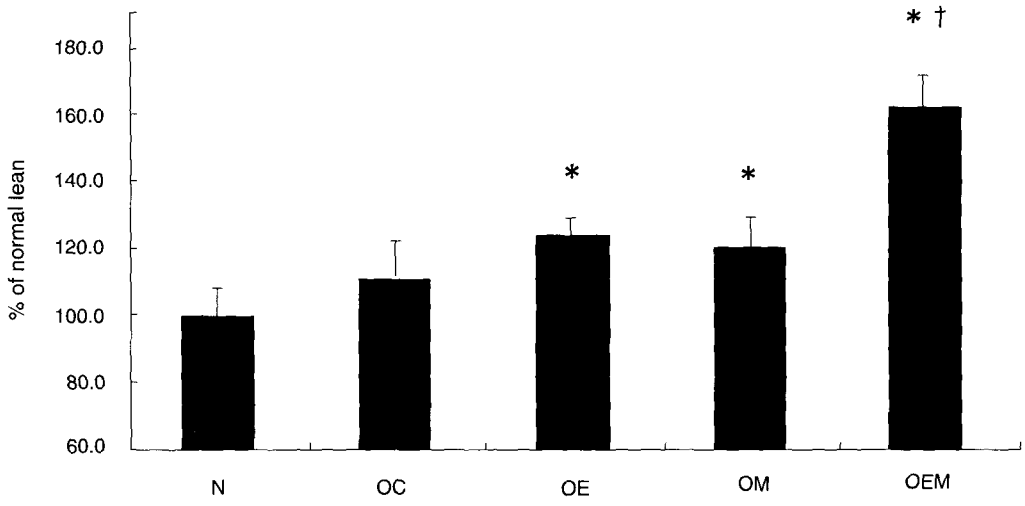

Fig. 3. GLUT-4 protein concentration in myocardium among groups
* : statistical significance as compared with N group(* : $p<.05$)
† : statistical significance as compared with OC group(† : $p<.05$)
N : normal lean, OC : obese control, OE : obese exercise
OM : obese medication, OEM : obese exercise & medication

Fig. 4. Representative Western blot of GLUT-4 protein in myocardium of ZDF Rat.

resistance. So even though they have enough insulin concentration, insulin does not work appropriately. When the GLUT-4 level, however, is elevated, it activates the glucose metabolism, which suppresses the insulin resistance. Currently there have been 5 kinds of glucose transporting protein found in vertebrates, and 2 of them (GLUT-1 and GLUT-4) are in muscle cells. In this study, exercise or PMT administration made the GLUT-4 concentration increase($p<.05$). Therefore, it followed reduction of insulin resistance and activated the glucose metabolism. Again the OEM group showed more increase than either the OE or OM groups by itself and was the only significant increase group($p<.05$) compared with the OC group.

One of the major physiological functions of insulin is to regulate the glucose absorption in myocardium, skeletal muscle or fatty cells for maintaining the glucose homeostasis[24-28]. Myocardial VAMP-2 in the OEM group was significantly increased($p<.05$) compared with the OC group. Many factors such as vSNARE(VAMP-2, cellubrevin), tSNARE and SNAPs are involved to transform the GLUT-4 protein to move

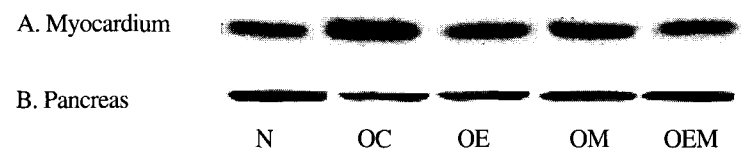

Fig. 5. *Representative Western blot of VAMP-2 protein in myocardium and pancreas of ZDF Rat.*

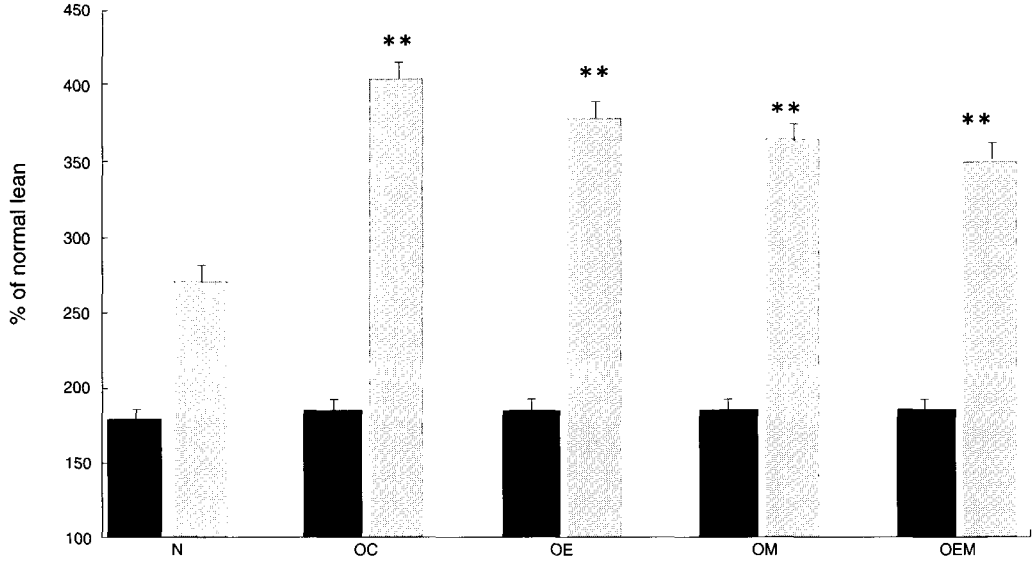

Fig. 6. VAMP-2 expression in myocardium among groups
* : statistical significance as compared with N group(* : $p<.05$)
† : statistical significance as compared with OC group(† : $p<.05$)

into the cell[6-8]. They are conjugated with GLUT-4 protein to make synthetic compounds and affect the glucose transport[3,4]. And also in the pancreas β-cell the OEM group only showed the significant decrease($p<.05$) of VAMP-2. These results ensure that insulin secretion function of pancreas β-cell is lowered. As blood insulin was decreasing compared with OC, VAMP-2 in the OEM group was increased significantly($p<.05$) to secrete more insulin. That suggests PMT administration helps to activate the decreased function of insulin secretion.

From this study I can suggest that PMT administration or exercise could cause loss of body weight in ZDF rats while reducing insulin resistance

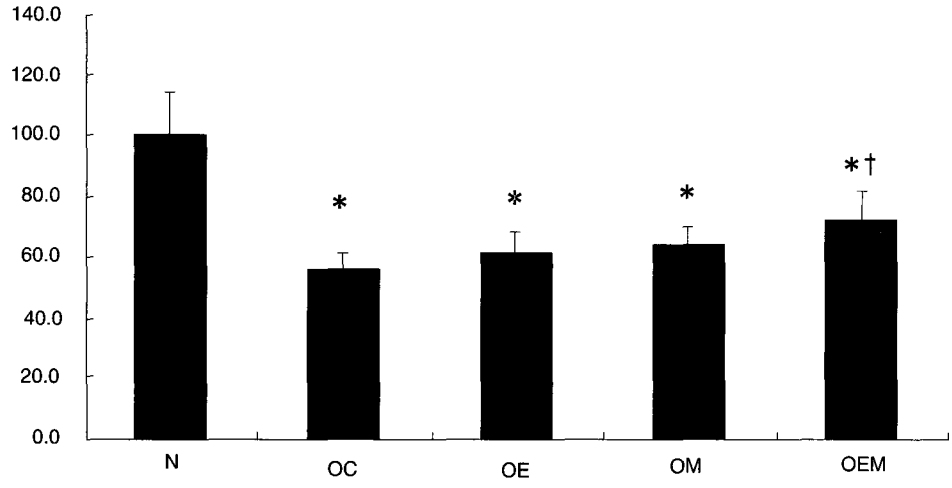

Fig. 7. VAMP-2 expression in pancreas among groups
* : statistical significance as compared with N group(* : $p<.05$)
† : statistical significance as compared with OC group(† : $p<.05$)

blood insulin levels became lowered and blood glucose absorbed faster. In addition, as pancreas β-cell recovered the insulin secretion function VAMP-2 concentration in pancreas increased. GLUT-4 was the most activated substance among the glucose transporting proteins when PMT and exercise worked on myocardium cell membranes, which are one of the target organs of insulin. Further investigation should be planned to examine the other herbal medications for the glucose metabolism on cell membrane.

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