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Morbidity of Diabetes Mellitus in Cynomolgus Monkeys

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Abstract

Object: To determine the morbidity of diabetes mellitus in cynomolgus monkey by performing the Oral Glucose Tolerance Test (OGTT).

Method: OGTTs were performed in 600 male cynomolgus monkeys, aging 4-18 years. The values of plasma glucose (PG) and insulin (Ins) were detected.

Result: Significant differences were found in the values of PG and Ins among groups with different ages. The values of PG of 4 pathological courses of DM were: Normal glucose tolerance (NGT) group: Fasting plasma glucose (FPG) < 6.10 mmol/L and 2hPG < 7.8 mmol/L; Impaired fasting glucose (IFG) group: 6.10 mmol/L ≤ FPG < 7.0 mmol/L and 2hPG < 7.8 mmol/L; Impaired glucose tolerance (IGT) group: FPG < 7.0 mmol/L and 7.8 mmol/L ≤ 2hPG < 11.1 mmol/L; diabetes mellitus (DM) group: FPG ≥ 7.0 mmol/L or 2hPG ≥ 11.1 mmol/L. The morbidity of diabetes mellitus in the tested cynomolgus monkeys was 11.5%.

Conclusion: Our findings pave the ways for a wide range of screening of DM in cynomolgus monkeys. Meanwhile, we also provide the reference to preclinically test new antidiabetic drugs in cynomolgus monkeys.

Keywords: Cynomolgus monkeys; Spontaneous diabetes mellitus; OGTT; Plasma glucose

Introduction

Diabetes mellitus (DM) is a metabolism syndrome with abnormally high blood glucose levels [1]. Type 2 DM (T2DM) is a complex metabolism syndrome and the major symptom is the increased plasma glucose with the impaired response to insulin and islet β cells dysfunction. In current researches on T2DM, rodent models are often used [2]. However, the genetic relationship of human and rodent is far and life of rodent is short. Therefore, rodents are not suitable for studying DM mechanism and complication. Cynomolgus monkeys are similar to humans in phylogeny, omnivorous diet, structures of organization, physiological and metabolism functions [3]. Both humans and cynomolgus monkeys are susceptible to the development of diabetes and dyslipidemia in middle age.

The oral glucose tolerance test (OGTT), also referred to as the glucose tolerance test, is a commonly accepted method to measure the body's ability to metabolize glucose [4]. The test can be used to diagnose diabetes, gestational diabetes or prediabetes. Moreover, OGTT is also useful in assessing insulin release and insulin sensitivity [5]. Therefore, although OGTT is more time-consuming and complicated, the test is better able to diagnose high blood glucose after a glucose challenge than the fasting blood glucose test. In the present study, we screened spontaneous diabetes mellitus of cynomolgus monkeys by OGTT.

Materials and Methods

Experimental animal

Six hundred male cynomolgus monkeys, aging from 4 to 18 years, were obtained from Guangxi Weimei Biotechnology Co., Ltd. (Nanning, China). Animals were roomed at 20°C-26°C, with the relative humidity 60%-80% and the time of illumination: 12h/12h. All experiments performed in this study were approved by the Committee on the Use of Live Animals in Teaching and Research of Yunnan Minzu University.

Animal groups

According to experiences, monkeys get sexual maturity and start mating at about 3.5 years old. Thus the monkeys were divided into the following 4 groups: Young (age ≤ 8); Adult (9 ≤ age ≤ 11); Middle-aged (12 ≤ age ≤ 14) and Aged (age ≥ 15).

OGTT

When carrying out the OGTT, these monkeys were kept fasting overnight. Blood sample were collected from the forearm vein into tubes containing EDTA- K_2 (0 min). After oral glucose (40%, 4 g/kg, 10 ml/kg) adopt same method was performed to collect blood sample at 5, 15, 30, 60, 120 and 180 min, followed by jiggling the tubes about 1 min, and a rapid centrifugation at 4000 rpm for 10 min at 4°C to collect plasma samples. The concentrations of plasma insulin and glucose were determined by using enzyme linked immunosorbent assay (ELISA) kits (Siemens Healthcare Diagnostics Inc).

According to the screening method of spontaneous DM from the domestic and foreign studies, as well as the diagnostic criteria of WHO and American Diabetes Association on DM, we divided these monkeys

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into 4 groups: normal glucose tolerance (NGT) group, impaired fasting glucose (IFG) group, impaired glucose tolerance (IGT) group and DM group. Meanwhile, the cut-off points of different DM process were determined.

Statistical analysis

All data were expressed as means ± S.E.M. Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL). Comparison between two groups was analyzed using Student's *t*-test. Comparison among three or more groups was analyzed using one-way ANOVA; $P < 0.05$ was considered statistically significant.

Results

According to the relevant literatures the fasting plasma glucose (FPG) of cynomolgus monkeys over 6.00 mmol/L are likely DM, and fasting insulin (Fins) ≥ 60.00 mIU/L belongs to hyperinsulinism [6]. There were 36 monkeys FPG ≥ 6.00 mmol/L, and 174 monkeys Fins ≥ 60.00 mIU/L. Thus, we concluded these 174 monkeys had insulin resistance. All these 210 monkeys were selected to perform OGTT.

Values of FPG and Ins in groups with different ages

Significant differences were observed in FPG and Ins among monkeys with different ages (Table 1).

FPG and 2hPG cut-points

The glucose cut-off point of different courses of DM in cynomolgus monkeys were determined referring to the Human's Standard of DM provided by American Diabetes Association [1]. Based on the cut-off points, we divided these monkeys to following four groups: NGT (FPG < 6.1 mmol/L, 2hPG < 7.8 mmol/L) group, IFG (6.1 ≤ PG < 7.0 mmol/L, 2hPG < 7.8 mmol/L) group, IGT (FPG < 7.0 mmol/L, 7.8 ≤ 2hPG < 11.1 mmol/L) group, and DM (FPG ≥ 7.0 mmol/L or 2hPG ≥ 11.1 mmol/L) group. Table 2 displayed the different values of FPG and Ins in various developmental stages of DM in cynomolgus monkey.

Time-dependent changes of PG and Ins

As shown in Figures 1 and 2, the changes of PG in each group were raised to the crest values at 60 min, and recovered to close to the basal level 3 h later. The levels of PG in IFG, IGT and DM groups were all significantly higher than that of NGT group. In NGT and DM groups, the levels of Ins reached the highest points at 120 min, showed the similar changes.

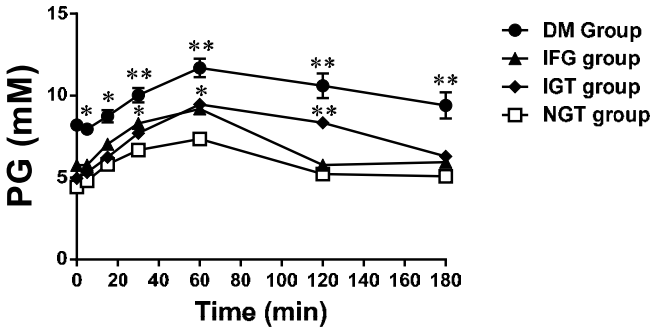

Figure 1: Time-dependent changes of PG. Values are means ± S.E.M. PG: plasma glucose; DM: diabetes mellitus; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; NGT: normal glucose tolerance. $^{*}P < 0.05$, compared with NGT group; $^{**}P < 0.01$, compared with NGT group.

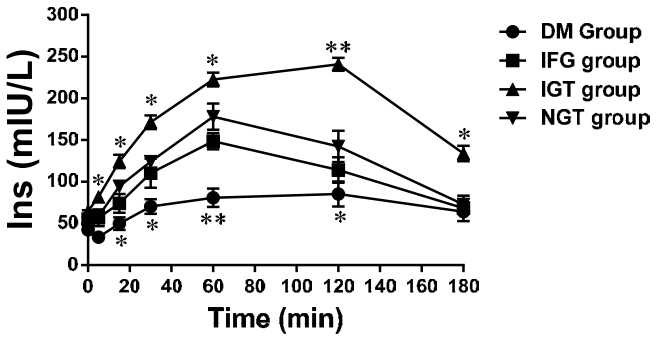

Figure 2: Time-dependent changes of Ins. Values are means ± S.E.M. Ins: insulin; DM: diabetes mellitus; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; NGT: normal glucose tolerance. $^{*}P < 0.05$, compared with NGT group; $^{**}P < 0.01$, compared with NGT group.

Age	Total	FPG (mmol/L)	Ins (mmol/L)
≤ 8	113	3.97 ± 0.18*	172.12 ± 26.80*
9-11	253	3.26 ± 0.08**	171.60 ± 38.10
12-14	171	3.52 ± 0.09#	182.61 ±16.00##
≥ 15	63	4.06 ± 0.21	282.00 ± 31.00***

*: $P \leq 0.01$, compared with Age ≥ 15 group; **: $P \leq 0.01$, compared with Age ≤ 8 group; #: $P \leq 0.01$, compared with 9 ≤ Age ≤ 11 group; ##: $P \leq 0.01$, compared with Age ≤ 8 group; ***: $P \leq 0.01$, compared with 12 ≤ Age ≤ 14 group.

Table 1: Values of FPG, and Ins in different groups with different ages.

Group	Total	PG (mmol/L)		FPG (mmol/L)	Ins (mIU/L)
		FPG	2hPG		
NGT	98	PG < 5.43	PG < 7.40	4.06 ± 0.05	41.10 ± 3.37
IFG	17	5.43 ≤ PG < 6.20	PG < 7.40	4.78 ± 0.08*#	59.72 ± 9.43*#
IGT	26	PG < 6.20	7.40 ≤ PG < 9.20	5.10 ± 0.03*#	56.22 ± 9.78*#
DM	69	PG ≥ 6.20	PG ≥ 9.20	4.06 ± 0.05	41.32 ± 6.00

*: $P \leq 0.05$, compared with NGT group; #: $P \leq 0.05$, compared with DM group.

Table 2: FPG and Ins in different developmental stages of DM.

Discussion

Diabetes is a serious chronic disease to human's health. Some animal models of diabetes have the same characteristics as human diabetes in many aspects, such as the developmental and various pathological changes. Appropriate animal models of diabetes play an important role in the study of the pathogenesis, complications, treatment, and prevention of diabetes. Nonhuman primates can serve as an excellent model for DM because of their close phylogenetic relationship to human [7,8]. Furthermore, the lives of nonhuman primates are long enough so that we have enough time to sufficiently observe and research the disorder of glycometabolism, lipid and protein metabolisms, as well as the pathological changes in diabetic animal [9,10].

In the present study we regard FPG ≥ 6.20 mmol/L as diagnostic criteria of DM, which is close to the reported standard criterion of DM in cynomolgus monkey. Meanwhile, in the present study, we observed a rate of DM (11.5% of total monkeys), which is much higher than the prevalence of DM in our country (3%) [11,12]. This result might because the proportion of the tested cynomolgus monkeys with middle and old age, which belongs to the DM pathogenic high risk group [13,14], is comparatively large.

OGTT is a good method for examining the function of glycoregulation since it can not only detect PG concentration at every

time point to reflect the characteristic of glucose tolerance, but also can detect insulin concentration at every time point to reflect the function of islets β cell [15]. By performing OGTT, we got the time-dependent curves of plasma glucose and insulin. Thus, we established a spontaneous diabetic cynomolgus monkey model, and determined the cut-off points of plasma glucose at every course of DM. Our findings pave the ways for a wide range of screening of DM in cynomolgus monkeys.

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