Short Communication

The Regulatory Effect of D-Alloxan on Glucose Metabolism and Lipid Metabolism in Diabetic Mice

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To investigate the regulatory effect of d-alloxan on glucose metabolism and lipid metabolism in diabetic mice. Twenty five mice were divided into control group, glucose group, fructose group, d-alloxan group and cellulose group. The results showed that the blood glucose of mice in each group reached the peak at 90 min and the blood glucose concentration of d-alloxan group was significantly lower than that of other groups ($p<0.05$). The blood glucose concentration of fructose group was significantly higher than that of other groups ($p<0.05$). The blood glucose concentration before and after meal in d-alloxan group was significantly lower than that in other groups ($p<0.05$), the liver glycogen concentration before and after meal was significantly higher than that in other groups ($p<0.05$), the insulin level was higher than that in other groups, but the difference was not significant ($p>0.05$), the muscle glycogen concentration before and after meal was not significant ($p>0.05$). The results showed that the triglyceride concentration of d-alloxan group was significantly lower than that of other groups ($p<0.05$). While it was not significantly lower than that of control group ($p>0.05$) and there was no significant difference in total cholesterol level between groups ($p>0.05$). The free fatty acid concentration in glucose group, fructose group and d-alloxan group was lower than that in control group and cellulose group ($p<0.05$). Compared with the control group, the low density lipoprotein concentration of the other four groups was significantly lower ($p<0.05$) and the low density lipoprotein concentration of the d-alloxan group was significantly lower than that of the other groups ($p<0.05$) and there was no significant difference between them ($p>0.05$). The results showed that the content of succinate dehydrogenase in the blood and tissues of d-alloxan group was significantly higher than that of other groups ($p<0.05$) and the level of hepatic lipase in d-alloxan group was higher than that of other groups, but there was no significant difference ($p>0.05$). Alloxan can effectively regulate blood glucose concentration, inhibit postprandial blood glucose rise, improve insulin level and liver glycogen level, but has no significant effect on muscle glycogen. It can be used as an ideal substitute of sucrose once the disorder of lipid metabolism is improved, the ability of fat metabolism is increased and antioxidation function is improved.

Key words: D-alloxan, diabetes, glucose, metabolism, lipid, antioxidant

Diabetes is a chronic endocrine and metabolic disorder caused by insulin resistance and insufficient insulin secretion[1]. In recent years, with the rapid development of economy, people's living standards have been continuously improved and their eating habits have changed, resulting in the increasing incidence of chronic diseases. Relevant studies show that the incidence of diabetes is increasing year by year and it has become a public health problem endangering human health[2]. Obesity caused by high calorie diet is the main factor leading to diabetes. Therefore, low calorie and nutritious food is favored in the market[3]. Sucrose, a traditional sweetener, can increase fat production and insulin secretion[4]. In 2011, Food and Drug Administration (FDA) approved d-alloxan as a food additive. D-alloxan is a new functional monosaccharide with special health functions. Its sweetness is 70 % of sucrose and its heat absorption rate is 0.3 % of sucrose. It does not produce energy after eating and produces very little heat[5]. The research on d-alloxan in China started late, but its biological function and clinical research have been gradually deepened. This study will explore the regulatory effect of d-alloxan on glucose metabolism and lipid

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metabolism in diabetes mice. It is reported as follows; there were 25 male C57 mice, which were 8-9 w old with a body weight of 36-49 g. The experimental animals were provided by Shanghai Shrek experimental animals Co., Ltd. with license no. SCXK (Shanghai) 2007-0005. The feeding temperature was 22.65°±2.49° and the relative humidity was 56.97 %±15.39 %. The day and night were alternated for 12 h and the food was freely available. This study conforms to the ethical standards formulated by the experimental animal ethics committee of our hospital. After 1 w of adaptive feeding, the mice were randomly divided into 5 groups; control group, glucose group, fructose group, d-alloxan group and cellulose group. The control group was fed with AIN-76A experimental animal feeding formula (casein 200 g, methionine 3 g, corn starch 550 g, sucrose 100 g, cellulose 50 kg, soybean oil 50 g, inorganic salt mixture 35 g, vitamin 10 g, choline chloride 2 g and antioxidant 0.01 g). The amount for the glucose group was increased by 50 g glucose on the basis of the control group. For fructose group, 50 g fructose was added to the control group; for the d-alloxan group, add 50 g alodonose on the basis of the control group and 50 g cellulose on the basis of the cellulose group. Each group was fed once in the morning and evening, with free diet and killed after 8 w of continuous feeding. The glucose group, fructose group, d-alloxan group and cellulose group were fed with high-fat diet on the above basis for 28 consecutive d. The fasting blood glucose content in the tail vein of mice in each group was measured with a total glucose meter. When the fasting blood glucose was <16.7 mmol/l, the model was successful. After 7 w of feeding, the blood glucose was measured. At 6:00 AM and 8:00 AM, blood was taken from the tail artery of mice and the blood glucose was measured. Mice in each group were gavaged with 10 % glucose solution and 2 kg glucose. After intragastric administration, blood was taken from the caudal artery of small rats at 0, 30, 60, 90 and 120 min respectively, and blood glucose was measured. After being fed for 8 w, the mice were killed after anesthesia. The chest cavity of the mice was opened, the heart blood was taken, and other tissues and organs were removed. The mice were washed with normal saline and weighed. After quick freezing with liquid nitrogen, they were placed in an environment of -80° for storage. The whole blood of mice was collected and centrifuged at the speed of 3000 r/min using a low-temperature high-speed centrifuge. The obtained serum was stored at -80°. Glucose concentration, insulin concentration and glycogen content in serum and tissues were detected by kit. Experimental animals, grouping and establishment of animal model of diabetes are consistent with the above. The mice were forbidden to eat for 12 h before being killed. After anesthesia, the chest cavity of the mice was opened, the heart blood was taken, and the liver and other tissues were removed. After washing with normal saline, weigh, fix part of the liver lobules and fat in 4 % neutral formaldehyde, take part of the liver lobules for preservation and Ribonucleic Acid (RNA) preservation solution, and place the remaining tissues in the environment of -80° after quick freezing with liquid nitrogen for standby. The whole blood of mice was collected and centrifuged at the speed of 3000 r/min using a low-temperature high-speed centrifuge. The obtained serum was stored at -80°. Take the liver tissue preserved in RNA preservation solution, add 1 ml TRIzol™ solution and extract the total RNA in liver tissue by TRIzol™ one-step method. The RNA was concentrated by isopropanol precipitation method, and NucleoSpin RNA clean-up KITS was used for column purification of total RNA, quantitative determination by spectrophotometer, and further detection by formaldehyde denatured agarose gel electrophoresis. The levels of Total Cholesterol (TC), Triglyceride (TG), Free Fatty Acid (FFA) and Low Density Lipoprotein-Cholesterol (LDL-C) were detected by blood biochemical index kit. The levels of Succinate Dehydrogenase (SDH) and Hepatic Lipase (HL) were detected according to the instructions of the kit. Statistical Package for the Social Sciences (SPSS) 21.0 statistical software was used for data analysis. The measurement data±standard error was expressed by ($\bar{x}$±s); for data comparison, t test was conducted and $p<0.05$ is the difference with statistical significance. The results showed that the blood glucose of mice in each group reached the peak at 90 min, the blood glucose concentration of d-alloxan group was significantly lower than that of other groups ($p<0.05$), and the blood glucose concentration of fructose group was significantly higher than that of other groups ($p<0.05$) as shown in Table 1. The results showed that the concentrations of serum glucose, insulin and liver glycogen in each group increased significantly after meal. D-alloxan group mice had significantly lower blood glucose concentration before and after meal than other groups ($p<0.05$), significantly higher liver glycogen concentration before and after meal than other groups ($p<0.05$), higher insulin level than other groups, but there was no significant difference ($p>0.05$), and there was no significant difference in muscle glycogen concentration before and after meal ($p>0.05$) as shown

in Table 2. The results showed that the TG concentration of d-alloxan group mice was significantly lower than that of other groups ($p<0.05$), the TG level of fructose group and cellulose group was not significantly lower than that of control group ($p>0.05$), and there was no significant difference in TC level among groups ($p>0.05$). The concentration of d-alloxan group FFA in glucose group, fructose group and d-alloxan group was lower than that in control group and cellulose group ($p<0.05$), and the concentration of d-alloxan group was the lowest ($p<0.05$). Compared with the control group, the concentration of LDL-C in the other four groups decreased significantly ($p<0.05$), and the concentration of d-alloxan group LDL-C was significantly lower than that in the other groups ($p<0.05$). There was no significant difference between d-alloxan group and cellulose group ($p>0.05$) as shown in Table 3. The results showed that the content of SDH in blood and tissues of d-alloxan group mice was significantly higher than that of other groups ($p<0.05$) and the level of d-alloxan group was higher than that of other groups, but the difference was not significant ($p>0.05$) as shown in Table 4. Diabetes is a metabolic disease characterized by elevated blood glucose. Its clinical manifestations are polyuria, polydipsia and weight loss[6]. In recent years, with the rise of people's living standards and the increase of population aging, the incidence rate of chronic diseases such as diabetes has increased year by year. The main factor leading to the disease is the excess energy caused by the use of too much carbohydrate. Therefore, new low calorie sweeteners have become a hot spot in food and medical research[7]. D-alloxan is a reducing monosaccharide, which can be used as a substitute for sucrose. As a sweetener, d-alloxan has been widely used in food[8]. Animal studies have found that d-alloxan has no obvious metabolism in the body. High levels can be detected in the liver, kidney and bladder by intravenous injection of 14C d-alloxan in mice. However, d-alloxan can be rapidly excreted from the body through urine, indicating that d-alloxan can hardly be stored in the body. After oral d-alloxan is absorbed by the small intestine, more than 70 % of it is excreted by urine. The remaining unabsorbed part is reabsorbed by the large intestine and finally fermented in the cecum. Studies have shown that d-alloxan can be absorbed by the body and enter metabolism, which can effectively control the occurrence of obesity[9]. Insulin resistance can lead to hepatic glucose metabolism disorder and oral glucose tolerance can be used to judge islets beta (β). According to the standard of cell and glucose metabolism, this study observed the change of blood glucose concentration by intragastric administration of 10 % glucose solution in mice. The results showed that the blood glucose of mice in each group reached the peak at 90 min, and the blood glucose concentration of d-alloxan group was significantly lower than that of other groups ($p<0.05$), indicating that d-alloxan can improve the glucose load of mice and increase the glucose control function of the body. In this study, it was found that d-alloxan can significantly reduce postprandial blood glucose in mice and has an auxiliary effect on the treatment of diabetes. D-alloxan can increase the absorption of sugar in peripheral tissues, but it will not cause the increase of muscle glycogen. D-alloxan can increase liver glycogen, thus reducing blood glucose. FFA is a neutral fat decomposition product. When the liver sugar that generates energy from body activities is exhausted, FFA can be used as energy. The concentration of FFA is related to fat metabolism, glucose metabolism and endocrine[10]. Related research shows that obesity and the abnormal receptor can lead to the increase of FFA level, which is an index reflecting the disorder of blood lipid metabolism[11]. LDL-C is a lipoprotein particle that carries cholesterol into peripheral tissues. When LDL-C oxidizes in excess, it can lead to cholesterol deposition and atherosclerosis. LDL-C is the gold index to judge atherosclerosis and regulate lipid[12]. The results showed that the concentrations of d-alloxan group FFA in glucose group, fructose group and d-alloxan group were lower than those in control group and cellulose group ($p<0.05$), and the concentration of d-alloxan group was the lowest ($p<0.05$). Compared with the control group, the concentration of LDL-C in the other four groups decreased significantly ($p<0.05$), and the concentration of d-alloxan group LDL-C was significantly lower than that in the other groups ($p<0.05$). There was no significant difference between d-alloxan group and cellulose group ($p>0.05$). It indicates that d-alloxan has certain anti atherosclerotic effect. Tricarboxylic acid is the final metabolic pathway of sugars, lipids and amino acids. SDH is a marker enzyme reflecting the function of mitochondria. It is also an enzyme on the cell membrane in the tricarboxylic acid cycle pathway, which can judge the extent of tricarboxylic acid cycle[13,14]. HL is one of the main members of lipase, which is synthesized by hepatocytes and participates in lipoprotein metabolism. HL is closely related to the metabolism of plasma lipoproteins. The increase of HL level can lead to metabolic disorder and the occurrence of diabetes[15]. The results of this study show that the concentration of d-alloxan group

SDH is significantly higher than that of other groups, indicating that d-alloxan can enhance the ability of oxygen metabolism and promote the decomposition of fat and protein. D-alloxan group has the highest concentration, but it is not significant, indicating that d-alloxan can promote fat metabolism and reduce the level of cholesterol in plasma. In conclusion, d-alloxan can effectively regulate blood glucose concentration, inhibit postprandial blood glucose rise, and improve insulin level and liver glycogen level. It has no significant effect on muscle glycogen. It can improve the disorder of lipid metabolism, increase the body's ability of fat metabolism, and improve the body's antioxidant function. It can be used as an ideal substitute for sucrose.

TABLE 1: CHANGES OF BLOOD GLUCOSE CONCENTRATION OF MICE IN EACH GROUP AFTER INTRAGASTRIC ADMINISTRATION OF GLUCOSE (x̄±s)

Group	Time after gavage					AUC (h/mg/100 ml)
	0	30	60	90	120	
Control	92.53±6.21*#	133.54±9.54#α	144.59±10.65	173.85±7.51#	161.25±10.55*#	17262±826#
Glucose	89.65±6.24*#α	135.62±10.59#α	159.64±9.28*#	170.65±9.64#	151.64±11.25*#	17557±732#
Fructose	96.54±6.25*	162.95±11.52*	176.54±11.35*	198.65±6.55*	154.29±10.69*#	19817±648*
D-alloxan	78.64±5.36α	132.65±9.54α	142.68±11.25#	169.65±9.21#	148.52±12.36#	16649±719#
Cellulosic	89.64±5.45#α	154.62±12.55*#	186.57±15.36*	192.65±4.62*	172.48±12.25*	19725±1013*

Note: The values in the same column in the table have different symbols, indicating significant differences p<0.05 and the same letter means no significant difference p>0.05

TABLE 2: COMPARISON OF BLOOD GLUCOSE, INSULIN AND GLYCOGEN INDEXES BEFORE AND AFTER MEAL IN EACH GROUP (x̄±s)

Group	Time	Blood sugar (mg/100 ml)	Insulin (ng/ml)	Hepatic glycogen (mg/g)	Muscle glycogen (mg/g)
Control	06:00	142.15±10.12*#	25.68±4.38#	7.21±1.49#	0.23±0.08
	08:00	156.48±9.42*	33.54±6.59*#	8.97±2.64*#	0.32±0.12
Glucose	06:00	142.97±7.68*#	25.61±5.64#	7.60±1.48#	0.25±0.06
	08:00	156.94±7.69*	33.24±6.95*#	9.12±2.11*#	0.32±0.10
Fructose	06:00	141.33±12.22*#	25.21±4.62#	8.71±1.42*#	0.25±0.09
	08:00	158.69±8.54*	33.87±5.46#	9.31±2.41*#	0.32±0.11
D-alloxan	06:00	134.12±6.23#	26.12±4.65*#	9.20±1.92*#	0.31±0.04
	08:00	146.59±10.36*#	43.25±8.65*	12.35±1.79*	0.34±0.04
Cellulosic	06:00	141.59±10.55*#	24.68±5.36#	7.86±2.59#	0.32±0.07
	08:00	157.64±3.25*	35.54±6.24*#	9.96±0.35*#	0.34±0.06

Note: The values in the same column in the table have different symbols, indicating significant differences p<0.05, and the same letter means no significant difference p>0.05

TABLE 3: COMPARISON OF TG, TC, FFA AND LDL-C LEVELS IN DIFFERENT GROUPS OF MICE (x̄±s)

Group	TG (mmol/l)	TC (mmol/l)	FFA (mmol/l)	LDL-C (mmol/l)
Control	1.43±0.47*	3.45±0.91	0.95±0.22*	0.58±0.11*
Glucose	1.35±0.24*#	2.93±0.74	0.61±0.06#	0.52±0.08*#
Fructose	0.95±0.31*#	2.43±0.68	0.58±0.07#	0.38±0.11#α
D-alloxan	0.82±0.12#	3.32±0.74	0.41±0.04#	0.37±0.05α
Cellulosic	1.21±0.31*#	3.53±0.69	0.93±0.07*	0.027±0.04α

Note: The values in the same column in the table have different symbols, indicating significant differences p<0.05, and the same letter means no significant difference p>0.05

TABLE 4: COMPARISON OF SDH AND HL LEVELS IN MICE OF EACH GROUP ($\bar{x}$±s)

Group	SDH (U/ml)	SDH (U/mg prot)	HL (U/mg prot)
Control	21.42±3.22#	2.74±0.85#	1.32±0.29
Glucose	21.74±4.72#	3.16±1.12*#	1.22±0.35
Fructose	19.75±4.96#	2.97±1.17*#	1.45±0.34
D-alloxan	34.85±4.99*	5.41±0.92*	1.68±0.38
Cellulosic	22.23±4.12#	3.57±0.84*#	1.45±0.28

Note: The values in the same column in the table have different symbols, indicating significant differences $p<0.05$, and the same letter means no significant difference $p>0.05$

Conflict of interests:

The authors declared no conflict of interests.

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