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The Combination of L-lysine and Alloxan Induces Acute Necrotizing Pancreatitis in Rats

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Abstract

This study was designed to evaluate the L-lysine effect on the pancreas of induced diabetic rats. Adult male Sprague-Dawley rats were grouped in four groups (N = 6 rats per group) and studied for 20 days. Group I: Control (saline solution, i.p.). Group II: Rats treated with 10 mM L-lysine. Group III: Rats treated with 120 mg/kg alloxan (diabetic control). Group IV: Rats treated with alloxan plus L-lysine. Blood was taken from aorta to determine glucose, triglycerides, and cholesterol. As spected, glucose levels were increased in rats of Group III. A loss of body weight was also observed ($p < 0.001$). Glucose concentration was higher in rats of Group IV on day 10 of the experiment compared with those of diabetic control group. The results shown here indicate that the pancreas was affected by L-lysine administration to intact animals. The pancreatic damage was increased in those animals previously injected with alloxan. It is clear that a single administration of L-lysine caused hyperglycemia. This effect is stressed when L-lysine is combined with alloxan. These results permit us to state a new model of acute pancreatitis in rats.

Keywords: Acute Necrotizing Pancreatitis; Alloxan; Hyperglycaemia; L-lysine; Rat

Introduction

Pancreatitis, also known as the inflammation or infection of pancreas, can cause hypoxia, kidney failure and other severe complications [1]. Acute pancreatitis is an exceptionally grave disorder characterized by the necrosis of pancreas parenchyma, fat and vessels resulting from the intrapancreatic activation of pancreatic enzymes [2]. Over decades, several animal models had been developed in order to improve the clinical prevention and treatment of acute pancreatitis [3-6]. Theoretically, a reliable experimental model ought to be easily produced, to reproduce the disease in term of etiology, histology, symptomatology and effectiveness in treatment, which means the acute pancreatitis animal models should mimic remarkable pathophysiological changes of human pancreatitis [7]. Ethionine [8] and arginine-induced pancreatitis [9] models are some of the examples.

On the other hand, lysine has been one of the main targets of the non-enzymatic glycosylation or glycation studies [10], especially under hyperglycemia conditions, leading to the production of advanced glycation end-products [11]. Previously, we used L-lysine to study the in-vitro glycation process [12], and alloxan to induce diabetes in rats [13]. Here, the combination of L-lysine and alloxan on the rat pancreas was evaluated. Glucose, triglycerides and cholesterol were also measured.

Materials and methods

Reagents

Alloxan, droperidol, ketamine, L-lysine, aminophenazone, phenol, 4-chlorophenol, H_2O_2, xylol, hematoxylin and eosin were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Formaldehyde and ethanol were purchased from Merck &

Co. (E. Merck, D-6100 Darmstadt, Germany).

Animals

Adult male Sprague-Dawley rats weighing 335 ± 18 g were used in this study. Animals fed *ad libitum* were grouped in four groups (N = 6 rats per group) that were studied during 20 days. Group I was the control group treated with 1.0 mL 0.154 M NaCl (i.p.). Group II were normal rats administered 1.0 mL 10 mM L-lysine in 0.154 M NaCl (i.p.). Group III rats were diabetic controls treated with 120 mg/kg alloxan (i.p.). Group IV rats were diabetized by 120 mg/kg alloxan as in Group III, but they were also administered 1.0 mL 10 mM L-lysine in 0.154 M NaCl (i.p.) as did those in Group II. On the day 10, half of the animals were anesthetized by 0.4 mL droperidol and 0.6 mL ketamine (i.m.), and blood was taken from aorta to determine glucose, triglycerides and cholesterol. Blood samples were centrifuged at 3,000 rpm for 15 minutes, and the serum was separated to perform the clinical determinations. At the end of the 20-day-period, this process was repeated with the other half of animals. Body weight was registered on the days 8, 10, 12, 18 and 20. A DU-64 Beckman Spectrophotometer was used to register the absorbance when it was necessary.

Glucose

The method of Trinder [14] was performed in the determination of glucose levels using 4-aminophenazone and phenol. The absorbance was read at 540 nm.

Triglycerides

The triglycerides determination was carried out according to the method of Wahlefeld [15] using 4-aminophenazone and 4-chlorophenol. The absorbance was registered at 620 nm.

Cholesterol

Cholesterol levels were detected performing the method of Siedel [16] using aminophenazone, phenol and H2O2. The absorbance was read at 540 nm.

Histology Analysis

Pancreas from the rats sacrificed on the day 10 were dissected and fixed in 10% formaldehyde, and embedded in paraffin for the histology analysis. After dehydrated by xylol and ethanol, tissue slices of 7 μm thickness were stained with hematoxylin and eosin before observed by light microscopy.

Statistical Analysis

For data obtained from the 4 groups, the mean ± S.D. were calculated. Significance among these groups was calculated by one-way ANOVA test and Tukey-Kramer test for multiple comparisons using program R (the R Foundation for Statistical Computing, Version 2.2.0), $P < 0.05$ denoted statistical significance.

Results

Body weight

No significant difference was shown in body weight among the Group I, II and III during the 20-day-period, however, body weight of the rats treated with both alloxan and L-lysine (Group IV) significantly decreased on the day 8 and 10. These rats started to recover their body weight after the day 12 (Table 1).

Day	Body weight (g)			
	Group I (Control)	Group II (10 mM L-lysine)	Group III (Alloxan*)	Group IV (Alloxan+L-Lysine)
0	329.6 ± 24.6	344.3± 19.8	336.8 ± 8.6	332.6 ± 18.6
8	356.0 ± 29.4	359.3 ± 23.4	361.8 ± 4.7	294.1 ± 28.4
10	376.0 ± 43.8	365.5 ± 14.8	362.5 ± 3.5	268.0 ± 33.9
12	354.3 ± 10.5	360.3 ± 18.5	382.5 ± 3.5	323.6 ± 50.8
18	366.6 ± 17.2	367.3 ± 20.0	396.0 ± 16.9	363.1 ± 11.0
20	362.8 ± 14.5	369.1 ± 25.2	361.0 ± 9.8	360.6 ± 13.0
*120 mg/kg (i.p.)				

Table 1: Changes in body weight in rats treated with alloxan and L-lysine.

Biochemical Parameters

Hyperglycemia was produced with the concentration of alloxan administered. Rats treated with L-lysine (Group II) or alloxan (Group III) did not present significant variations on glucose concentrations (Figure 1). The increase of glucose levels was even higher in Group IV when both L-lysine and alloxan were injected. At the end of the 20-day-experiment, glucose serum concentration trended to be normal (Figure 1).

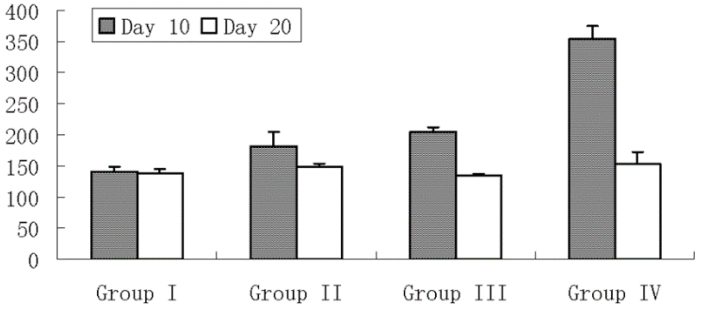

Figure 1: Glucose concentration (mg/dL) after the administration of 120

mg/kg (i. p). Group I (Control), Group II (10 mM L-lysine), Group III (Alloxan), Group IV (Alloxan + L-Lysine).

The top levels of triglycerides were detected in diabetic rats induced by alloxan (Group III) on the day 10; however, this value dramatically decreased and reached the bottom on the day 20. In contrast, rats treated with L-lysine only (Group II) maintained normal as the control (Group I), but reach higher levels at the end of the experiment (Figure 2). The effect of L-lysine and alloxan on triglyceride serum levels was also proved by the results from Group IV, when both of the chemical agents were administered, the triglyceride concentrations on the day 10 and 20 were between the levels of Group II and III.

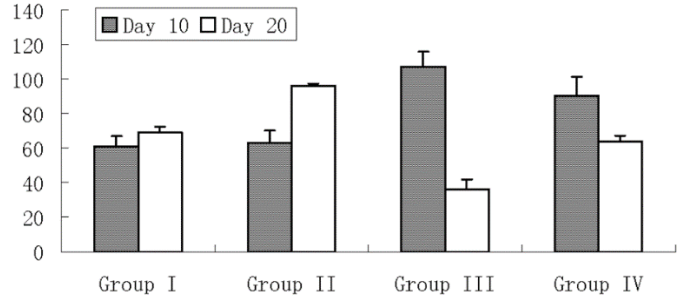

Figure 2: Triglyceride concentration (mg/dL) after the administration of alloxan.

No significant difference was detected in the cholesterol serum levels among the four groups in the conditions of this experiment (Figure 3).

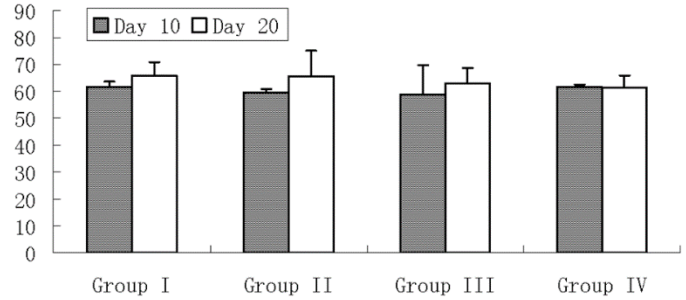

Figure 3: Serum cholesterol levels in treated and non-treated rats with alloxan and L-lysine.

Histology Analysis

Under the light microscope, alterations of the normal morphology could be observed in pancreas (Figure 4). When the rats were treated with L-lysine (Group II), the acinar cells of pancreas lost their angular shape, the nucleus were shrunken and their diffuse granularities were lost. Marginal hyperchromatosis was also remarkable in the acinar cells. Endocrine cells of the islets of Langerhans were slightly decreased in number. Necrosis and steatosis occurred in pancreas of alloxan-treated rats (Group III), especially in Langerhans islets. A great number of zymogen granules disappeared. Edema was evident in intercellular spaces. When both of L-lysine and alloxan were administered (Group IV), pancreatic necrosis had markedly disrupted the acinar architecture, the Langerhans islets also appeared affected.

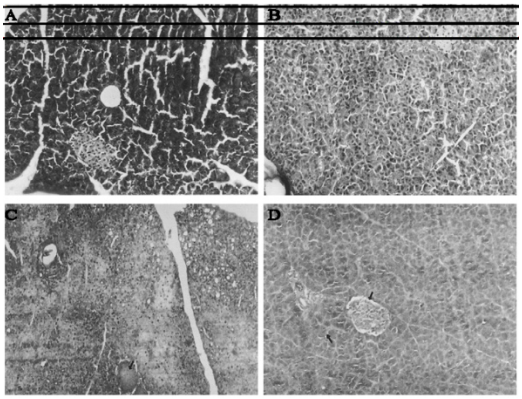

Figure 4: Light microscopy analysis (H & E 20X) showed that the Langerhans islets were destroyed (C) after alloxan administration when compared with the Control (A). Endocrine and exocrine cells were affected by L-lysine (4B). Alloxan and L-lysine destroyed exocrine or endocrine cell structures (4D).

Discussion

Early findings suggested that an excess of L-arginine can be used to produce pancreatitis in rats [8]. In this study, we design a new animal model of acute necrotizing pancreatitis by using L-lysine in synergy with alloxan. Milder dose (120 mg/kg) of alloxan was administered only because higher dosage increases the death rate among the animals. Body weight, biochemical parameters including glucose, triglycerides and cholesterol were analyzed. Changes in the pancreas morphology were confirmed by light microscopy.

The administration of L-lysine together with alloxan reduced the body weight of treated animals, but their weight was recovered after the day 12 as previously reported [17]. Rats treated with L-lysine or alloxan only showed no significant difference in body weight, probably because they recovered even faster when affected only by one of these chemicals.

Alloxan is toxic to the pancreatic β-cells because of the production of free radicals, especially reactive oxygen species derived from oxygen through its partial reduction and cause damage to β-cells. As a response, the enzyme poly (adenosine diphosphate-ribose) polymerase is activated to repair the deoxyribonucleic acid; however, the excess of its activation may cause the depletion of nicotinamide adenine dinucleotide pool, interfering with adenosine triphosphate synthesis, which finally leads to cell death [18]. In this study, diabetic conditions were clearly produced by the effect of alloxan, as the glucose serum levels increased in alloxan-treated rats. Light microscopy analysis also showed that the Langerhans

islets were destroyed (Figure 4C).

On the other hand, L-lysine administration also significantly reduced the number of endocrine cells in Langerhans islets; resulting in an increase of serum glucose concentrations. No significant difference in glucose serum levels was shown between alloxan-treated and lysine-treated rats on the day 10.

L-Lysine did not only cause severe damage to the pancreatic endocrine cells, but also to the exocrine cells (Figure 4B). The administration of both L-lysine and alloxan destroyed either exocrine or endocrine cell structures, diffused marginal hyperchromatosis could be observed in all the acinar cells associated with tissue necrosis, which means that the acute necrotizing pancreatitis was induced in these rats (Figure 4D). Triglyceride concentrations were higher than the normal levels on the day 10. Because no significant difference was shown between the L-lysine treated group and the control group, this increase should be caused by alloxan. No changes in cholesterol levels were detected during this experiment, so L-lysine and alloxan did not affect the cholesterol metabolism. In general, the information on L-lysine metabolism in the pancreas is scarce, but its effects would be related in part to cadaverine formation, however, it remains to be demonstrated. Cadaverine is a diamine which is produced from lysine decarboxylation catalyzed by lysine decarboxylase (Figure 5).

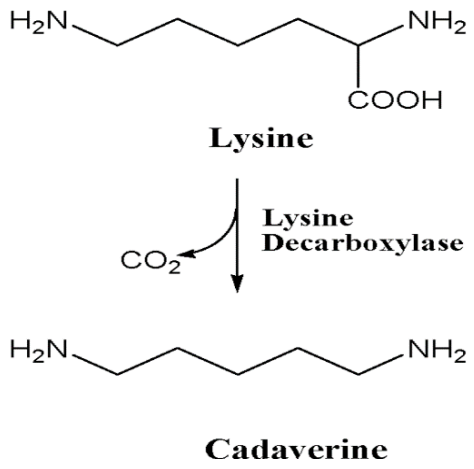

Figure 5: Cadaverine (1, 5 diaminopentane) is formed from lysine decarboxylation.

The *in vivo* cadaverine functions in macromolecular metabolism are not well known. It has been demonstrated that this amine has similar effects to other polyamines such as putrescine, spermidine and spermine, on the protection of deoxyribonucleic acid against thermal denaturing, protects T5 bacteriophage against denaturing effect of urea, and stimulates the *in vitro* protein biosynthesis [19,20].

It is clear that in general, a parallelism exists between the polyamine levels and macromolecular content in tissue under development, as it has been observed in several seeds under germinating process [21], which are in accordance with other biological systems that present rapid growth. However, it has also been demonstrated that when the polyamines were administered in excess, they could lead to several adverse effects similar to that observed from an excess of L-arginine, the precursor in the putrescine, spermidine and spermine biosynthesis in extrahepatic tissues, such as the pancreas [22].

Conclusions

In conclusion, the results shown here provide evidence that L-lysine is toxic to pancreatic exocrine and endocrine cells. The administration of L-lysine and alloxan induces the acute necrotizing pancreatitis in rats.The precise mechanism by which L-lysine affects the pancreatic function remains to be investigated. However, these results permit us to state a new model combining low concentrations of L-lysine and alloxan to induce acute pancreatitis in rats, which may be useful for investigating the mechanisms and therapy for this disease. This model is economical and reproducible. In addition, these results can alert on the intake of L-lysine by diabetic patients who can make the diabetic complications worse than before, so it could be likely that L-lysine affect the pancreatic function by multiple mechanisms.

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