·Basic Research·

Effect of alloxan time administerDrug on establishing diabetic rabbit model

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Abstract

• AIM: To explore the effect of alloxan time administerDrug on establishing diabetic rabbit model.

• METHODS: Thirty-six healthy rabbits, weighed 2-2.5kg, were randomly divided into one time administerDrug group (Group A, n=12), two times administerDrug group (Group B, n=12) and three times administerDrug group (Group C, n=12). Every rabbit was injected with alloxan of 150mg/kg. The three groups were measured for fasting blood-glucose. The success rate and death rate of each group were also calculated.

• RESULTS: The success rate of diabetic rabbit model in Group B was higher than that in Group A (P<0.01) but its death rate was lower than that of Group A (P<0.01); the success rate of diabetic rabbit model in Group C was highest but the death rate was the lowest in the three groups.

• CONCLUSION: Separate administration of alloxan can improve success rate in establishing diabetic rabbit model, decrease the death rate and keep the stability of model.

• KEYWORDS: alloxan; diabetes; rabbit; death rate; success rate

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INTRODUCTION

As an endocrine metabolic disease caused by deficiency and/or biological effect barrier in insulin secretion and with hyperglycemia as the main feature, diabetes gains an increasing incidence and poses a serious threat to people's health. Currently, diabetic animal models established are mainly these with β cells destructed by chemical drugs, and especially, modeling induced by alloxan (ALX) and streptozotocin is most commonly used [1]. Being toxic agent of islet β cells, alloxan damages β cell through generating superoxide free radical to injure cellular DNA, activate the activity of poly ADP-ribose polymerase, decrease contents of coenzyme Ⅰ, impair the function of mRNA, reduce insulin before the synthesis of β-cell, and cause insulin deficiency finally. The alloxan-induced diabetic animal model is similar to human diabetes of type Ⅰ. Although alloxan diabetic animal model is commonly used for evaluating the efficacy and safety of anti-diabetic drug [2], and alloxan is cheap, it is difficult to grasp safe dose of alloxan in the method previously adopted, thus the application of alloxan-induced diabetic animal model is affected due to its low success rate and high death rate [3]. This experiment probes into the best way to establish model of alloxan-induced rabbit by observing the success rate, death rate, blood glucose stability, *etc.* of alloxan-induced experimental rabbit with the same dose but different time administerDrugs.

MATERIALS AND METHODS

Materials Thirty-six healthy grown-up female white rabbits (Laboratory Animal Center of Xi'an Jiaotong University), weighed 2-2.5kg. ALX (manufactured by Sigma Company): Before application, make it up into 50g/L solution with sterilized normal saline for injection and sterilize & filtrate by 0.22μm cellulose membrane. Determination of blood glucose and urine sugar: Accu-CHEK Active glucometer and blood glucose test paper manufactured by Germany Company Roche. Three random groups based on time administerDrug, each of 12 rabbits. Group A was one time administerDrug group, in which, rabbits were prevented from eating in 12 hours before administration, and then newly prepared 50g/L ALX solution was slowly injected via ear vein at 150mg/kg; Group B was two times administerDrug group, in which, rabbits were fasted for 12 hours first, and then newly prepared 50g/L ALX was slowly injected via ear vein at 50mg/kg; one week later, rabbits were fasted for 12 hours before the second administration, and then newly prepared 50g/L ALX solution was rejected again by 100mg/kg; Group C was three times administer

Table 1 Comparison on success rate and death rate of laboratory animal in each group

Variable	Group A	Group B		Group C		
		B1	B2	C1	C2	C3
Laboratory animal(*n*)	12	12		12		
Dead animal(*n*)	11	0	5	0	1	0
Death rate(%)[b]	91.67	0	41.67	0	8.33	0
Molded animal(*n*)	1	0	4	0	2	5
Success rate(%)[b]	8.33	0	33.33	0	16.67	41.67
Final success rate(%)	8.33	33.33		58.33		

[b]P<0.01, comparison on success rate and death rate of laboratory animal in the 3 groups differs significantly

Drug group, in which, newly prepared 50g/L ALX solution was injected for three times respectively by 50mg/kg at the interval of one week, and rabbits were fasted for 12 hours before each administration.

Methods Before administration, fasting blood glucose of all rabbits was measured for three times from 8:00-8:30 *a.m.* every other day, and the mean value was taken as base value. At least 72 hours after ALX injection, fasting blood glucose from 8:00-8:30 *a.m.*was tested 2 times per week. If blood glucose could more than triple the base value for 2 continuous weeks and remained stable, the modeling was successful. After that, fasting blood glucose from 8:00-8:30 *a.m.* was tested once every week for 7 weeks to observe the fluctuation of blood glucose.

Statistical Analysis Chi-square test was performed by statistical software SPSS 12.0 for the comparison on death rates and success rates of the 3 groups of rabbits, with P < 0.05 as significant test criterion.

RESULTS

Success Rate and Death Rate of Each Group Among laboratory rabbits of the 3 groups, in Group A, 4 died within 72 hours after injection, and totally 11 died in one week; when fasting blood glucose from 8:00-8:30 *a.m.* was measured 72 hours after injection, 7 among the 8 survived rabbits turned up with fasting blood sugar being more than 3 times of the base value, and 1 week later, the only survived laboratory rabbit reached the standard for diabetic animal model, therefore, the ultimate success rate was 8.33% and the death rate was 91.67% . In Group B, upon the first injection (B1), 0 died, 1 had blood glucose went up, and standard for diabetic animal model was not met. Upon the second injection (B2), 3 died within 72 hours, and respectively 1 died in the fifth and seventh day; among the 7 alive rabbits after 1 week, 4 became stable diabetic animal models, as a result, the eventual success rate was 33.33% and the death rate was 41.67%. In Group C, upon the first injection (C1), 0 died but all failed to meet the standard for diabetic animal model; upon the second injection (C2), 1 died but two became diabetic animal models; upon the third injection (C3), 0 died, and 1 week later, totally 7 turned into stable diabetic animal models, consequently, the final success rate was 58.33% and death rate was 8.33%. And comparison on success rate and death rate of laboratory animal in the 3 groups differs significantly (P<0.01, Table 1).

Final Base Values of Blood Glucose in the Three Groups of Laboratory Rabbits Base values of fasting blood glucose: 4.7mmol/L (min.), 7.9mmol/L (max.), 6.33mmol/L (mean). The results of blood glucose assay for the 3 groups of laboratory rabbits at different time after injection was shown in Table 2. The state of rabbits in Group A was very poor: the death rate within 1 week topped 91.67%, results of blood glucose assay for the died rabbits were quite different with part blood glucose exceeded 35mmol/L and some other extremely low, and insulin or glucose rescue therapy was carried out via vein according to the results of blood glucose, but the effect was not obvious. Upon B1, most of the rabbits showed a normal diet and mental state. But after B2, they were of poor mental state, ate less, were lazy to move, lost weight, and totally 5 died with apparent increase of blood glucose before death. Condition upon C1 was similar with Group B; after C2 and C3, most rabbits showed poor mental state, ate less and were lazy to move first, then there appeared other obvious symptoms of diabetes such as polydipsia, polyuria, weight loss, *etc.*

DISCUSSION

As a metabolic disease with high morbidity, diabetes is of extensive and severe complications as well as disability rate and lethality rate, and the research on diabetes has become hot spots of the world. The application of diabetic animal models can overcome limitations of research on human body and contribute significantly to diabetes research. The most common method for simulating diabetes is to selectively destroy pancreatic β cell that secrete insulin by chemical drugs such as ALX and STZ.

Compared with other methods or drugs, established ALX diabetic animal model has obvious advantages and

Table 2 Comparison on blood glucose of the three groups of laboratory rabbits at different time mmol/L,mean±SD)

Time	A	B1(*n*=12)	B2	C1(n=12)	C2	C3(*n*=11)
Before injection	6.26±1.25(*n*=12)	6.37±1.53	8.66±3.02(*n*=12)	6.19±1.49	8.33±2.86(*n*=12)	15.05±4.36
72 hours	23.43±11.57(*n*=8)	8.49±2.83	16.47±5.38(*n*=9)	7.98±3.11	13.96±5.79(*n*=12)	19.51±7.72
1 week	24.60±0(*n*=1)	8.66±3.02	17.85±4.92(*n*=7)	8.33±2.86	15.05±4.36(*n*=11)	20.36±8.78
2 weeks			18.02±5.14(*n*=7)			19.50±8.98
3 weeks			18.41±7.31(*n*=7)			20.08±9.39
5 weeks			18.35±7.62(*n*=7)			18.57±8.90
7 weeks			17.90±6.97(*n*=7)			18.49±7.61

characteristics: the former refers to low price, fast modeling, higher success rate of ALX-induced diabetic model than streptozotocin [4], stable model of ALX-induced diabetic animal and long-term survival without using insulin. The latter is relation of success rate to the dose used. However, studies have shown that ALX dosage is of positive correlation to the level of hyperglycemia, the toxicity enhances as the dose has been increased[5], it is easy to bring about toxic impairment in liver and kidney while causing diabetes, killing the animal and reducing the final success rate subsequently. Zhang *et al*.[6] also found that alloxan was not highly selective to pancreatic function *in vivo* of mouse but greatly toxic to kidney. With the increase of ALX dose, diabetes caused became increasingly serious, and death rate of animal rose significantly. Some studies reported [7] while establish diabetic rabbit model with high dose (160mg/kg) or medium dose (130mg/kg), the death rate of animal were estimated to be 50.0% or 12.5%, and it was also reported that the average death rate was 30% [2]. Therefore, when using ALX-induced diabetic animals, an important research subject is how to improve the success rate of modeling and significantly reduce the death rate of animal.

In the document, ALX dosage varies greatly (100-200mg/kg) [1]. This study refers to the ALX dosage described in most of the documents with total amount of 150mg/kg, and discusses the effect of separate injection on the success rate and death rate of diabetic rabbit models by dividing them into one time administerDrug, two and three times administerDrug groups. The results show (Table 1) that in Group A, 72 hours after the injection, the success rate was 87.5% but the death rate was very high, and within 1 week, 11 died, recording a death rate of 91.67%. Therefore, once injection is inadvisable because centrally released toxicity from a large number of drugs to the liver and kidney disables animals to tolerate, kills them and results in the success rate of only 8.33%. In Group B, although no animal was modeled upon B1, the death rate was 0; upon B2, the final success rate was significantly higher than that of Group A(P<0.01), and death rate was 41.67%, significantly lower than that of Group A(P< 0.01). This was possibly because animals' tolerance of hepatotoxicity and renal toxicity was improved after injecting small dose of drug for the first time, the death rate was reduced, and the success rate was not significantly reduced due to separate impairment of islet β cell by ALX. Moreover, three times of injection in Group C can further improve the tolerance of animals and reduce death rate, and the improved survival of molded animals brings a significantly higher final success rate than that of Group A and Group B (P<0.01). Therefore, it can be concluded that while establishing ALX diabetic rabbit model with total dosage of 150mg/kg, three times of injection exhibits the highest success rate and lowest death rate.

The objective of establishing diabetic rabbit model is to raise blood glucose and stabilize it at a certain level, because if blood glucose is too high, animals are likely to die, and if the blood glucose is too low, the model becomes unstable. As can be seen from Table 2, models in Group B and Group C maintain relatively stable levels of blood glucose in the 7 weeks' observation, indicating that ALX-induced diabetic rabbit model is stable. Of course, stability in a longer term requires further observation and study.

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