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Optimized alloxan-induced diabetic rabbit model

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Abstract

• AIM: To explore the frequency of drug injection of alloxan diabetes on the established model of rabbit.

• METHODS: Thirty-six healthy rabbits, weighing 2-2.5kg, were randomly divided into one time drug injection group (group A, $n=12$), two times drug injection group (group B, $n=12$) and three times drug injection group (group C, $n=12$). Each rabbit was injected with a total amount of 150mg/kg of alloxan. Fasting blood glucose was measured. 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 ($P<0.01$) and 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 and the death rate was the lowest in three groups ($P<0.01$).

• CONCLUSION: Multiple administration of alloxan can improve success rate in establishing diabetic rabbit model with decreased death rate and increased stability.

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

As an endocrine metabolic disease caused by insulin secretory deficiency and/or biological effect abnormality with the main feature of hyperglycemia, diabetes gains an increasing incidence and poses a serious threat to human health. Currently, established animal models of diabetes are mainly produced by administration of β cells destructive chemical drugs, especially the models induced by alloxan (ALX) and streptozotocin [1]. Being toxic agent of islet β cells, alloxan damages β cell through generating superoxide free radical to injure cellular DNA, activate poly ADP-ribose polymerase, decrease coenzyme Ⅰ contents, impair mRNA function, reduce proinsulin synthesized by β-cell, and cause insulin deficiency finally. The alloxan-induced diabetic animal model is similar to human diabetes of type Ⅰ. Although alloxan-induced diabetic animal model is commonly used for evaluating the efficacy and safety of anti-diabetic drugs [2], and alloxan is cheap, it is difficult to control safe dose of alloxan with common methods. Therefore, the application of alloxan-induced diabetic animal model is limited due to its low success rate and high death rate [3]. This experiment aims to discuss the optimized way to establish alloxan-induced rabbit model of diabetes by observing the success rate, death rate, blood glucose stability etc. with the same total dose but different times of drug administration.

MATERIALS AND METHODS

Materials Thirty-six healthy white adult female rabbits (Laboratory Animal Center of Xi'an Jiaotong University) were included, weighing 2-2.5kg each.

Reagents and Preparation (1) ALX (Sigma Company): Before application, 5% ALX solution was reconstituted with sterilized normal saline, and filtrated by 0.22μm cellulose membrane for sterilization. (2) Determination of blood glucose and urine sugar: Accu-CHEK Active glucometer and blood glucose test paper were manufactured by Germany company Roche.

Experimental Group Rabbits were randomly divided into 3 groups based on times of drug administration ($n=12$). Group A was one time drug administration group, in which rabbits were fasted for 12 hours before administration, followed by injection of 150mg/kg newly prepared 5% ALX solution via ear vein. Group B was two times drug administration group, in which rabbits were fasted for 12 hours, and then injected with 50mg/kg newly prepared 5% ALX; 1 week later, rabbits were fasted for 12 hours before the second administration, and then injected with 100mg/kg newly prepared 5% ALX solution. Group C was three times drug administration group, in which 50mg/kg newly prepared 5% ALX solution was injected for three times respectively at the interval of 1 week, and rabbits were fasted for 12 hours before each administration.

Result Evaluation Before administration, every other day, fasting blood glucose of all rabbits was measured between 8:00 a.m. to 8:30 a.m. for three times, and the mean value was considered as base value. At least 72 hours after ALX injection, fasting blood glucose between 8:00 a.m. to 8:30 a.m. was tested 2 times per week. If blood glucose was higher than triple the base value for 2 continuous weeks and remain stable, the modeling was successful. After that,

fasting blood glucose between 8:00 a. m. to 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 rate and success rate 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 of injection, and totally 11 died in the first week; when fasting blood glucose between 8:00 a. m. to 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 8.3% and the death rate was 91.7%. In group B, upon the first injection (B1), 0 died, 1 had increased blood glucose, but did not qualify for the standard of diabetic animal model. Upon the second injection (B2), 3 died within 72 hours, and respectively 1 died on 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 33.3% and the death rate was 41.7%. In group C, upon the first injection (C1), 0 died but all failed to meet the standard of 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 58.3% and death rate was 8.3%. The success rate and death rate of laboratory animal in 3 groups differed significantly (Table 1, $P<0.01$).

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]	91.7	0	41.7	0	8.3	0
Molded animal(n)	1	0	4	0	2	5
Success rate(%)[b]	8.3	0	33.3	0	16.7	41.7
Final success rate(%)	8.3	33.3		58.3		

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

Final Base Values of Blood Glucose in the 3 Groups of Laboratory Rabbits Base values of fasting blood glucose: 4.7mmol/L (min.), 7.9mmol/L(max.), 6.33mmol/L(mean). Results of blood glucose assay for the 3 groups of laboratory rabbits at different time after injection were illustrated in Table 2.

The reaction of rabbits in the three groups upon administration The state of rabbits in group A was very poor: the death rate within 1 week was 91.7%. Results of blood glucose assay for the died rabbits were quite different, in which some blood glucose exceeded 35mmol/L and some other were extremely low. 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 dietary and mental state. But after B2, they were of poor mental state, low activity, and lost weight. Totally 5 died with apparently increased blood glucose before death. Condition upon C1 was similar with group B; after C2 and C3, most rabbits showed poor mental state and low activitiy, followed by 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 high disability rate and lethality rate. 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 inducing diabetes is to selectively destroy pancreatic β cell that secrete insulin by chemical drugs such as STZ and ALX. Compared with other methods or drugs, ALX-induced diabetic animal model has obvious advantages and characteristics: it provides low cost, fast modeling [4]. The ALX-induced diabetic animal is stable during long-term survival without the use of insulin. Also, its success rate is related to the dose used. However, studies have shown that ALX dosage is of positive correlation with the level of hyperglycemia. Its toxicity is enhanced with increasing dose[5], and 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 of mouse *in vivo* but greatly toxic to kidney. With the increase of ALX dose, diabetes became increasingly serious, and death rate of animal rose significantly. Wang *et al*[7] reported that while establish diabetic rabbit model with high dose (160mg/kg) or medium dose (130mg/kg), the death rate of animal was 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 literature, ALX was used with greatly varied dosage (100mg/kg-200mg/kg)[1]. This study refered to the ALX dosage described in most of the previous studies with total amount of 150mg/kg, and discussed the effect of separate injections on the success rate and death rate of diabetic rabbit models by dividing them into one time, two and three times drug administration groups. The results showed (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, resulting in a death rate of 91.7%. Therefore, once injection is inadvisable because concentrately released toxicity from a large amount of drugs to the liver and kidney disables animal's tolerence and kills them. So the success rate of group A was only 8.3%. In group B, although no animal was modeled upon B1, the death rate was 0; upon B2, the

Table 2 Comparison on blood glucose of the three groups of laboratory rabbits at different time (mmol/L, $\bar{x} \pm s$)

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

final success rate was significantly higher than that of group A ($P<0.01$), and death rate was 41.7%, which was significantly lower than that of group A ($P<0.01$). This was possibly because animals' tolerance of hepatotoxicity and renal toxicity was improved after injection with small dose of drugs 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 improve the survival of molded animals significantly 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 maintained relatively stable levels of blood glucose during the 7 weeks' observation, indicating that ALX-induced diabetic rabbit model is stable. Of course, stability in a longer term observation and further observations are required.

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改良四氧嘧啶制作兔糖尿病模型的实验研究

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摘要

目的:通过改变四氧嘧啶给药次数探索改良制作兔糖尿病模型的方法。

方法:选取健康大耳白兔36只,体质量为2~2.5kg,注射四氧嘧啶剂量均为150mg/kg,随机分为3组,每组12只,分别为一次给药组(A组),分两次给药组(B组)和分三次给药组(C组)。观测各组空腹血糖值,计算各组成模率和死亡率。

结果:B组兔糖尿病模型成模率显著高于A组($P<0.01$),死亡率显著低于A组($P<0.01$);C组兔糖尿病模型成模率显著高于B组($P<0.01$),死亡率显著低于B组($P<0.01$)。

结论:四氧嘧啶分次给药能提高制作兔糖尿病模型的成模率,降低死亡率并且可以保持模型的稳定。

关键词:四氧嘧啶;糖尿病;兔;死亡率;成模率