Pakistan Journal of Pharmacology
Vol.22, No.2, July 2005, pp.41-45

ALLOXAN INDUCED DIABETES IN RABBITS

SAADIA SHAHZAD ALAM, ABDUL HAMEED KHAN, GHAZANFAR ALI SIRHINDI AND SAADATULLAH KHAN
Department of Pharmacology, Department of Hematology,
Shaikh Zayed Federal Postgraduate Medical Institute Lahore, Pakistan

ABSTRACT:

Alloxan monohydrate is a common drug used for developing experimental diabetes in animals, as streptozotocin, though less toxic than alloxan is considerably costlier. This study aimed to provide an exact and detailed account of alloxan induced diabetes in rabbits as none was available in both local and international literature and in Pharmacology textbooks. This resulted in extremely high mortality in experimental animals i.e., rabbits, leading to inevitably wasted research time and effort for a researcher.

Twenty-four healthy rabbits of a local strain weighing 1000 to 1800 g were obtained and kept at the animal house of the Department of Pharmacology Shaikh Zayed Hospital, Lahore. They were injected with varying doses of Alloxan monohydrate (from 75mg - 110mg)/ kg body weight in order to induce diabetes. The blood glucose RBS levels were estimated 8 days after injecting the alloxan and subsequently randomly at intervals of five to ten days till the rabbits became diabetic. These doses were found to lower the rabbit mortality by 25% as compared to (upto 75%) that was encountered with a single standardized dose of 160mg/kg body weight suggested by Akhtar *et al*. (1982) or 80mg/kg body weight (Puri and Prabhu 2002). It was therefore concluded that our proposed regime may be beneficial for future researchers aiming to develop a similar animal model.

Keywords: Alloxan monohydrate, streptozotocin, RBS (random blood sugar).

INTRODUCTION

Diabetes mellitus can be chemically or surgically induced in different animal species. Chemical induction of diabetes can be achieved by injecting uric acid, dial uric acid, dehydro ascorbic acid, quinoline and magnesium. However, the most commonly used means of chemical induction of diabetes has been either alloxan or streptozotocin, as their diabetogenic dose is usually 4 to 5 times less than their lethal dose. However Guinea pigs are totally insensitive to alloxan (Gordsky *et al.*, 1982).

Alloxan (mesoxal urea) was the first chemical used to induce experimental diabetes. It was found by Leibig in mucus excreted during dysentery (Merck Index, 1976). The diabetogenic dose of alloxan vary considerably amongst species, age and metabolic state of the animal. Nephrotoxicity is also a side effect (Bonar 1980). Alloxan diabetes can be prevented by sulphhydryl containing compounds such as glutathione, cystine and dimercaprol prior to alloxan administration.

It's monohydrate form as alloxan monohydrate is less toxic than its tetrahydrate form. Therefore alloxan monohydrate was selected for induction of alloxan diabetes in rabbits.

MATERIALS AND METHODS

Animals

Healthy rabbits of a local strain weighing 1000 to 1800 g were obtained and kept under

observation for one week before experimentation in the animal room of the Shaikh Zayed Federal Post Graduate Medical Institute, Lahore for a week prior to experimentation. The animals were fed green fodder, wheat grains and grams, ad libitum. Fresh and wholesome water was also provided ad libitum.

Chemicals

1. Alloxan monohydrate – Acros Organics, New Jersey, USA. (NH-CO-NH-CO-CO.H_2O).

2. Blood Glucose determination Kit – Cenix Diagnostics Gmbh, Dresden, Germany.

Procedure for Injecting Alloxan Monohydrate and for Collecting Blood

The procedure for injecting alloxan monohydrate into the rabbits was as described by Akhtar *et al.* (1982). The rabbit was held in a steel holder specially designed for blood collection. Upto 10 ml of drug could be injected intravenously into the marginal ear veins of the rabbit using an insulin needle mounted on a 10 cc syringe or less, if required, following dabbing of xylene to that particular vein.The xylene would make the ear veins prominent and easy to inject into. 2 cc of blood was drawn from the marginal ear veins of the rabbits to determine random blood sugar levels.

Calculation of Alloxan Dose for Rabbits

Twenty-four rabbits weighing 1000-1800 grams were made diabetic by injecting 80 mg/kg body weight of alloxan monohydrate (Puri and Prabhu, 2002). However, the problem of mortality (upto 75%) was noticed. A re-evaluation of the doses was done and 50, 60 and 70 mg/kg body weight were all tried and found to be ineffective. Given below is the dose regime that was found most effective, with the minimum mortality upto 25% which could not be lowered any further:

Table-1
Doses of Alloxan for Rabbits Given per kg Body Weight

Weight of the Rabbit	Dose/kg
1 kg – 1.2 kg	75 mg/kg
1.21kg – 1.3kg	80mg/kg
1.31kg – 1.35kg	85mg/kg
1.36kg – 1.4kg	90mg/kg
1.41kg – 1.5kg	95mg/kg
1.51kg – 1.6kg	100mg/kg
1.61kg – 1.7kg	105mg/kg
1.71kg – 1.8kg	110mg/kg

Prior to each injection, 2 grams of glucose/kg body wt dissolved in 10 cc of distilled water, was administered orally to each rabbit to counteract the anticipated alloxan hypoglycemia. The dose of alloxan monohydrate for each rabbit was selected very carefully based on not only the weight but also the general condition of the animal. The rabbits between 1.6 to 1.8 kgs did not sustain the alloxan administration well and showed greater mortality than the others; probably because of being aged (rabbits show a directly proportional age/weight relationship).

The required dose was dissolved in 8 cc of distilled water in a petri dish and was injected into the marginal ear veins by a insulin needle mounted on a 10 cc syringe contrary to the tuberculin syringe method (Butt, 1962).

8 days after administration of alloxan, the surviving rabbits having a RBS of more than 200 mg/dl were taken as diabetic and employed for further testing. In case of the animal being found non-diabetic, a repetition of alloxan doses even lower than those administered previously was done at an interval of 5 to 10 days each. For example, a rabbit previously given 75 mg/kg was given 55 mg/kg the second time and 30 mg/kg the third time – if found non diabetic. Each time re-evaluation of RBS, weight and general health status of the rabbit was done before re-injecting alloxan monohydrate. Most of the

Table-2
Blood Glucose Levels of Rabbits before and after Alloxan Administration

S. Number of Rabbit	RBS Levels Before Alloxan (Mg/Dl)	RBS Levels After Alloxan (Mg/Dl)
1	110.33	472.55
2	118.18	455.81
3	109.09	496.21
4	123.55	396.72
5	100.07	413.95
6	149.76	237.03
7	129.76	225.46
8	129.95	388.97
9	106.41	529.41
10	116.57	618.75
11	102.67	230.66
12	111.76	296.65
13	142.87	698.65
14	151.87	311.53
15	92.51	503.84
16	81.28	263.10
17	149.41	529.41
18	124.70	618.75
19	101.76	230.66
20	103.04	296.65
21	118.27	698.65
Mean ± SD	117.80±19.32	424.44 ± 155.89
SEM	4.21	34.01

rabbits became diabetic following a third administration of alloxan and a final total dose not exceeding 200 mg/kg. Some required 4 attempts and final doses upto 240 mg/kg. However, a few hardy ones (two) were able to sustain extremely high total doses of 280 mg/kg and upto 5 attempts and were still not diabetic upon completion of data collection.

All the surviving diabetic rabbits had blood sugar levels ranging from 200 mg/dl to 699mg/dl. As they survived without insulin injections, the rabbits had developed Type-2 diabetes which was the requirement of our animal diabetic model.

Symptoms following Administration of Alloxan in Rabbits

Induction of alloxan diabetes by beta cell necrosis of the islets of langerhans required few minutes to few hours to many days to be expressed. As it caused **B** cell necrosis there was a massive release of pre-formed insulin from the dying beta cells. A confirmation of the hypoglycemia was done by measuring RBS (found to be as low as 15 mg/dl) of the animals just prior to their deaths. These seizures were found to occur within the first few minutes, a few hours or even upto two days, of injecting alloxan monohydrate.

RESULTS

The random blood sugar levels of 21 rabbits were recorded before and after alloxan administration and were found to be as shown in Table-2.

STATISTICAL ANALYSIS

A statistical analysis was done using SPSS version 10.The paired t-test was applied and showed highly significant increase (p value < 0.001) in blood sugar random levels of the rabbits after alloxan administration as compared to before alloxan administration.

DISCUSSION

Following its administration, alloxan is concentrated in the islets of Langerhans and in the liver where it is reduced to dialuric acid. This acid is unstable in aqueous solutions and undergoes oxidation back to alloxan, accompanied by generation of O_2, H_2O_2 and OH radicals by fenton type reaction (Uchigata, 1982).

Latest research has found alloxan to be an effective pro-oxidant selectively cytotoxic to ß cells of the pancreatic islets of langerhans (Shanti, 1994). Hydroxy radicals generated cause single stranded breaks in the islets cell DNA. There is a two-fold increase in lipid conjugated dienes, the primary products of lipid peroxidation (Shanti and Ramakrishnan 1994). The glutathione and catalase activity which can scavange these free radicals are present in large amounts in the liver. On the contrary, the beta islet cells of the pancreas have low quantities of these enzymes and are extremely vulnerable to free radical injury (Halliwell 1989). Alloxan induced experi-mental diabetes is also associated with marked reduction of anti-oxidant enzyme superoxide dismutase activity in islets cells. In anti-oxidant enzyme superoxide dismutase activity (Halliwell, 1989).

Other reputed theories of its B cytotoxi-city are:

1. Alloxan induced diabetes is also suggested to result from initial islet cell inflammation followed by activation of macrophages and lymphocytes might be the source of cytotoxic oxygen radicals (Trivedi *et al.,* 2004).
2. Alloxan has been shown to inactivate a calcium and calmodulin dependent protein kinase which reduces insulin secretion (Katzung 1993).
3. (Gillman *et al.,* 1990) claim that structural similarity between alloxan and D glucose may be responsible for its affinity with the **B** receptor on the **B** cell.
4. Alloxan binds almost instantly to islets cell membranes and causes rapid in vitro or in vivo inhibition of the insulin secretory mechanism (Gordsky, 1982).
5. According to (Burger, 1960) zinc removal from insulin in chelate form may be the reason for its diabetogenic effect.

CONCLUSION

It was concluded from this study that alloxan monohydrate cannot be administered as a single standardized dose per kg body weight (160mg/kg) as suggested by Akhtar *et al.* (1982), or the 80mg/kg body weight dose recommended by Puri and Prabhu (2002). The requirement is for careful dose selection based on body weight as determined by us in Table-1.

The general condition of the rabbit must be borne in mind as well. Additionally before injecting alloxan, oral administration of 2gms of glucose/kg body weight dissolved in 10cc of distilled water reduces both immediate and delayed animal mortality. Thus only after fulfillment of these requirements can research time be saved and accurate research results be obtained.

REFERENCES

Akhtar, M.S., Athar, M.A. and Yaqub, M. (1981). Effect of Momordica charantia on blood glucose levels of normal alloxan diabetic rabbits. *Planta Med*., **32**: 103-105.

Bonar, J.R. (1980). Diabetes. 2nd ed. Medical Examination Publishing Company Inc., pp.25-7.

Burger, A. (1960). Medicinal Chemistry. 2nd ed. New York: Inter Science Publishers, Inc. p.62.

Butt, T.A. (1962). The hypoglycemic response to the glucagon in normal and alloxan diabetic rabbits (M.Phil. thesis), University of Karachi, p.57.

Gillman, A.G., Rall, T.W., Nies, A.S. and Taylor, P. (1991). Pharmacological basis of therapeutics. 8th ed. New York, Macmillan Publishing Co., pp.1463-1585.

Gordsky, G.M. (1982). *Diabetes.* **31**(Suppl): 45-53.

Halliwell, B. and Gutteridge, J.M.C. (1989). Free Radicals in Biology and Medicine. 2nd ed. Oxford: Clarenden Press.

Katzung, B.G. (1993). Basic and Clinical Pharmacology. Philadelphia: WB Saunders, pp.586-598.

Merck Index (1976). 9th ed. Merck & Company, Inc. Rahway NJ, USA, p.274.

Puri, D., Prabhu, K.M. and Murthy, P.S. (2002). Mechanism of actions of a hypoglycaemic principle isolated in Fenugreek seeds. *Indian J Physiol Pharmacol*., 46.

Shanti, V.P. and Ramakrishnan, P. (1994). Mechanism of the antioxidant effect of Bordetella pertussis extract. *Indian J Biochem Biophy*., **31**: 398-402.

Trivedi, N.A.,Mazumdar, B., Bhatt, I.D. and Hemavathi, K.D. (2004). Effects of shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. *Indian Journal of Pharmacology*, **36**(6): 373-376.

Uchigata, Y., Yamamoto, H., Kawamura, A. and Okamoto, H. (1982). *J Biol Chem.,* **257**: 6084-88.