

Alloxan Not an Ideal Drug for Diabetes Induction

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Abstract

Background : Alloxan-induced diabetes model is used as a “study tool” to elucidate the pathophysiology of the disease and much more as a “search engine” for antidiabetic compounds with better therapeutic characteristics. It was the first agent used in the category of chemically induced diabetes to create a model of insulin dependent diabetes mellitus. Other chemicals being: Streptozocin, Dexamethasone, Insulin antibodies – induced diabetes

Methods: Albino rats were divided into four groups with ten rats in each group. alloxan monohydrate 2%, solution which was dissolved in 0.9% of sodium chloride (normal saline) as a diluent and given intraperitoneally to rats and blood glucose estimation made by using glucometer.

Total 40 albino rats were taken and divided into 4 groups

- Group A - 10 rats receiving normal saline
- Group B - 10 rats received alloxan at a dose of 150 mg/kg
- Group C - 10 rats received alloxan at a dose of 160 mg/kg
- Group D - 10 rats received alloxan at a dose of 170 mg/kg

Result: Highest rate of mortality and alopecia were noted in group D receiving alloxan at a dose of 170 mg/kg whereas highest percentage of fluctuation in fasting blood glucose range was seen in group C receiving alloxan at a dose of 160mg/kg.

Conclusion: Such unpredictable response shows that alloxan is not ideal drug for induction of diabetes in experimental animal. Mortality, fasting blood glucose returning to non diabetic range and alopecia are the chief drawbacks

Keywords: alloxan , alopecia, diabetes

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I. Introduction

The pancreas is an organ composed of [98%] exocrine and [2%] endocrine cells. Islets of Langerhans, which form the endocrine part of pancreas, consist of four types of cells α cell, β cell, δ cell and PP cells, which secrete glucagons, insulin, somatostatin and pancreatic polypeptide¹.

Glucose stimulates the β cell to release insulin, which then promotes glucose uptake and storage in various tissues. Diabetes mellitus is a disease characterized by derangement in carbohydrate, protein and fat metabolism caused by complete or relative insufficiency of insulin secretion and/ or insulin action.²

In 1980 von mering was working on the absorption of fat from the intestine after removing the pancreas of a dog³. The dog developed polyuria and polydipsia and was found to have diabetes mellitus. Many experiments on rabbits and dogs followed, although history has given special place to Marjorie, one of the dogs used by banting and best in their seminal experiments on the isolation and purification of insulin in the 1920s⁴. This makes Marjorie one of the most famous experimental animal model in history.

Chemically induced type-1 diabetes is the most commonly used animal model of diabetes. Chemical agents which produce diabetes can be classified into 3 categories and include agents that:

1. Specifically damage β cell
2. Cause temporary inhibition of insulin production/ or secretion
3. Diminish the metabolic efficacy of insulin in target tissues

In general, chemicals is the first category are of intrest as they reproduce lesions resembling IDDM.

Alloxan is a cyclic urea analog. The compound has the molecular formulae, $C_4H_2N_2O_4$ and a relative molecular mass of 142.06. Alloxan was first used in 1818 by Brugnatelli and described by Frederick Wohler and Justin Liebig in 1838⁵. Its use in induction of diabetes in experimental animals was first reported by Dunn and McLetchie in their study in which they successfully induced diabetes in experimental rabbits⁶. This

discovery made, several researchers to use alloxan-induced diabetes model as a “study tool” to elucidate the pathophysiology of the disease and much more as a “search engine” for antidiabetic compounds with better therapeutic characteristics. It was the first agent used in the category of chemically induced diabetes to create a model of insulin dependent diabetes mellitus. Other chemicals being:

1. Streptozocin
2. Dexamethasone
3. Insulin antibodies – induced diabetes

Mechanism of action

The mechanism by which it induces diabetes is not well defined. Alloxan is highly reactive molecule and readily reduced to diuleric acid, which is then auto-oxidized back to alloxan resulting in the production of free radicals⁷. These free radicals damage the DNA of beta cells and cause cell death. Second mechanism proposed for alloxan is its ability to react with protein SH groups, especially the membrane proteins like glucokinase on the beta cells, finally resulting in cell necrosis. However, there are major species differences in response of alloxan.

Aims and Objectives

1. To detect mortality rate in alloxan induced diabetic rats
2. To detect fluctuation in fasting blood glucose range in alloxan induced diabetic rats
3. To detect anomalies in alloxan induced diabetic rats.

II. Material And Methods

The present study was conducted as pilot study in the department of pharmacology, MGM Medical College, Jamshedpur. The interventions and investigation to be carried out in albino rats was done by adopting the terms prescribed by CPCSEA guidelines⁸. Healthy male wistar rats weighing between 150-200 grams were taken for the present study. All albino rats were kept in clean and dry cages, with 12 h: 12 h light-dark cycle at room temperature and humidity. They were acclimatized to the available housing condition and were fed with standard laboratory diet consisting of soaked black gram (Kala Chana) and water was given *ad libitum*. The cages were floored with a layer of saw dust for absorption of urine of rats. This was done because after induction of diabetes by alloxan there would be excess of urination of rats

Inclusion Criteria:

- 1) Normal rats: Adult male albino rats weighing 150– 200g.
- 2) Diabetic rats: Alloxan induced diabetic male rats with blood glucose more than 250 mg/dL.

Exclusion criteria:

- 1) The albino rats which do not fall in the above mentioned weight are excluded from study.

III. Methodology

Animals were divided into four groups with ten rats in each group. Total of 40 healthy male albino rats weighing 150-200g were included in the study. The methods employed in this study to induce diabetes was chemical method using alloxan monohydrate 2%, solution which was dissolved in 0.9% of sodium chloride (normal saline) as a diluent given intraperitoneally to rats and blood glucose estimation made by using glucometer. Fasting blood glucose levels was measured before starting the treatment and after alloxan administration at 24 hr , 48 hr and 72hr by using glucometer. Rats with blood glucose level between 250-300 were included in the study.

Method of blood collection

Blood samples were collected from the tail of rat, since it is the most venous part of body of rat. The tail of rat was cleaned with spirit cotton and then with the help of sterilized blade, it was cut 0.5mm just enough to allow one drop of blood to ooze out and was collected directly on the strip placed in the glucometer (Dr. Morepen Gluco One BG-03 Gluco meter).

Total 40 albino rats were taken and divided into 4 groups

- a. Group A - 10 rats receiving normal saline
- b. Group B - 10 rats received alloxan at a dose of 150 mg/kg
- c. Group C - 10 rats received alloxan at a dose of 160 mg/kg
- d. Group D - 10 rats received alloxan at a dose of 170 mg/kg

All the rats were kept for next 24 hr on 10% glucose to prevent hypoglycemia. After 72 hrs fasting blood glucose was checked using accucheck glucometer. Rats with fasting blood glucose more than 200 mg/dl was

considered to have developed diabetes. Thereafter fasting blood glucose was recorded at day 3, 7, 14, 21 and 28 and 35 day.

Observation

1. Mortality

Group A – No mortality was observed in control group

Group B – 20% of mortality

Group C – 42% of mortality

Group D – 70% of mortality

2. Fluctuation in fasting blood glucose range

In diabetic rats fluctuating fasting blood glucose with high blood sugar returned to non diabetic range at different time period.

Stable diabetes developed for 35 days in

Group A – control group

Group B – 5%

Group C – 20%

Group D – 10%

3. Alopecia

Group A- no alopecia

Group B – no alopecia

Group C – no alopecia

Group D – 10 % of rats developed alopecia

IV. Discussion

The efficacy, safety and toxicity of alloxan for induction of experimental diabetes has been challenged by a number of researchers.. Jain and Arya have demonstrated several anomalies and inconsistencies in alloxan-induced diabetes model, that lead us to raise a level of concern and attention regarding its use in induction of diabetes⁹. The autoreversal and unstability of alloxan-induced hyperglycemia is particularly of utmost concern. Alloxan is also known to cause multiphasic glucose response characterized by inconsistent increase and decrease in blood glucose concentration¹⁰. Even if stability is achieved, the duration of such stable hyperglycemia is on the average less than a month and this period is not adequate for proper evaluation of a test drug. Moreover, alloxan does not exactly induce the human type 2 diabetes mellitus which accounts for about 90–95% of all diabetic cases¹¹. Alloxan has been observed to stimulate a type 1 form of diabetes when used in animals. This form of diabetes is often associated with high level of ketoacidosis that arguably is partly responsible for the high animal mortality rate (30–60%)¹².

V. Conclusion

Such unpredictable response shows that alloxan is not ideal drug for induction of diabetes in experimental animal. Mortality, fasting blood glucose returning to non diabetic range and alopecia are the chief drawbacks. although many reports shows a high success rate in diabetes induction by rapid i.v injection but this too is associated with significant high mortality.

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