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Research Article

Peculiar glycemic patterns in alloxan-induced diabetes animal model

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Background: Laboratory investigations on diabetes mellitus (DM) have employed various animal models. Alloxan, a β-cell toxin, has widely been used to induce DM in animals. The current study reports peculiar glycemic patterns during alloxan DM induction.

Method: Thirty eight, six-month old *Sprague Dawley* rats were assigned to two groups: control (n=14) and experimental (n=24). Alloxan (125 mg/kg) was administered intraperitoneally to the experimental group, and an equal volume of normal saline to the control. Using a glucometer, fasting blood glucose (FBG) levels were assessed on days 0, 3, 6, 13, 20 and 27.

Results: At day 3, FBG levels were higher in the experimental group (19.26±1.77 vs. 3.21±0.04 mmol/l, $p<0.01$). Thereafter, FBG levels in the experimental group reduced gradually till day 27, though remaining higher than the control (7.59±0.86 vs. 3.22±0.13 mmol/l, $p<0.01$). The FBG levels in the control oscillated between 3.14±0.04 and 3.26±0.05 mmol/l. Out of the 24 rats administered with alloxan, only 10 were diabetic at day 27.

Conclusion: Induction of diabetes in rats using alloxan (125mg/kg) leads to variability in glucose levels. A number of animals greater than required may be necessary to accommodate failed diabetes induction. Follow-up for at least one month is necessary before confirming induction.

Key words: animal model, alloxan, diabetes induction

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1. Introduction

Animals are commonly used for experimentation in academic and research institutions (Kimwele et al, 2011). The reliability of a study is partly determined by the animal model used. The researcher must therefore have a fine understanding of animal models when designing animal studies. The current study focuses on animal models used in investigating diabetes mellitus (DM), and its management. These animal models differ significantly and no single one has been reported to accurately represent the essential pattern of type 2 DM in humans, in whom the disease is often preceded by obesity and various molecular changes. Animal models are developed by techniques such as pancreatectomy, chemical induction, genetic engineering, molecular biology and islet cell transplantation (Junod et al, 1969; Lenzen et al, 1988; Lanza et al, 1999; Rees and Alcolado, 2005; Frode and Medeiros, 2008).

Advances in genetic engineering have led to breeding of specific animal strains which are susceptible to development of type 1 and 2 DM. These include the 'non-obese diabetic mouse' and 'bio-breeding rat' for type 1 DM (Rees and Alcolado, 2005). Molecular biological techniques such as gene targeting and transgenic transfer have also been used. Animal models with specific gene alterations or with modified genes incorporated within their genome have been developed (Frode and Medeiros, 2008). However, commonly used genetic models deviate from human type 2 DM, because they contain mutations which are rare in human type 2 DM (Coleman, 1978; Surwit et al, 1984; Kuhn et al, 1987). Islet cell transplantation studies have also been

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conducted in animal models, notably dogs and rodents (Lanza et al, 1999).

In the majority of studies, chemical-induced diabetes models have been utilized (Frode and Medeiros, 2008). Such chemicals include alloxan (Lenzen, 2008), and streptozotocin (Junod et al, 1969). As at 2010, streptozotocin had reportedly been used in 69% of chemical-induced diabetes animal models, whereas alloxan was the second most commonly used chemical at 31% (Etuk, 2010).

Alloxan is a urea derivative which causes selective necrosis of the pancreatic islet β-cells (Etuk, 2010). It is used experimentally to induce type 2 DM in animals such as rabbits, rats, mice and dogs. The experimental dose of the drug needs careful consideration in order to avoid excessive pancreatic tissue damage. The most frequently used intravenous dose of alloxan in rats is 65 mg/kg, but its effective dose must be higher when it is administered intraperitoneally or subcutaneously (Antia et al, 2005). With the rising use of alloxan-induced DM models, different dosages and different methods of inducing diabetes have been reported. Though most methods have demonstrated success, a handful have reported failed induction or variation in blood glucose following alloxan administration (Etuk, 2010). The current study was thus carried out to assess the pattern of blood glucose following intraperitoneal administration of alloxan (125 mg/kg).

2. Materials and Methods

2.1 Study animals

Thirty eight (38), six month old female *Sprague Dawley* rats weighing between 200 - 250 g were obtained from the Biochemistry animal laboratory, University of Nairobi. The animals were then housed in plastic cages in the animal house, Department of Medical Physiology, University of Nairobi, where a twelve hour light/dark cycle was maintained. The animals were fed on commercial pellets from a local supplier (Unga Feeds Ltd). Water was provided *ad libitum*. The rats were randomly assigned to control (n = 14) and experimental (n = 24) groups. The animals were allowed a 15 day acclimatization period after which baseline blood glucose and body weights were taken. Thereafter, diabetes was induced on the experimental group using alloxan (125 mg/kg), while the control group received an equal volume of normal saline.

2.2 Induction of diabetes mellitus

The experimental animals were fasted for 16 hours before inducing DM. The animals were intraperitoneally injected with a single dose of alloxan (125 mg/kg), dissolved in 10% sodium chloride (Lenzen et al, 1988; Reyes et al, 2006; Lenzen, 2008). The alloxan was obtained from the Department of Medical Physiology and stored at 4 °C. The blood glucose levels were assessed on days 0, 3 and 6 following administration of alloxan. Thereafter, the animals were followed up for 3 weeks with a weekly assessment of the blood glucose levels. The blood sample was obtained by sequential snipping of the tail (Fluttert et al, 2000). A glucometer (On Call® Plus, Acon Industries, Inc. 4108, San Diego, USA) was used to measure the blood glucose levels. Animals were considered diabetic if fasting blood glucose (FBG) level was above 7.1 mmol/l (Kwanghee et al, 2009).

2.3 Ethical considerations

The study protocol was approved by the Postgraduate Research Committee, Department of Medical Physiology, University of Nairobi. Animals were handled in accordance with the guidelines of the US National Research Council for the care and use of laboratory animals (National Academy Press, 1996).

2.4 Data and statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Student's t-test was employed to test statistical significance using SPSS Version 16.0. Significance levels were set at $p < 0.05$.

3. Results

Baseline FBG levels (day 0) were 3.06 ± 0.16 mmol/l and 3.24 ± 0.05 mmol/l in the experimental and control groups respectively. However, the FBG level increased significantly in the experimental group (19.26 ± 1.77 vs. 3.21 ± 0.04 mmol/l, $p < 0.01$) at day 3. Thereafter, the FBG level in the experimental group reduced gradually till day 27, though still remaining higher than the control group (7.59 ± 0.86 vs. 3.22 ± 0.13 mmol/l, $p < 0.01$). On the other hand, the FBG levels in the control group oscillated between 3.14 ± 0.04 mmol/l and 3.26 ± 0.05 mmol/l for the duration of the study (**Figure 1**).

Of the 24 experimental rats, four (16.67%) maintained normal blood glucose levels whereas 2 (8.33%) became glucose intolerant at day 6. Two rats (8.33%) achieved high glucose levels (> 30 mmol/l) at day 6 and died before day 27 (**Table 1**). Sixteen (66.67%) rats were 'diabetic' at day 6, though one of these died before day 27. Five (5) of the sixteen (16) rats recovered and became normoglycemic by day 27. Thus, of the 24 rats administered with alloxan, only 10 had successfully induced diabetes by day 27. On the other hand, all control rats (n=14) were alive and normoglycemic at day 27.

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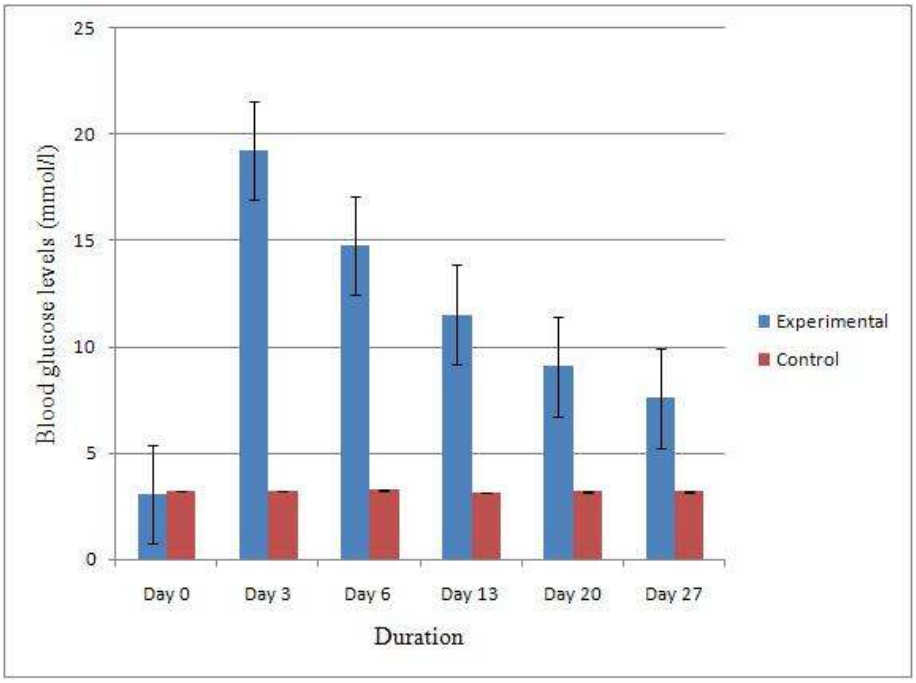

Figure 1: Effect of Alloxan on Fasting Blood Glucose level in rats (error bars ± SEM)

Table 1: Glycemic and Death outcomes of rats following alloxan administration

FBG at day 6	Number of rats	Outcome at day 27
Normal ($3.2 \leq x \leq 6.0$)	4	Alive
Glucose intolerant ($6.1 \leq x \leq 7.0$)	2	Alive
Diabetes mellitus(DM) ($7.1 \leq x \geq 10$)	3	Alive
DM ($10.1 \leq x \geq 20$)	7	Alive
DM ($20.1 \leq x \geq 30$)	6	5 alive, 1 died
DM (> 30)	2	Died

FBG, Fasting blood glucose; DM, Diabetes mellitus

4. Discussion

Alloxan is a known β-cell toxin that has widely been used in inducing DM (Lenzen et al 1988). Effects of alloxan (125 mg/kg) noted in the present study were: elevation of blood glucose levels to values still within the normal range, induction of glucose intolerance and ultimately induction of DM. This variation in response to the same dose of alloxan (125 mg/kg), demonstrates inter-individual dissimilarity among the rats with respect to resistance to the diabetogenic effect of alloxan. Such dissimilarity within a species may demonstrate cytological variation in the number and properties of β-cells. This may partly explain the varied response to similar environmental conditions in the development of DM among the general population. Besides the varied response, these results corroborate previous findings on the role of alloxan in the destruction of β-cells, thereby reducing pancreatic ability to secrete insulin (Lenzen, 2008).

Of the twenty four (24) experimental rats, only 16 (66.7%) were 'diabetic' at day 6, a number that later fell to 10 (41.7%) by the end of the induction period (day 27). This was probably due to the recovery of partially destroyed β-cells. This gradual decrease in blood glucose levels in the experimental group could also be due to hormones counter regulatory to hyperglycaemia (Gelfand et al, 1984). In administering alloxan to induce DM, it is thus prudent to begin with a larger number of animals so as to take care of such variation. Furthermore, it is appropriate to follow up the animals for up to a month before any experimentation, to account for possible β-cell recovery.

Several mechanisms have been proposed to explain the diabetogenic effect of alloxan. Alloxan induces the formation of reactive oxygen species (superoxide radicals, hydrogen peroxide and hydroxyl radicals) which mediate cellular damage (Lenzen, 2008). This cellular damage may in addition induce auto-immune reactions against the β-cells. Secondly, alloxan disrupts

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the formation of microtubules as well as destroying those already formed (Schmidt et al, 1990). Thirdly, alloxan is thought to inhibit the enzyme O-linked N-acetyl glucosamine transferase (Konrad et al, 2002), which is very abundant in the β-cells and catalyzes protein O-glycosylation.

Alloxan and streptozotocin produce diabetes mellitus by selectively destroying pancreatic β-cells, and thus cause type 1 diabetes (Rakieten et al, 1963; Wilson et al, 1984). Type 2 diabetes can be induced with these drugs by manipulating the dosages and timings of administration to destroy only a portion of β-cells (Portha et al, 1989; Serradas et al, 1991; Beppu et al, 1993). These animal models, however, conceptually deviate from the pattern of type 2 DM in humans, in whom the disease is often preceded by obesity. Furthermore, insulin resistance in humans is a complex process that involves several mediators and other mechanisms outside the pancreas. In addition, rats have dissimilar pancreatic islet β-cell pathology compared to humans. This has implications since β-cell destruction is crucial in the development and progression of DM.

The use of alloxan has been demonstrated to produce similar results in both female and male *Sprague Dawley* rats (Bernard, 1980). Furthermore, young animals have been reported to cope better than old animals with alloxan-induced stress (Bernard, 1980). The relatively old age of the animals used (6 months old) in the current study could have partly contributed to the deaths observed. In addition, it is known that the experimental dose of alloxan must be carefully selected to avoid excessive pancreatic tissue damage. As multiple methods and dosages of alloxan continue to be used for DM induction, we recommend their standardization for ease of replication.

5.0 Conclusion

Induction of diabetes in rats using alloxan (125 mg/kg) leads to variability in glucose levels. A larger number of animals may be required during administration of alloxan to take care of variation in DM induction. A follow-up period of up to one month may be necessary before confirming diabetes induction.

Conflict of Interest declaration

The authors declare no conflict of interest

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