Original Article

Pre-diabetic Clinical Changes Induced by Low Doses of Alloxan-Streptozotocin Cocktail in Rabbits

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ABSTRACT

Background & Objectives: **Alloxan & streptozotocin are used for inducing diabetic models. Their combination has been used to reduce the individual chemical dosage and minimize the side effects. Present investigation was aimed at studying pre-diabetic clinical changes induced by low doses of Alloxan-STZ cocktail in rabbits.**

Materials and Methods: **New Zealand White rabbits, 1-1.5 kg body weight, were administered alloxan (@50 mg/kg b.w.) and STZ (@ 35mg/kg b.w.) cocktail, as single intravenous dose. Blood glucose levels were monitored (0 h, 20 min, 1 h, and then hourly up to 9 h) and clinical signs noted. Rabbits surviving up to 9 hours were given glucose therapy.**

Results: **The cocktail caused immediate transient hypoglycaemia, followed by hyperglycaemia, and then progressively severe hypoglycaemia. Hypoglycaemia caused characteristic behavioural alterations from lethargy, through aesthesia, muscular weakness to recumbency. Severely affected rabbits revealed intermittent convulsions and died in coma.**

Conclusion: **Low dose Alloxan-STZ cocktail induced triphasic immediate response in rabbits. The behavioural changes reflected glycaemic status serving as a guide for institution of glucose therapy.**

Key words: **Alloxan, Streptozotocin Prediabetic State, Rabbit**

Introduction

Experimental studies in animals have been used extensively for elucidating pathogenesis of diabetes mellitus (DM), its complications, and studying new treatments including drug testing, islet cell transplantation and preventative strategies. Most experiments are carried out on rodents. Either spontaneously or induced models have been used. Drug induced models are more frequently used and the most

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widely used drugs are streptozotocin (STZ) and Alloxan, which induce hyperglycaemia through selective destruction of insulin producing β-cells (1).

Alloxan, an oxidized product of uric acid, is a β-cytotoxic glucose analogue (2), and is commonly used for development of animal model of Type-I DM (T1DM) (3). Alloxan exerts its diabetogenic action when it is administered parenterally (intravenously, intraperitoneally) or subcutaneously. The dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status (4).

Streptozotocin (STZ) is an *N*-methyl-*N*-nitrosoureido D-glucosamine derivative originally isolated from *Streptomyces achromogenes*. It is specifically toxic to pancreatic beta-cells (3, 4) and is being used to induce both T1DM and type 2 DM (T2DM). It is preferred over alloxan as a β-cytotoxic agent because of its more specific action; comparatively broader dose range and longer half-life (15 min); producing sustained hyperglycaemia for longer duration; developing well characterized diabetic complications with fewer incidences of ketosis; and reduced mortality (1). However, its sensitivity has been reported to vary with species, strain, sex and nutritional state. Also, batch differences in activity have been reported (1, 5).

Rabbits are increasingly used as experimental diabetic models especially in pharmacological studies (6). However, in contrast to other rodent species, alloxan has been the chemical of choice for this species because of the relative ineffectiveness of STZ for induction of diabetes or development of well characterized diabetic complications (1). Further, beta-cytotoxicity induced by these drugs (especially alloxan) causes sudden release of insulin leading to severe hypoglycaemia and even mortality if glucose therapy is not given (7, 8). Hence combination of drugs viz. STZ and nicotinamide in adult rats (9) and Gottingen pig (10); and alloxan and STZ in dogs (11), has been used to reduce the individual chemical dosage and minimize the side effects.

The present investigation was aimed at studying pre-diabetic clinical changes induced by low doses of Alloxan-STZ cocktail in rabbits.

Materials and Methods

New Zealand white rabbits of three months age and weighing about 1 to 1.5 kg were utilized in the study. The experimental protocols involved in this study were approved by the Institutional Animal Ethics Committee, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K vide No. AU/FVS/Estt/C-09/7983-88 dated 19-01-2010 and conforms to the guidelines for the Care and Use of Laboratory Animals. All the animals were acclimatized for a period of 7 days prior to the commencement of the experiments. Rabbits were maintained under standard conditions in cage system and offered feed and water *ad libtum*. Commercially procured rabbit feed and greens were given twice a day (morning and evening).

Alloxan monohydrate and STZ were procured from Sigma-Aldrich and administered as a cocktail to 18 rabbits intravenously through ear vein using insulin syringe. Alloxan was given @ 50 mg/kg body weight in 1mL sterile water, followed immediately by STZ @ 35mg/kg body weight in 1mL freshly prepared citrate buffer (containing 100 mM citric acid and 100 mM sodium citrate at pH 4.6). Rabbits were fasted for 18 hours prior to drug administration.

After administration of beta-cytotoxic drugs, rabbits were monitored for immediate effects vis-à-vis changes in blood glucose levels, and clinical signs including changes in behavior, appearance, activity, water/feed intake, urination/defaecation or any other deviation. The blood glucose levels were recorded using glucometer (Accu-Chek, Roche diagnostics India Pvt. Ltd., Mumbai) before treatment (0 h) and after drug adminstration at 20 min, 1 h, and then up to 9 h at hourly intervals. At this stage rabbits were given 5 mL of 25% dextrose intraperitoneally, and 10% glucose in drinking water up to 24 hours post-treatment.

The data were analyzed by one-way ANOVA using SPSS software and values expressed as mean ± SE.

Results

The fasting blood glucose (FBG) level prior to administration of alloxan-STZ cocktail was 113.05 ± 3.381 mg/dL (96 to 146 mg/dL). At 20 min after treatment the blood glucose level 96.44 ± 1.838 mg/dL (85 to 115 mg/dL), decreased significantly (P<0.05). Thereafter, significant (P<0.05) increase was observed peaking at 3 h. At 1 h it approached the normal fasting value (115.77 ± 2.156 mg/dL; 100 to 130 mg/dL), and at 2 and 3 h reached to 132.50 ± 1.977 mg/dL (120 to 149 mg/dL) and 144.22 ± 2.101 mg/dL (127 to 160 mg/dL), respectively. A significant (P<0.05) drop in the blood glucose levels was recorded from then onwards up to 9 h after which glucose therapy was given. The values were 116 ± 2.750 mg/dL (100 to 135 mg/dL), 103.05 ± 3.031 mg/dL (81 to 120 mg/dL), 87.50 ± 2.230 mg/dL (65 to 108 mg/dL),68.88 ± 3.250 mg/dL (48 to 95 mg/dL), 58.50 ± 3.160 mg/dL (40 to 82 mg/dL), and 51.00 ± 3.004 mg/dL (30 to 71 mg/dL), respectively, at 4, 5, 6, 7, 8 and 9 h post-treatment (Fig. 1).

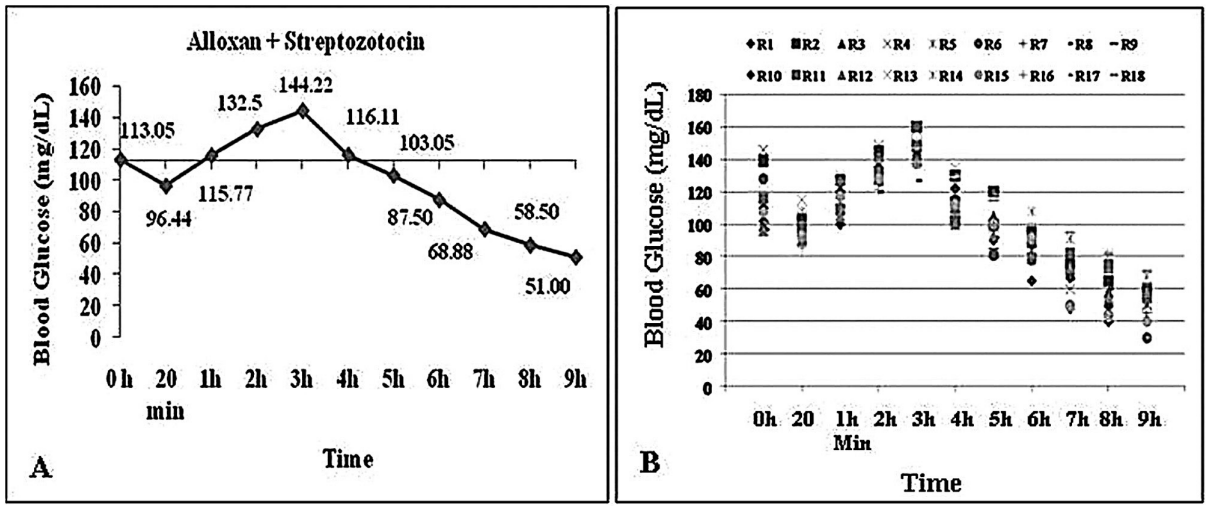

Fig. 1: General (A) and individual (B) trend of immediate changes in blood glucose levels of rabbits following administration of single intravenous dose of alloxan@ 50 mg/kg b.w. and streptozotocin @ 35 mg/kg b.w. cocktail

Clinically the rabbits were normal up to first 5 hours after which the hypoglycaemic signs appeared. The rabbits looked lethargic, dull, and depressed. Later a sudden increase in activity was noted. They dashed aimlessly either spontaneously or in response to noise and when approached. It was followed by appearance of muscular weakness which progressed from neck to hind legs. The rabbits sat quietly showing lordosis and did not move even when prompted, but ears were held upright (Fig. 2a). Some rabbits showed slight tilting of head. The blood glucose level was 70 to 90 mg/dL at this stage. In four rabbits severe hypoglycaemic signs were noted. At 7 to 8 h the rabbits showed drooping of head with chin touching the ground and limbs folded (Fig. 2b). This was followed by abduction of forelimbs and resting on chest on the ground thereby assuming a sprawling posture with limbs spread out laterally, chin and thorax touching the ground in three rabbits and the remaining one rabbit revealed lateral recumbency with intermittent paddling of limbs. At this stage blood glucose ranged from 40 to 50 mg/dL. The first three rabbits were also laterally recumbent within 15 to 20 minutes. The rabbits showed marked rotation of head and frequently rolling movements on longitudinal axis of the body. When held in

air, head downwards, rotation of body axis in the direction that of rotation of head was evident at lumbar and thoracic region (Fig. 2c). Accucheck revealed the blood glucose levels low (< 30 mg/dL) at this stage.

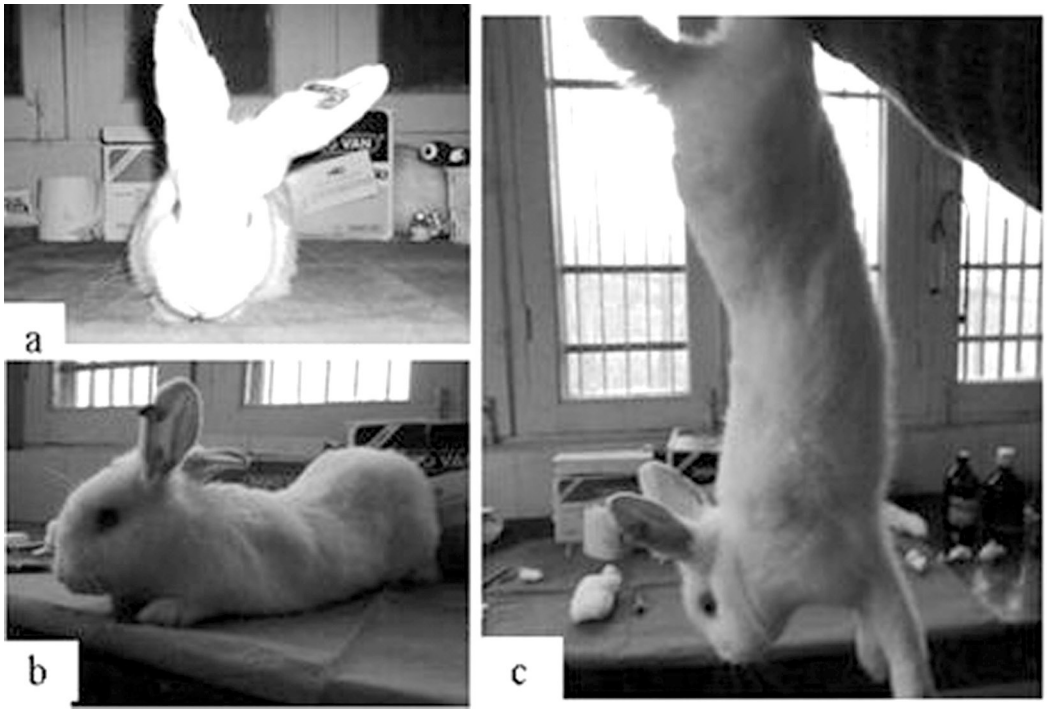

Fig. 2: a)Rabbits following administration of single intravenous dose of alloxan (@50 mg/kg b.w.) - streptozotocin (@35mg/kg b.w.) cocktail sitting quietly, with curved back ;b) drooped head with chin touching the ground and limbs folded ;c) rotation of body axis, in the direction of rotation of head, at lumbar and thoracic region

At 9 h post-treatment all the four severely hypoglycaemic rabbits were administered 10mL of 25% dextrose intravenously through ear vein while the remaining rabbits were administered with 5mL of 25% dextrose intraperitoneally. Within 5 minutes after glucose administration rabbits started to move and restored feeding with intermittent rest. The rabbits appeared normal after about two hours of glucose therapy. However, in two of the severely affected rabbits, hypoglycaemic state relapsed causing death within 24 hours.

Discussion

The triphasic response caused by intravenous administration of alloxan - STZ cocktail has been reported in dogs (11). The effect seemed to be essentially alloxan mediated, which has been found to induce triphasic response in all the susceptible animals including rabbits (4, 8). Both alloxan and streptozotocin are glucose analogs and their uptake in pancreas is mediated by GLUT2 receptors (7, 12). Immediately following uptake by pancreatic beta-cells, alloxan induced transient stimulation of insulin secretion leading to brief hypoglycaemia (3), which in turn favour beta-cytotoxic activity of both the drugs (3, 4). The increase in blood glucose levels after initial hypoglycaemia may be ascribed to direct effects of alloxan and STZ causing impaired glucose oxidation, inhibition of insulin biosynthesis and secretion, as well as to hypoinsulinaemia mediated increased release of glucose into blood from liver due to loss of negative neural control on gluconeogenesis (3, 13).

The progressive hypoglycaemia observed following the brief hyperglycaemia may be ascribed

to temporary return of β-cell responsiveness to glucose, followed by drug induced beta-cytolysis (14). Alloxan elevates cytosolic free Ca^{2+} concentration in pancreatic β-cells facilitating insulin release (15). Both alloxan and STZ cause altered redox potential and generation of ROS leading to cytolysis (4). Further, STZ has been found to cause DNA alkylation (16), protein glycosylation (17), resulting in beta-cytotoxicity and apoptosis (18). High insulin levels within the islet exerts paracrine inhibitory effects on α-cells (19) whereas high insulin in blood results in CNS mediated negative regulation of counter-regulatory hormones including glucagon, epinephrine, norepinephrine and cortisol (20, 21). Moreover, insulin acts on the hypothalamus to regulate hepatic glucose production (22) further favoring development of hypoglycaemia.

The pattern of glucose response to alloxan-STZ cocktail was similar in all rabbits. Glucose therapy was given at 9 hours post-drug administration. Anderson *et al.* (11) observed a marked drop in blood glucose in dogs from 5 mM/L (= 90 mg/dL) to 3-4 mM/L (=54-72 mg/dL) at 9 hours post administration of alloxan-STZ cocktail. The nature and progression clinical signs simulated to that observed in alloxan induced hypoglycaemia and severity was inversely related to blood glucose levels (8). In alloxan or STZ induced hypoglycaemia, feedback responses are partially compromised warranting exogenous glucose infusion. Individual variations observed in the rate and severity of changes in blood glucose levels may be attributed partially to the inherent differences in anti-oxidant levels and counter-regulatory process.

The behavioral changes observed are in congruence with the observations in human and other animals (20, 23, 24) and have been attributed to catecholamines (norepinephrine and epinephrine), in an adrenergic receptor-mediated manner (23) and neuroglycopaenia associated impairment of psychomotor functions (20, 25, 26). This is supported by the observed prompt recovery following intravenous glucose therapy (27). In our previous study with high dose of alloxan the hypoglycaemic changes progressed much faster warranting glucose therapy by 5h post treatment (8). However, the progression of hypoglycaemia was slower with low dose alloxan-STZ cocktail.

Conclusion

Low dose alloxan-STZ cocktail induced triphasic immediate response in rabbits. The behavioral changes reflected glycaemic status serving as a guide for institution of glucose therapy.

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