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COMPARATIVE EVALUATION OF DIABETOGENIC POTENTIALS OF ALLOXAN, STREPTOZOTOCIN AND THEIR COCKTAIL IN RABBITS (*Oryctolagus cuniculas*)

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ABSTRACT

Comparative diabetogenic potentials of alloxan, streptozotocin (STZ) and alloxan-streptozotocin cocktail in rabbits was investigated. 'New Zealand White' rabbits, 1-1.5 kg body weight (b.w.), were administered 100 mg alloxan kg^{-1} b.w and 65 mg STZ kg^{-1} b.w, individually and 50 mg alloxan kg^{-1} b.w. and 35 mg STZ kg^{-1} b.w. cocktail, as single intravenous dose. Fasting blood glucose levels were monitored on day 0, 3, 5, and 7. Rabbits with fasting blood glucose levels > 200 mg dL^{-1} were considered diabetic. Alloxan caused early induction of sustained hyperglycaemia while relatively resistant rabbits revealed mild hyperglycaemia on day 3 followed by progressive recovery. In contrast, alloxan-STZ cocktail caused a progressive increase in hyperglycaemic animals upto 7 days. However, the overall diabetogenic efficiency was less than alloxan alone. The hyperglycaemia was mild to moderate when alloxan-STZ cocktail was used as compared to that induced by alloxan alone which resulted in moderate to severe hyperglycaemia. STZ failed to induce significant and sustained hyperglycaemia in rabbits. It may be concluded that low dose alloxan-STZ cocktail is a choice for the induction of diabetic rabbit model whereas rabbits appeared relatively resistant to diabetogenic effects of STZ. Further, spontaneous progressive recovery warrant extra caution in evolving and extrapolating results while using such models.

Keywords: Alloxan, cocktail, diabetes mellitus, rabbits, streptozotocin

INTRODUCTION

Animal models, either spontaneous or induced, are extensively used in studies focused on understanding diabetic complications and therapeutics. While rodents are the choice animals, β-cytotoxic drugs - streptozotocin (STZ) and alloxan are more frequently and widely used drugs for inducing hyperglycaemia through selective destruction of insulin producing β-cells. Both, alloxan and STZ are glucose analogue and are avidly taken up by β-cells of islets of Langerhans through GLUT-2 receptors. Alloxan typically induces Type-I Diabetes Mellitus (T1DM), whereas STZ induces both T1DM and type 2 DM (T2DM) (Tripathi and Verma, 2014). STZ 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 periods; developing well characterized diabetic complications with fewer incidences of ketosis; and reduced mortality. However, its sensitivity reportedly varies with species, strain, sex and nutritional state. Also, batch differences in activity have been reported (Mir *et al.*, 2015).

Rabbits (*Oryctolagus cuniculas*) are increasingly used as experimental diabetic models in pharmacological studies. In contrast to other rodent species, alloxan has been the chemical of choice for this species (Wang *et al.*, 2010). Although STZ has been used (Mir *et al.*, 2008), some workers have reported its relative ineffectiveness for induction of diabetes or development of well characterized diabetic complications (Srinivasan and Ramarao, 2007). Further, β-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 (Mir *et al*., 2013a,b, 2015). Hence, combination of drugs *viz.*, STZ and nicotinamide in adult rats and Gottingen pig and alloxan and STZ in dogs have been used to reduce the individual chemical dosage and minimize the side effects (Srinivasan and Ramarao, 2007). However, such combinations have not been reported in rabbit models. Studies on early glycaemic changes following administration of low dose of alloxan and STZ cocktail in rabbits have revealed a triphasic response simulating the effects of alloxan (Mir *et al.*, 2014). The present investigation was aimed at comparative evaluation of the diabetogenic potentials of alloxan, STZ, and their low dose cocktail in rabbits.

MATERIALS AND METHODS

'New Zealand White' rabbits of 3 months age and weighing 1.0 - 1.5 kg were utilized in this study. The experimental protocols involved were approved by the Institutional Animal Ethics Committee, Faculty of Veterinary Sciences & Animal Husbandry, SKUAST-K vide No. AU/FVS/Estt/C-09/ 7983-88 dated 19 Jan., 2010 and conforms to the guidelines for the Care and Use of Laboratory Animals. All the animals were acclimatized for 7 days prior to the experimentation. Rabbits were maintained under standard conditions in cage system and offered feed and water *ad libitum*. Commercially procured rabbit feed and greens were given twice a day (morning & evening).

Alloxan monohydrate and streptozotocin (STZ) were procured from Sigma-Aldrich. For studying diabetogenic effects 18, 13 and 18 rabbits, respectively, were administered with alloxan, STZ and alloxan-STZ cocktail as a single intravenous dose. Rabbits were fasted for 18 hr prior to the drug administration. Individually alloxan was given @ 100 mg kg^{-1} body weight (b.w.) in 1 mL sterile water as slow intravenous injection and STZ @ 65 mg kg^{-1} b.w. in 1 mL freshly prepared citrate buffer (containing 100 mM citric acid and 100 mM sodium citrate at pH 4.6) as slow intravenous injection. In rabbits receiving cocktail of the two drugs, alloxan was given @ 50 mg kg^{-1} b.w. in 1 mL sterile water, followed immediately by STZ @ 35 mg kg^{-1} b.w. in 1 mL freshly prepared citrate buffer (Mir *et al.*, 2014). Fasting blood glucose levels were recorded on day 0, 3, 5 and 7 of the study using one touch glucometer. Diabetogenic response was evaluated on the basis of sustained or progressive hyperglycaemia upto 7 days. Rabbits with blood glucose levels > 200 mg dL^{-1} at day 7 were considered diabetic. The data were analyzed by ANOVA followed by Duncan's test, using SPSS software and values expressed as mean±SE (Snedecor and Cockran, 1989).

RESULTS AND DISCUSSION

Diabetogenic response of β-cytotoxic drugs was evaluated on the basis of sustained or progressive hyperglycaemia upto 7 days where rabbits with blood glucose levels > 200 mg dL^{-1} were considered diabetic. Although the response in individual rabbits differed widely, the mean blood glucose values showed a progressive increase (Table 1). The mean blood glucose levels on day 3, 5 and 7 in alloxan and alloxan-STZ cocktail treated rabbits were comparable within groups, but significantly ($p \leq 0.05$) higher when compared with the initial pretreatment mean fasting glucose level and those

Table 1: Changes in blood glucose (mg dL^{-1}) upto 7days following administration of single intravenous dose of alloxan (@ 100 mg kg^{-1} b.w.), STZ (@ 65 mg kg^{-1} b.w.) and alloxan (@ 50 mg kg^{-1} b.w.)-STZ (@ 35 mg kg^{-1} b.w.) cocktail in rabbits (mean±SE)

Groups	Day of treatment			
	Day 0	Day 3	Day 5	Day 7
Alloxan (ALL)	113.63 ± 3.64^{aA}	258.56 ± 29.29^{bA}	297.00 ± 36.85^{bA}	324.88 ± 45.20^{bA}
(N = 16)	(98.00 - 149.00)	(89.00 - 510.00)	(95.00 - 630.00)	(95.00 - 690.00)
Streptozotocin	112.54 ± 3.02^{aA}	115.00 ± 11.76^{aB}	161.85 ± 9.93^{bB}	95.39 ± 3.12^{aB}
STZ (N = 13)	(97.00 – 128.00)	(58.00 – 231.00)	(120.00 – 250.00)	(81.00 – 118.00)
ALL + STZ	112.31 ± 3.68^{aA}	166.13 ± 10.75^{bB}	187.63 ± 14.68^{bcB}	217.94 ± 22.48^{cC}
(N = 16)	(96.00 - 146.00)	(89.00 - 255.00)	(95.00 - 320.00)	(101.00 - 467.00)
Control	115.30 ± 4.95^{aA}	114.900 ± 3.87^{aB}	119.50 ± 3.80^{aB}	116.00 ± 4.61^{aB}
(N = 10)	(98.00 - 145.00)	(95.00 - 132.00)	(100.00 - 137.00)	(98.00 - 142.00)

Means in different rows having at least one common 'lower case alphabet' superscript, and in different columns with at least one common 'upper case alphabet' superscript do not differ significantly at $P \leq 0.05$; Values in brackets are range.

of control. In STZ-treated rabbits, it was significantly higher on day 5 (161.85±9.93 mg dL^{-1}) when compared with day 0, 3 and 7.

Pretreatment fasting blood glucose levels of 3 groups were comparable between groups. The mean blood glucose levels on day 3, 5 and 7 in alloxan treated groups were significantly higher than control, STZ and alloxan-STZ cocktail group rabbits. Further, alloxan-STZ cocktail group had significantly higher mean blood glucose level on day 7 in comparison to control and STZ group.

Out of the 16 alloxan-treated rabbits that survived initial hypoglycaemia 11 animals showed consistent hyperglycaemia with blood glucose levels >200 mg dL^{-1} on day 3, 5 and 7, giving 68.7% efficacy for inducing diabetes (Table 2). On day 3 out of the rest 5 rabbits 3 (18.7%) showed blood glucose levels between 150 to 200 mg dL^{-1} followed by progressive recovery of one by day 5 and the other 2 by day 7 when the blood glucose levels were <150 mg dL^{-1}. Out of 13 STZ-treated rabbits, on day 3 and 5 blood glucose >200 mg dL^{-1} were observed in 1 (7.7%) and 2 (15.4%) rabbits, respectively, and between 150 to 200 mg dL^{-1} in 1 (7.7%) and 5 (38.5%) rabbits, respectively. However, no rabbit was hyperglycaemic on day 7. Following administration of alloxan-STZ cocktail on day 3, 5 and 7 blood glucose levels > 200 mg dL^{-1} were noticed in 3, 5 and 10 rabbits, and from 150 to 200 mg dL^{-1} in 8, 6 and 1 rabbits.

Alloxan @100 mg kg^{-1} b.w. caused early induction of sustained hyperglycaemia while relatively resistant rabbits revealed mild hyperglycaemia on day 3 followed by progressive recovery. In contrast, alloxan-STZ cocktail induced a progressive increase in hyperglycaemic animals upto 7 days. However, the overall diabetogenic efficiency was less than alloxan alone. The hyperglycaemia was mild to moderate when alloxan-STZ cocktail was used as compared to that induced by alloxan alone which resulted in moderate to severe hyperglycaemia. STZ failed to induce significant and sustained hyperglycaemia in rabbits. Though early mild to moderate hyperglycaemia was noted in some rabbits yet it was followed by complete recovery on day 7.

Alloxan-induced diabetes was recognized early (day 3) with progressive hyperglycaemia and 68.7% rabbits showed moderate to severe hyperglycaemia. In rabbits, alloxan has been the chemical of choice for inducing diabetes (Wang *et al.*, 2010). Individual variation in susceptibility to diabetogenic action of alloxan may be attributed to variable susceptibility of individual islets evidenced histopathologically by changes ranging from degranulation to complete necrosis of β-cells (Mir *et al.*, 2013b). Certain species like musk shrew and cats reportedly are absolutely resistant to development of hyperglycaemia with general toxic effects prevailing at higher doses of alloxan (Ohno *et al.*, 1998). In other species, including rabbits, the state of resistance seems to be relative with higher doses resulting in increased diabetics and more severe hyperglycaemia. In rabbits suboptimal dose (@ 60 mg kg^{-1}) has been found to induce mild glucose tolerance (Puri, 2006).

Table 2: Induction of diabetes mellitus in rabbits following the administration of single intravenous dose of alloxan (@ 100 mg kg^{-1} b.w.), STZ (@ 65 mg kg^{-1} b.w.) and alloxan (@ 50 mg kg^{-1} b.w)-STZ (@ 35 mg kg^{-1} b.w) cocktail (Values are percentage & number)

Blood glucose (mg dL^{-1})	Day 3			Day 5			Day 7		
	<150	150-200	≥200	<150	150-200	≥200	<150	150-200	≥200
Alloxan (ALL) (N = 16)	12.50 (2)	18.75 (3)	68.75 (11)	18.75 (3)	12.50 (2)	68.75 (11)	31.25 (5)	0.00 (0)	68.75 (11)
Streptozotocin STZ (N=13)	92.30 (12)	7.69 (1)	0 (0)	46.15 (6)	38.46 (5)	15.38 (2)	100.00 (13)	0.00 (0)	0.00 (0)
ALL + STZ (N=16)	31.25 (5)	50.00 (8)	18.75 (3)	31.25 (5)	37.50 (6)	31.25 (5)	31.25 (5)	6.25 (1)	62.50 (10)
Control (N=10)	100.00 (10)	0.00 (0)	0.00 (0)	100.00 (10)	0.00 (0)	0.00 (0)	100.00 (10)	0.00 (0)	0.00 (0)

Values in braces represent absolute number

STZ induced mild hyperglycaemic state upto day 5 with 15.4% diabetics. However, 4 rabbits showed hypoglycaemia on day 3 and none was hyperglycaemic by day 7. Single intravenous doses of STZ have been used for induction of IDDM in adult rats (Patel *et al*., 2006) and mice (Sharma *et al.,* 2006*)*. Reports for rabbits are inconsistent, varying from relative resistance (Rees and Alcolado, 2005) to development of diabetes (Eman and Eman, 2011). The discrepancies may partly be attributed to cut off glucose levels for diabetics and partly to the time post-treatment for evaluation. Mir *et al.* (2008) reported induction of diabetes in rabbits using STZ (@ 65 mg kg^{-1} b.w single i.v. dose). However, the authors evaluated diabetic status on day 2 and rabbits with blood glucose >150 mg dL^{-1} were considered diabetic. Several studies in humans and rodents suggested that age is a factor in susceptibility to STZ, possibly related to changes in glucose transport (Yang and Wright, 2002; Dufrane *et al*., 2006). Kramer *et al.* (2009) reported that GLUT2 expression was diminished in very young and very old rhesus monkeys compared with juvenile and adult which showed moderate to strong GLUT2 expression. STZ has been reported to lack demonstrable diabetogenic effect on the human fetal β-cell and malignant insulinomas (Tuch *et al*., 1989). Contrary to earlier reports of extended mild hyperglycaemic phase with slow recovery in rabbits (Mir *et al.*, 2008), none of the STZ treated rabbits was hyperglycaemic by day 7. Histopathological evaluation at later periods during present study revealed variably affected islets (unpublished). It could be concluded that early release of insulin by degranulation and partly by lysis of β-cells in some islets might have resulted in the observed hypoglycaemia. The hyperglycaemia upto day-5 probably reflects a transient refractory phase or recovery period with normoglycaemia being achieved by day-7. Thus, evaluation of susceptibility to diabetogenic effects of STZ in rabbits warrants deciphering biochemical and molecular events at different dose levels *viz-a-vis* its toxicological effects.

Alloxan-STZ cocktail caused progressive hyperglycaaemia upto day 7 when 62.5% rabbits were diabetic. Induction of diabetes mellitus by a single injection of alloxan-STZ cocktail has been reported in male beagle dogs (Stitt *et al.,* 1994) in an attempt to reduce the individual chemical dosage and hence minimize its side effects. In view of the relative resistance of rabbits to diabetogenic effects of STZ, the effects of cocktail seem to be principally mediated by alloxan. However, further studies are needed to decipher the possible mode of interaction. It may be concluded that low dose of alloxan-STZ cocktail is a choice for induction of diabetic rabbit model whereas rabbits appeared relatively resistant to diabetogenic effects of STZ. Further, spontaneous progressive recovery warrants extra caution in evolving and extrapolating results while using such models.

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