

The Influence of Glimpiride on the Biochemical and Histomorphological Features of Streptozotocin - Induced Diabetic Rabbits

Sajad Hussain Mir¹, M.M. Darzi², Fayaz Ahmad¹, M.Z. Chishti¹ and M.S. Mir²

¹Postgraduate Department of Zoology, University of Kashmir, Srinagar - 190006, Kashmir,

²Department of Veterinary Pathology, FVSC and AH, Shuhama (Alusteng),
SKUAST, Srinagar - 190006, Kashmir

Abstract: An experiment was conducted to study the efficacy of glimepiride (a new sulphonylurea) on the biochemical values and histomorphological features of streptozotocin-induced diabetic rabbits. Eighteen New Zealand white male rabbits were selected for the study. The rabbits were randomly divided into three Groups of six each. Group I was kept as normal healthy control (NC), Group II and Group III were made diabetic by single intravenous administration of streptozotocin (@ 65 mg/kg. b.w.). Glimepiride was given to Group III rabbits by gavage @ 2mg/kg. b.w. daily for twenty-one days whereas Group I and Group II rabbits received normal saline in the similar manner. The results indicated a significant decrease in the blood sugar ($P<0.01$), blood urea ($P<0.001$) and serum creatinine ($P<0.001$) in Group III rabbits. Further, the histomorphological study showed an increase in the percentage of beta cells in pancreatic islets and recovery of renal tubules in Group III rabbits. The findings indicate that glimepiride improves the biochemical values and ameliorates the histopathology of diabetic rabbits particularly restoring the morphology of beta cells of islets of Langerhan's and thus, seems to be encouraging.

Key words: Glimepiride, streptozotocin, diabetes, biochemistry and histopathology

Introduction

Streptozotocin induces severe and irreversible hyperglycemia in experimental animals (Mitra *et al.*, 1996). The action of streptozotocin in beta cells is accompanied by characteristic alterations in blood insulin and thereby glucose concentrations (West *et al.*, 1996). It impairs glucose oxidation (Bedoya *et al.*, 1996), decreases insulin biosynthesis and secretion (Nukatsuka *et al.*, 1990) and alters the histomorphology of most of the organs (Mir *et al.*, 2008). The microvascular and macrovascular complications in diabetes are the major causes of morbidity and death in diabetic subjects (Nagappa *et al.*, 2003). The search for more effective and safer hypoglycemic agents therefore, has continued to be an area of search of interest (Krishna *et al.*, 2004).

Sulphonylurea have represented the backbone of NIDDM therapy for more than 30 years (Groop, 1992). The insulinotropic effect of sulphonylurea is augmented by glucose and they apparently increase beta cell sensitivity to glucose and non-glucose stimuli (Pfeifer *et al.*, 1980).

Glimepiride has been developed for glycemic control in diabetic patients and represents the third generation sulphonylurea. It effectively inhibits the development of oxidative stress in diabetes (Krauss *et al.*, 2004) by possessing a potent extrapancreatic effect on glucose metabolism and may directly stimulate glucose transport activity through phospholipid signalling pathway (Takada *et al.*, 1996).

The present study was designed to investigate the effect of glimepiride on biochemical values and histopathological features of streptozotocin induced diabetic rabbits.

Materials and Methods

The study was conducted in 18 New Zealand white male rabbits of almost uniform age acclimatized to prescribed standard laboratory conditions (Anonymous, 2000). Twelve rabbits were made diabetic by single dose of streptozotocin (@ 65mg/kg. b.w.) dissolved in 1ml of freshly prepared citrate buffer, pH 4.5 and administered intravenously following twelve hours fasting whereas remaining six were kept as control that received equal volume of normal saline.

When diabetes mellitus was well established in rabbits which was confirmed by fasting hyperglycemia, the rabbits were divided into three groups of six each. Group I comprised of normal untreated rabbits (NC), Group II comprised saline treated diabetic rabbits (DC) and Group III comprised diabetic rabbits that received glimepiride (@ 2mg/ kg.b.w.) daily for 21 days. The assessment of treatment was based on blood sugar, blood urea and serum creatinine levels estimated on day 7th, 14th and 21st. At the end of 21st day the rabbits were sacrificed for histological examination of pancreas and kidneys.

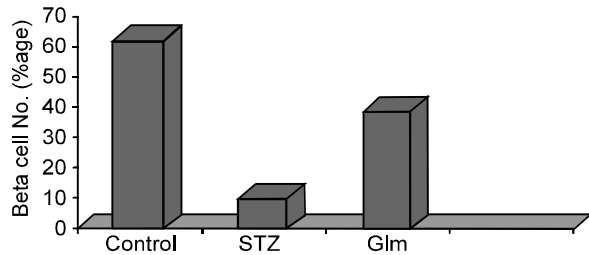
Analytical procedure: The blood sugar of rabbits was estimated by Glucometer Gx (Bayer Diagnostic India, Ltd.), blood urea by "DAM Method" and serum creatinine

Mir et al: Efficacy of Glimepiride

Table 1: Effect of glimepiride on blood sugar (f), blood urea and serum creatinine of streptozotocin-induced diabetic rabbits

Factor Group	DAYS											
	Initial Value			7th			14th			21st		
	BS (F)	BU	SC	BS (F)	BU	SC	BS (F)	BU	SC	BS (F)	BU	SC
NC	94±	19.5±	1.25±	99±	19±	1.05±	105±	21.03±	1.29±	102±	9.75±	1.21±
	6.06	0.64	0.20	5.95	1.46	0.20	4.66	1.58	0.25	3.191	0.84	0.04
DC	292±	53±	3.32±	285±	52.9±	3.12±	210±	46.6±	3.00±	192±	40.1±	2.80±
	10.60	2.11	0.16	9.46	1.80	0.16	8.83	1.25	0.15	9.41	1.16	0.14
Glimepiride treated	281±	48.25±	3.13±	154±	30.25±	2.46±	119±	24.25±	1.48±	86±	18.75±	0.98±
	9.11	1.54	0.08	4.96	1.79	0.05	4.29	0.84	0.17	8.39+	0.84++	0.05+

Values represent mean±SEM; BS(F): Blood Sugar (Fasting); BU: Blood Urea; SC: Serum Creatinine; NC: Non-diabetic control rabbits; DC: Diabetic rabbits treated with normal saline; +p < 0.01, ++p < 0.001 compared with DC.



Control = Non-diabetic control rabbits
 STZ = Stropozo tocin-induced diabetic rabbit
 GLM = Glimepiride treated diabetic rabbit

Fig 1: Comparative percentage of beta cells in different groups of Rabbits.

by 'Alkaline Picrate Method" using commercially available kits. Histological examination was done by fixing pancreas and kidneys of rabbits in 10% formalin, processed and embedded in paraffin wax. Tissue blocks were sectioned 5 micron thick and stained with Harris Haematoxylin and Eosin (Luna, 1968). However, to demonstrate pancreatic islet cells, a modification of Gomori's staining technique (Scott, 1952) was used. For quantitative assessment of beta cells, cells of approximately four islets of each tissue and forty islets of each group were counted under trinocular microscope at a magnification of x1000.

Statistical analysis: All values were expressed as mean and analyzed using students 't' test (Prasad, 2000). 'P' value was obtained from the distribution of 't' probability chart.

Results

Diabetes mellitus in rabbits was induced by single intravenous administration of streptozotocin that was confirmed by elevated levels of fasting blood glucose, blood urea and serum creatinine levels. The subsequent effects of streptozotocin induced diabetes resulted in degenerative and lytic changes in pancreatic islets and kidneys of rabbits.

Glimepiride treatment effectively improved the biochemical levels in diabetic rabbits and the drug was

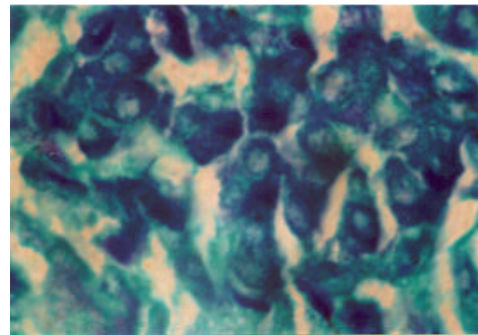


Fig. 2: Pancreatic islet of glimepiride treated diabetic rabbit showing beta cells with deep purple granules [modified Gomori's aldehyde fuchsin (Scott, 1952) x 1000].

not found to cause hypoglycemia. Table 1 shows the efficacy of glimepiride on blood glucose, blood urea and serum creatinine levels of diabetic rabbits. The histological study of the pancreatic sections by using special stain showed restoration of beta cell morphology in Group III rabbits (Fig. 2) that was also evident by the comparative percentage of beta cells with Group II rabbits (Fig. 1). Further, recovery of renal morphology was observed histologically in Haematoxylin and Eosin stained sections of Group III rabbits.

Discussion

The induction of diabetes mellitus in rabbits was confirmed by elevated levels of fasting blood glucose. Streptozotocin is well known for its selective pancreatic islet beta-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Papaccio et al., 2000) and induces severe and irreversible hyperglycemia (Mitra et al., 1996). Intravenous administration of streptozotocin (@ 65 mg/kg b.w.) in the present study effectively induced diabetes mellitus in rabbits and is in consonance with earlier methods of induction (Tawfeeg and Sherif, 2001; Mir et al., 2008). A decline of blood sugar level following glimepiride treatment observed in the present study is in total

agreement with earlier workers (Takada *et al.*, 1996; Krauss *et al.*, 2004). Sulphonylurea bind to specific receptors on beta cells resulting in closure of potassium ATP channels and subsequently open calcium channels leading to an increase in cytoplasmic calcium that stimulates insulin release (Pilipson and Steiner, 1995). There is much controversy about the mode of action of sulphonylurea and specifically whether they lower blood glucose through extra pancreatic mechanisms other than stimulation of insulin secretion (Groop, 1992). However, studies suggest that glimepiride has a potent extra pancreatic effect on glucose metabolism and may directly stimulate glucose transport activity through phospholipid signaling pathway (Takada *et al.*, 1996). Further studies have shown that glimepiride has more rapid onset than previous sulphonylureas (both glyburide and glipizide) and consequently less risk of hypoglycemia (Geisen, 1988).

The degenerative changes in pancreatic beta cells of streptozotocin-induced diabetic rabbits observed in the present study can be attributed to the production of free radicals (Teshamariam, 1994) that damage beta cells selectively (Pusztai *et al.*, 1996). However, an increase in the number of beta cells in the islets of Langerhan's in glimepiride-treated diabetic rabbits in comparison to saline treated diabetic rabbits can be attributed to the fact that glimepiride affect the activation of the redox sensitive transcription factor NF(Kappa)B in vitro and in vivo and possesses the free radical quenching properties (Schiekofer *et al.*, 2003). Although the mechanism of beta cell neoformation is not clear but there is strong evidence that islet stem cells may exist in the pancreatic duct and that these ductal epithelial cells may be switched into a proliferative/regenerative phase leading to nesideoblastosis (neogenesis of islets) (Hellerstrom, 1984; Bonner-Weir *et al.*, 1993). Lipsett and Finegood (2002) reported beta cell neoformation from precursor cells in the pancreatic duct of diabetic rats. According to Waguri (1997) the beta cells can regenerate either through differentiation of the precursor cells from the pancreatic duct, or proliferation from existing or surviving mature beta cells.

In the present study the improvement in blood urea, serum creatinine and subsequent amelioration of histomorphological changes in kidneys of glimepiride treated rabbits can be attributed to the recovery of renal function (Tedong *et al.*, 2006), which is explained by the regenerative capability of the renal tubules (Kissane, 1985). Studies have shown that good metabolic control is beneficial in slowing the progression of nephropathy in diabetes and if the duration of diabetes is prolonged before reinstatement of normoglycemia, nephropathy is not easily reversed (Floreto *et al.*, 1998; Renu *et al.*, 2004). Tedong *et al.* (2006) have reported that the normoglycemia in diabetic rats with treatment therapies

could ameliorate the glomerular and tubular lesions that characterize diabetic nephropathy and subsequently recover renal morphology and function.

References

- Anonymous, 2000. Guidelines for care and use of animals in scientific research. Revised Edn. Indian National Scientific Academy, New Delhi.
- Bedoya, F.J., F. Solano and M. Lucas, 1996. N-monomethyl-arginine and nicotinamide prevent streptozotocin-induced double strand DNA break formation in pancreatic rat islets. *Experientia*, 52: 344-347.
- Bonner-Weir, S., L.A. Baxter, G.T. Schuppin and F.E. Smith, 1993. A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. *Diabetes*, 42: 1715-1720.
- Floreto, P., M.W. Steffes, E.R.D. Sutherland, C.F. Goetz and M. Mauer, 1998. Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N. Eng. J. Med.*, 339: 69-75.
- Geisen, K., 1988. Special pharmacology of the new sulphonylurea glimepiride. *Drug Res.*, 38: 1120-1130.
- Groop, L., 1992. Sulphonylurea in NIDDM. *Diabetes Care*, 15: 737-754.
- Hellerstrom, C., 1984. The life story of the pancreatic beta-cell. *Diabetologia*, 26: 393-400.
- Kissane, J.M., 1985. *Anderson's Pathology*, 8th Edn. Toronto: Washington Univ. School Med., pp: 54-759.
- Krauss, H., M. Grazymislawski, J. Kozlik, P. Sosnowski, J. Piatek, K. Mikrut, P. Mackowiak and J. Paluszak, 2004. The influence of glimepiride on the binding kinetics of insulin with its skeletal muscle and liver receptors in rats with short term and prolonged hyperglycemia induced by streptozotocin. *Med. Sci. Monit.*, 10: BR11-6.
- Krishna, B., S. Nammi, M.K. Kota and R.V. Krishna, Rao, 2004. Evaluation of hypoglycemic and antihyperglycemic effects of *Datura metel* Linn seeds in normal and alloxan-induced diabetic rats. *J. Ethnopharmacol.*, 9: 95-98.
- Lipsett, M. and D.T. Finegood, 2002. Beta-cell neogenesis during prolonged hyperglycemia in rats. *Diabetes*, 51: 1834-1841.
- Luna Lee, G., 1968. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology* 3rd Edn. McGraw-Hill Book Company, New York.
- Mir Sajad Hussain, A. Baqui, R.C. Bhagat, M.M. Darzi and A.W. Shah, 2008. Biochemical and histomorphological study of streptozotocin-induced diabetes mellitus in rabbits. *Pak. J. Nutr.*, 7: 359-364.

- Mitra, S.K., S. Gopumadhavan, T.S. Muralidhar and S.J. Seshadri, 1996. Effect of D-400 a herbomineral formulation on liver glycogen content and microscopic structure of pancreas and liver in streptozotocin induced diabetes in rats. *Indian J. Exp. Biol.*, 34: 964-967.
- Nagappa, A.N., P.A. Thakurdesai, N. Venkat, Rao and Jiwan Singh, 2003. Antidiabetic activity of *Terminalia catappa* Linn fruits. *J. Ethnopharmacol.*, 88: 45-50.
- Nukatsuka, M., Y. Yoshimura, M. Nishida and J. Kawada, 1990. Importance of the concentration of ATP in rat pancreatic beta cells in the mechanism of streptozotocin induced cytotoxicity. *J. Endocrinol.*, 127: 161-165.
- Papaccio, G., F.A. Pisanti, M.V. Latronico, E. Ammendola and M. Galdieri, 2000. Multiple low dose and single high dose treatments with streptozotocin do not generate nitric oxide. *J. Cell. Biochem.*, 77: 82-91.
- Pfeifer, M.A., J.B. Hatter, R. Graf and D. Porte, Jr., 1980. Potentiation of insulin secretion to non-glucose stimuli in normal man by a tolbutamide. *Diabetes*, 29: 335-340.
- Pilipson, L.H. and D.F. Steiner, 1995. Pas de deux or more: The sulphonylurea receptor and K⁺ channels. *Sci.*, 268: 372-373.
- Prasad, S., 2000. *Fundamentals of Biostatistics (Biometry)*. EMKAY Publications, Delhi.
- Pusztai, P., J. Prechl, A. Somogyi, E. Szaleczky and J. Feher, 1996. Animal models in research of the pathomechanisms of diabetes mellitus. *Orv Hetil*, 137: 1865-1869.
- Renu, A., N.A. Saiyada and S. Odenbach, 2004. Effect of reinstatement of good metabolic control on oxidative stress in kidney of diabetic rats. *J. Diabetes Compl.*, 5: 282-288.
- Schiekofer, S., G. Ruodofsky, Jr. and M. Andrassy, 2003. Glimepiride reduces mononuclear activation of the redox sensitive transcription factor nuclear factor kappa B. *Diabetes Obes Metab.*, 5: 251-261.
- Scott, H.R., 1952. Rapid staining of beta cell granules in pancreatic islets. *St. Technol.*, 27: 267-268.
- Takada, Y., Y. Takata, M. Iwanishi, T. Imamura, T. Sawa, H. Morioka, H. Ishihara, M. Ishiki, I. Usui, R. Temaru, M. Urakaze, Y. Satoh, T. Inami, S. Tsuds and M. Kobayashi, 1996. Effect of glimepiride (HOE 490) on insulin receptors of skeletal muscles from genetically diabetic KK-Ay mouse. *Eur. J. Pharmacol.*, 308: 205-210.
- Tawfeeg, A.O., Najjar and Sherif Y Saad, 2001. Cisplatin pharmacokinetics and its nephrotoxicity in diabetic rabbits. *Int. J. Exp. Chemotherapy*, 47: 128-135.
- Tedong, L., T. Dimo, P.D.D. Dzeufiet, A.E. Asongalem, D.S. Sokeng, P. Callard, J.D.F. Flejou and P. Kamtchouing, 2006. Antihyperglycemic and renal protective activities of *Anacardium occidentale* (Anacardiaceae) leaves in streptozotocin induced diabetic rats. *Afr. J. Trad. CAM*, 3: 23-35.
- Tesfamariam, B., 1994. Free radicals in diabetic endothelial cell dysfunction. *Free Radi. Biol. Med.*, 16: 383-391.
- Waguri, M., 1997. Demonstration of two different processes of beta cells regeneration in a new diabetic mouse model induced by selective perfusion of alloxan. *Diabetes*, 46: 1281-1290.
- West, E., O.R. Simon and E.Y. Morrison, 1996. Streptozotocin alters pancreatic beta cell responsiveness to glucose within six hours of injection into rats. *West Indian Med. J.*, 45: 60-62.