

Study of influence of nicorandil on hypoglycemic action of glibenclamide in alloxan induced diabetic rats

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ABSTRACT

Background: To assess the interaction between ATP - sensitive potassium channel opener nicorandil and potassium channel blocker glibenclamide in alloxan - induced diabetic rats and to evaluate whether nicorandil antagonizes the oral hypoglycaemic action of glibenclamide.

Methods: Insulin secretion involves ATP sensitive K⁺ channel blockade, the influence of ATP sensitive K⁺ channel opener was studied in combination with its blocker. Albino rats of wistar strain, weighing between 200 - 250 grams of either sex were used for the study. Diabetes was induced by injecting alloxan monohydrate 2% solution intra - peritoneally in a dose of 150 mg/kg body weight. Animals with fasting blood glucose between 200 - 300 mg/dl were selected and were divided into 3 groups of six animals each. Group I received 2% gum acacia, group II was given glibenclamide (0.5 mg/kg body weight) and group III was treated with nicorandil (0.3 mg/kg body weight) + glibenclamide (0.5 mg/kg body weight) respectively orally for 30 days. Fasting blood sugar was recorded in all rats on 1st, 3rd, 7th, 14th, 21st and 28th days.

Results: Results show that glibenclamide has significantly reduced the blood sugar levels (P<0.05), whereas when glibenclamide was combined with nicorandil there was a significant rise in blood sugar level (P<0.05).

Conclusions: The study shows that hypoglycaemic action of glibenclamide is antagonized by nicorandil as indicated by worsening of diabetes, probably by blocking the K⁺ channel closing action of glibenclamide. These findings suggest that K⁺ channel openers should be avoided with K⁺ channel blockers in presence of diabetes.

Keywords: Diabetes mellitus, Albino rats, Alloxan monohydrate, Fasting blood sugar, Glibenclamide, Nicorandil

INTRODUCTION

Diabetes and hypertension are the commonest clinical problems of the present day in past middle age group individuals and they may coexist. Further diabetes mellitus may increase the risk of macro vascular complications like hypertension, angina, myocardial infarction, cardiomyopathy, stroke and peripheral vascular disease, as well as microvascular diseases like retinopathy, nephropathy and neuropathy. The management of individual disease is much easier than when they exist together. As diabetes is most often associated with hypertension/angina in some patients, there is a need to prescribe vasodilators. Among vasodilators nicorandil is one drug which is used in angina and hypertension.

METHODS

Wide varieties of drugs are used in the management of hypertension and angina. Potassium channel openers (like nicorandil) are one of the groups of drugs that are useful in angina, which by opening the ATP sensitive potassium channels brings about hyper polarization and produces vasodilatation of vascular smooth muscles. This vasodilator effect of nicorandil is partly antagonized by glibenclamide.¹ Drugs that have their primary site of action at arteriolar level (calcium channel blockers and potassium channel openers) are not beneficial in the treatment of angina. However nicorandil also exerts a nitrate like effect, stimulating guanylyl cyclase to increase cyclic GMP, primarily in epicardial coronary arteries including stenotic segments producing

vasodilatation.² Hence this drug is used in the management of angina/ischemic heart disease. Further minoxidil and diazoxide, which are potassium channel openers have been used since long in severe hypertension and hypertensive emergencies. They also act by opening ATP sensitive potassium channel bringing about hyper polarization and vasodilatation. One of the commonest side effects of diazoxide is hyperglycemia. This is due to opening of potassium channel in beta cells producing hyper polarization that inhibits insulin release. Thus hyperglycemia is the main problem with use of diazoxide in type 2 diabetic patients who are on oral hypoglycemic drugs.³ Like diazoxide the novel potassium channel opener (nicorandil) also activates ATP sensitive potassium channels, which are present both on vascular smooth muscles and membrane of beta cells of pancreas. Hence nicorandil in addition to producing vasodilatation in coronaries may also inhibit insulin secretion.

Hence in a situation where type 2 diabetes is associated with ischemic heart disease/hypertension, there may be a chance that nicorandil may be co-prescribed with glibenclamide for management of angina and this may worsen diabetic control. Thus in anticipation of the above action of nicorandil, this study has been taken up in albino rats. Diabetes was induced with intra - peritoneal injection of 2% alloxan. The results were analysed by noting the effect of nicorandil on insulin secretory action of glibenclamide when it is administered orally.

Mechanism of action of glibenclamide

ATP sensitive potassium channels (K-ATP) determine the resting membrane potential in β -cells. Glucose stimulation of insulin secretion begins with its transport into the β -cells via a membrane transporter called GLUT-2. Glucose phosphorylation by glucokinase is the rate-limiting step that controls glucose regulated insulin secretion. Further metabolism of glucose - 6 phosphate via glycolysis generates ATP which inhibits the activity of an ATP- sensitive potassium channel. This channel consists of two separate proteins: one is the receptor for certain oral hypoglycemics (example: sulfonylurea, meglitinides), the other is an inwardly rectifying K+ channel protein. Inhibition of this k+ channel induces β -cell membrane depolarization, which opens voltage-dependent calcium channels, leading to calcium influx. This calcium signal induces insulin secretion. Second generation sulfonylurea's (like glibenclamide) are the commonly used oral hypoglycemic drugs in the management of type 2 diabetes mellitus which by closing the ATP sensitive potassium channels brings about partial depolarization and produce release of insulin from beta cells.

Mechanism of action of alloxan

The unique capability of alloxan to selectively destroy the pancreatic β -cell was first described by Dunn et al.⁴ The toxic effect of alloxan in these cells seems to be closely

related to increased cellular permeability and morphological abnormalities of plasma membrane of rodents exposed to alloxan has been described.⁵ Alloxan administration leads to a decrease in islet superoxide dismutase activity. Recent studies have suggested mitochondrial dysfunction in the pancreatic β -cells may also be involved in the diabetogenic action of alloxan. As a result, free calcium levels may be increased. Excess calcium concentration in the cell cytoplasm has been known to be noxious and leads to cell death.^{6,7} Alloxan has shown to cause IDDM in dogs, cats, sheep, rabbits, mice, monkeys, fish, turtles and birds.⁸ Only guinea pig is insensitive to the diabetogenic action of alloxan.

Administration of alloxan in to the peritoneal cavity of 2 day old rat has been reported to cause an experimental situation similar to that seen in NIDDM.⁶ Alloxan diabetic animals also possess diabetic complications such as cardiomyopathy, gastroenteropathy and autonomic neuropathy.⁹

Methods of study

Animals

Animals used are albino rats of wistar strain weighing between 200-250 grams of either sex. The animals were randomly selected from central animal facility, JSS Medical College, Mysore. Animals were divided into 3 groups, each group with 6 animals. Animals were fed with laboratory food and water. The institutional animal ethical committee approved the protocol of this study.

Drugs and chemicals

- 2% Alloxan monohydrate: for induction of diabetes, stored between 2 - 8 degree centigrade¹⁰
- 2% Gum acacia as suspending agent, a dried gummy exudate obtained from bark of acacia arabica¹¹
- Glibenclamide (0.5 mg per kg body weight): 2nd generation sulfonylurea, standard drug
- Nicorandil (0.3 mg per kg body weight): k+ channel opener, test drug.¹²

Equipment's

Mouth gag, polythene tube, tuberculin syringe, glucometer

Methodology

Animals were divided into three groups with six rats in each group and were fed with pellet diet and water and acclimatized to laboratory conditions before carrying out any experimental work. For measuring fasting blood glucose, blood was collected from the rat's tail vein by tail cutting method. Following overnight fasting diabetes was induced by injecting freshly prepared 2% alloxan monohydrate solution dissolved in 0.9% sodium chloride

intra-peritoneally in a dose of 150mg per kg body weight.¹³ Following injection, animals were carefully observed for the first 24 hours for any evidence of allergic reaction, behavioural changes and convulsions. No untoward reaction was observed in any animal. Fasting blood glucose was recorded daily morning at around 9.00 am for one week. Animals developed stable hyperglycemia after 4-5 days. Only those animals with blood glucose between 200-300 mg/ dl were selected for the study. Later they were divided into 3 groups, each group having 6 animals. The groups were named as control, standard and test group respectively.

Administration of drugs

Animals belonging to different groups were fed with the following agents.

Control group

6 alloxan induced diabetic rats, orally fed with 0.5 ml of 2% gum acacia for 30 days.

Standard group

6 alloxan induced diabetic rats, orally fed with 0.5 ml of 2% gum acacia + glibenclamide 0.5 mg / kg body weight for 30 days.

Test group

6 alloxan induced diabetic rats, orally fed with 0.5 ml of 2% gum acacia + nicorandil 0.3 mg/kg body weight + glibenclamide 0.5 mg/kg body weight for 30 days.

Fasting blood glucose was recorded in all the animals of each group on 0, 1st, 3rd, 7th, 14th, 21st and 28th day. Insulin

secreting action of the drug is assessed by its capacity to decrease FBS levels and the antagonism of insulin secretory action of the drug by increase in FBS levels.

The results will be analysed by calculating the mean values, the standard deviation, the standard error, the 't'-test, 'p'-value and the analysis of variance (ANOVA). P-Value is the standard table value of 't' at (12 - 2) = 10 degree of freedom for 0.05 (5%) level of significance, $P < 0.05 = \text{significant}$

$P > 0.05 = \text{Not significant}$

The formulas are enclosed in annexure.

RESULTS

Blood glucose comparison within each group

There was no significant difference in the mean values of blood glucose level from day '0' - day '28' in the diabetic control group ($p > 0.05$).

In the standard (glibenclamide) group the difference in the mean values of blood glucose level was found to be statistically significant between 0 to 1st day, 1st to 3rd day, 3rd to 7th day, 7th to 14th day and 21st to 28th day ($p < 0.05$).

In the test (glibenclamide + nicorandil) group the difference in mean values of blood glucose level was not found to be statistically significant ($P > 0.05$) between 0 to 1st day and 1st to 3rd day but later the difference was found statistically significant ($P < 0.05$) between 3rd to 7th day, 7th to 14th day, 14th to 21st day and 21st to 28th day.

Table 1: The FBS values in mg/dl of 1st animal of each group from 0 to 28th day.

Si. no.	Groups	0 day	1 st day	3 rd day	7 th day	14 th day	21 st day	28 th day
A-1	Diabetic control	281	278	280	277	282	279	280
B-1	Standard (G)	229	168	145	128	110	112	108
C-1	Test group (G + N)	248	223	243	309	323	328	334

Table 2: The FBS values in mg/dl of 2nd animal of each group from 0 to 28th day.

Si. no.	Groups	0 day	1 st day	3 rd day	7 th day	14 th day	21 st day	28 th day
A-2	Diabetic control	288	291	281	286	283	280	281
B-2	Standard (G)	267	204	167	162	156	130	118
C-2	Test group (G + N)	273	246	276	266	281	302	320

Table 3: The FBS values in mg/dl of 3rd animal of each group from 0 to 28th day.

Si. no.	Groups	0 day	1 st day	3 rd day	7 th day	14 th day	21 st day	28 th day
A-3	Diabetic control	240	238	240	242	239	237	235
B-3	Standard (G)	250	220	210	198	142	167	148
C-3	Test group (G + N)	268	301	286	305	308	312	336

Table 4: The FBS values in mg/dl of 4th animal of each group from 0 to 28th day.

Si. no.	Groups	0 day	1 st day	3 rd day	7 th day	14 th day	21 st day	28 th day
A-4	Diabetic control	230	232	228	228	224	226	229
B-4	Standard (G)	242	218	206	182	161	138	122
C-4	Test group (G + N)	248	246	248	309	316	321	332

Table 5: The FBS values in mg/dl of 5th animal of each group from 0 to 28th day.

Si. no.	Groups	0 day	1 st day	3 rd day	7 th day	14 th day	21 st day	28 th day
A-5	Diabetic control	282	278	283	280	276	281	279
B-5	Standard (G)	276	242	236	212	186	152	146
C-5	Test group (G + N)	277	276	278	300	307	312	329

Table 6: The FBS values in mg/dl of 6th animal of each group from 0 to 28th day.

Si. no.	Groups	0 day	1 st day	3 rd day	7 th day	14 th day	21 st day	28 th day
A-6	Diabetic control	280	281	278	279	275	276	274
B-6	Standard (G)	282	260	242	202	176	138	120
C-6	Test group (G + N)	272	245	238	302	312	333	342

Table 7: The mean values of blood glucose levels and Std. deviations in different groups of animals.

Groups	0 day	1 st day	3 rd day	7 th day	14 th day	21 st day	28 th day
Diabetic control	267±22.8	266±24.0	265±24.3	265±24.0	263±25.1	263±24.8	263±24.2
Standard	258±18.9	218±31.7	201±38.0	180±31.1	161±27.0	139±18.7	127±16.2
Test-group	264±12.9	256±25.5	261±20.0	298±16.0	307±14.0	318±11.5	332±7.3

Table 8: A comparison of percentage of reduction in blood glucose levels in case of standard and percentage of increase in blood glucose levels in case of combination of glibenclamide with nicorandil.

Group	1 st day	3 rd day	7 th day	14 th day	21 st day	28 th day	Mean
Standard ↓	15.17%	21.78%	29.96%	37.35%	45.91%	50.58%	33.45%
Test drug ↑ (G + N)	5.3% reduction	1.1% reduction	12.8% increase	16.2% increase	20.4% increase	25.7% increase	18.77 - 3.20 = 15.57%

Table 9: The mean values and standard deviation of blood glucose levels in standard (G) and test (G+N) groups and their significance.

Day	Standard (G)	Test group (G+N)	P- value	Inference
0	258±18.9	264±12.9	> 0.05	NS
1	218±31.7	256±25.5	< 0.05	S
3	201±38	261±20	< 0.05	S
7	180±31.1	298±16	< 0.05	HS
14	161±27	307±14	< 0.05	HS
21	139±18.7	318±11.5	< 0.05	HS
28	128±16.2	332±7.3	< 0.05	HS

P < 0.05 = Significant (S) or highly significant (HS); G = Glibenclamide; N = Nicorandil.

Blood glucose comparison between the groups

Between the standard (glibenclamide) and test (glibenclamide + nicorandil) group.

The difference between the mean values of blood glucose levels of standard and test group was found to be statistically significant on 1st and 3rd day and highly significant from 7th day to the 28th day. i.e., the test drug combination has caused significant rise in blood glucose level when compared to standard.

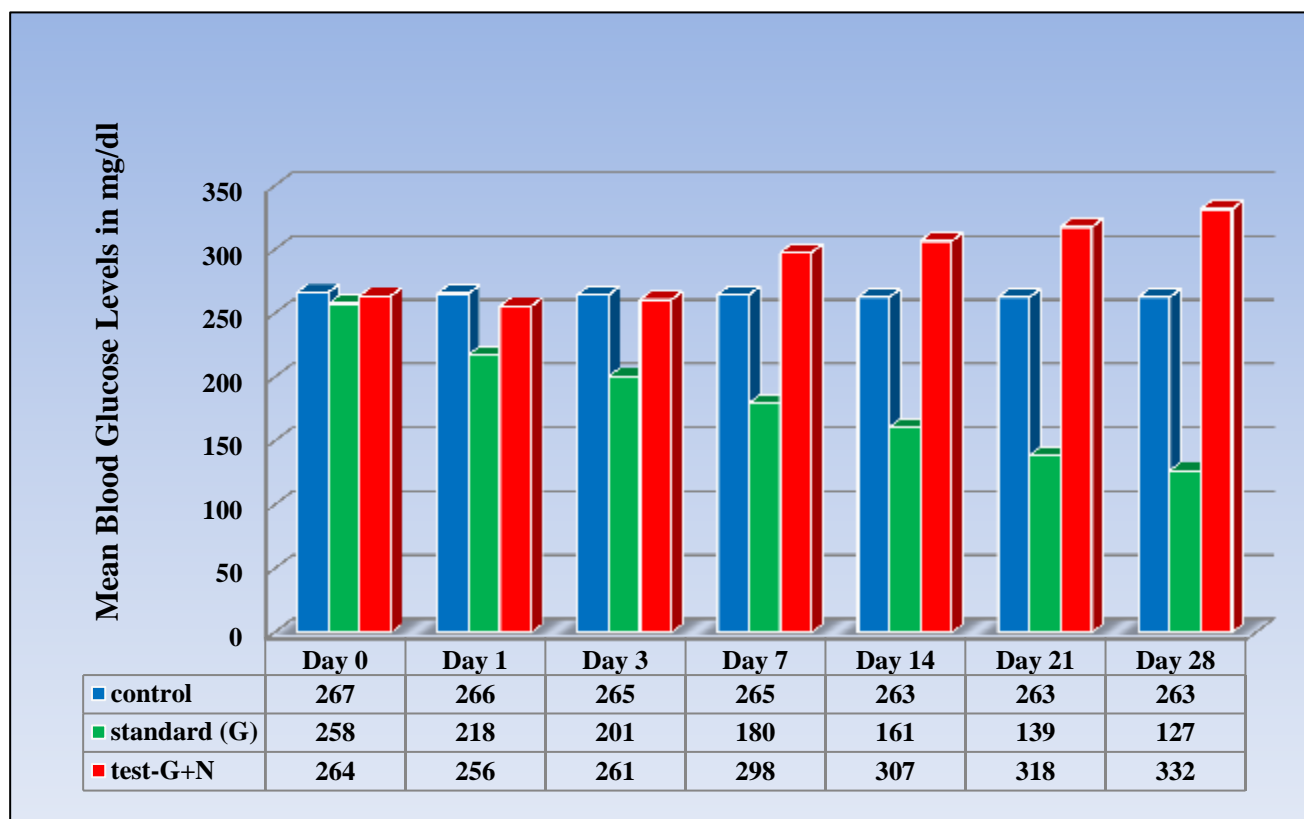


Figure 1: Multiple bar chart of comparison of mean blood glucose levels from day 0 to day 28.

DISCUSSION

Diabetes is most commonly associated with hypertension and ischemic heart disease, for which a combination of drug therapy is required. The introduction of insulin secretagogues like sulfonylureas and meglitinides has revolutionized the treatment of diabetes along with biguanides and thiazolidinediones, which reduce insulin resistance/produce insulin sparing effect.

In addition to nitrates, potassium channel openers are the other group of drugs that are useful in hypertension and ischemic coronary heart disease. It has been found that hyperglycemia is one of the commonest side effects of potassium channel opener like diazoxide due to inhibition of insulin release.³

In the light of this, the novel potassium channel opener nicorandil has been investigated to see whether it antagonizes the hypoglycemic effect of potassium channel blocker glibenclamide. The results obtained were compared with the hypoglycemic effect of the standard drug glibenclamide.

Albino rats of Wistar strain, weighing between 200-250 gms of either sex were used for the study. Chemical diabetes model was used as an experimental method for the study. In this method, alloxan monohydrate 2% solution was injected intraperitoneally in a dose of 150 mg/kg.b.wt as described by Dunn et al.⁴ In this study the

standard drug glibenclamide was given in the dose of 0.5 mg/kg.b.wt and the test drug combination of nicorandil in the dose of 0.3 mg/kg.b.wt + glibenclamide 0.5 mg/kg.b.wt was given orally for 30 days in different groups of diabetic animals.

The group in which glibenclamide was given showed a fall in blood glucose level. The mean fall in blood glucose level was 33.45% over 28 days which was found to be statistically significant.

The group in which both glibenclamide and nicorandil were given showed an initial fall in blood glucose level by 5.3% and 1.1% on 1st and 3rd day, which was found to be statistically not significant. Later the same group showed a mean rise in blood glucose level of 18.77% from 7th to 28th day, which was found to be statistically significant.

Statistical analysis was done using paired students 't' test, t-values obtained in each group were compared with P-value (standard table value of 't') at 10 degree of freedom for 0.05 (5%) level of significance, $P < 0.05$ (significant) and $P > 0.05$ (not significant).

The minimum number of animals required to conduct the study is 6 in each group and hence 6 animals were included in each group. The above results indicate that nicorandil opposes the hypoglycemic effect of glibenclamide in diabetic rats.

Because of pharmacokinetic and pharmacodynamic variations between animal and human species further studies are required to substantiate these results in human diabetic subjects where the minimum number of subjects required in each group is 30.

CONCLUSION

Thus from the above study it is concluded that nicorandil when added to glibenclamide in alloxan induced diabetic rats has shown the following effects;

- It has worsened diabetes by antagonizing the hypoglycemic effect of glibenclamide probably by blocking the potassium channel closing action of glibenclamide on ATP sensitive K⁺ channels on the surface of beta cells of pancreas.
- These findings suggest that potassium channel openers should be avoided when potassium channel blockers are being used as oral hypoglycemics in the management of diabetes. However if coronary vasodilator effect is required for angina in presence of diabetes, then other vasodilators like nitrates can be preferred.

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Annexure

Statistical formulae

If x_1, x_2, \dots, x_n are 'n' Observations [say x = blood glucose level in mg/dl] then,

- 1) The Arithmetic mean (AM) or \bar{x} is denoted by the formula

$$\bar{x} = \frac{X_1 + X_2 + \dots + X_n}{n} = \frac{\sum X}{n}$$

- 2) The Standard deviation (SD) is calculated by the formula

$$S.D = \sigma \text{ or } S = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

- 3) The Standard error (SE) is calculated by the formula

$$S.E. = \frac{S.D}{\sqrt{n}} = \frac{s}{\sqrt{n}}$$

The Statistic 't' is calculated by the formula

$$t = \frac{m_1 - m_2}{\frac{SE(m_1 - m_2)}{\sqrt{n_1 + n_2}}} = \frac{m_1 - m_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Where;

m_1 = mean of the 1st set of observations n_1

m_2 = mean of the 2nd set of observations n_2

s_1 = standard deviation of the sample of size n_1

s_2 = standard deviation of the sample of size n_2

Degree of freedom (d.f) is the number of independent variables: - Here d.f. = $(n_1 - 1) + (n_2 - 1) = (n_1 + n_2 - 2)$

P - value is the standard table value of 't' at $(n_1 + n_2 - 2) = 10$ degree of freedom for 0.05 (5%) level of significance.

$P < 0.05$ = significant or highly significant

$P > 0.05$ = not significant