

EFFECT OF AMIODARONE ON THE PHARMACODYNAMICS OF GLICLAZIDE IN ANIMAL MODELS

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ABSTRACT

Objective: The study was undertaken to find out the influence of amiodarone on the pharmacodynamic activity of gliclazide following single and multiple dose treatment in normal and diabetic rats, normal rabbits.

Methods: Studies were conducted in normal rats, diabetic rats and normal rabbits following oral doses of gliclazide, amiodarone and their combination with adequate washout between each period. Serial blood samples were collected through retro-orbital plexus in rats and marginal ear vein in rabbits at regular intervals and analyzed for glucose by glucose oxidase/peroxidase method.

Results: Gliclazide produced biphasic reduction of blood glucose levels in normal and diabetic rats with maximum activity attained at 2 h and 8 h. In rabbits, maximum hypoglycemic activity is observed at 4 h post dose. Amiodarone per se has negligible hypoglycemic activity, but in combination with gliclazide produced a marked reduction of blood glucose levels in rats/rabbits with more significant effect observed at multiple dose than at single dose treatment. A significant hypoglycemic effect is observed at the terminal elimination phase in combination treated rabbits following single and multiple dose amiodarone whereas negligible hypoglycemic activity at 24 h is observed following gliclazide alone. Literature indicates that amiodarone is known to be weak inhibitor of CYP2C9, CYP3A4 and CYP2D6 isozymes. Thus amiodarone appears to affect the isozymes responsible for gliclazide hydroxylation (CYP2C9 and CYP3A4) resulting in prolongation of its half-life and hypoglycemic activity.

Conclusions: There observed to be an interaction between the two drugs, but the interaction seems to be not of pharmacodynamic type. However the combination warrants close monitoring of blood glucose levels when such drugs are co administered.

Keywords: Gliclazide, Amiodarone, Pharmacodynamic, Drug interaction.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent elevation of blood glucose levels resulting from altered insulin secretion, resistance to insulin action, or a combination of both factors [1]. These defects result in altered metabolism of carbohydrates, lipids and proteins thereby leading to an increased risk of micro and macrovascular complications. DM is mainly of two types, type I and Type II. Type I or Insulin dependent diabetes mellitus (IDDM) is due to lack of synthesis of insulin and type II or noninsulin dependent diabetes mellitus (NIDDM) is due to lack of release of insulin from the β islets of langerhans of pancreas. Type II is more common than type I [2]. According to World Health Organization (WHO) estimate, there are 177 million people worldwide [3] suffering from diabetes among which 80-90% belongs to type-II diabetes. Type-II diabetes is associated with insulin resistance, visceral obesity, dyslipidemia and other related disturbances. The number of type II diabetes people is expected to increase because of modern life styles with high caloric diet and low energy expenditure leading to obesity and also due to medical advances that extend life span.

Chronic diabetes mellitus precipitates other disorders in the long run leading to existence of several disorders simultaneously. Such situations (existence of simultaneous multiple disorders) demand the use of more than one drug simultaneously known as polypharmacy which may precipitate drug interaction problems [4]. The drug treatment given to the patient is expected to improve the condition of the patient and should not lead to further deterioration. Hence care should be taken to avoid the possibility for over medication/ under medication / unwanted effects of the combinations particularly used in clinical disorders [5]. It is desirable if more information is generated on the safety of such drug combinations by conducting more studies in this area. Diabetes mellitus covers a wide range of heterogeneous diseases and involves management of its associated acute and chronic complications. Thus, there is every possibility of administering other drugs along with the primary anti-diabetic agent, which may be the cause for a drug-drug interaction to occur.

Oral hypoglycemic agents are used in the treatment of type II diabetes, among which gliclazide, a second-generation sulfonylurea derivative, is preferred in therapy because of its selective inhibitory activity towards pancreatic K⁺ adenosine triphosphate (ATP) channels, antioxidant properties, low incidence of severe hypoglycemia, and other hemobiological effects [6-7]. On the other hand amiodarone is a potent antiarrhythmic drug used in the treatment of atrial fibrillation and ventricular arrhythmias. Because of electrolyte imbalance, diabetic patients are at increased risk of atrial fibrillation which requires prompt treatment with antiarrhythmics such as amiodarone. Case studies in patients indicate that amiodarone is likely to cause hyperglycemia and hypertriglyceridaemia [8]. Literature studies indicate that amiodarone is an inhibitor of CYP3A4 and p-glycoprotein. Thus there is possibility of amiodarone interacting with other drugs that are substrates of CYP3A4 and p-glycoprotein.

There were very few *in vivo* drug-drug interactions that has been reported, but the potential for other interactions should be anticipated. This is especially important for drugs associated with serious toxicity, such as other antiarrhythmics and antidiabetics. If such combination is needed, reassessment of their dose and, where appropriate, plasma concentration need to be measured. Keeping in mind the long and variable half-life of amiodarone, potential for drug interactions with concomitantly administered drugs exists [9]. In such a situation, the drug-drug interaction studies are helpful to establish the safety of the drug combination. The influence of amiodarone on the pharmacodynamics of gliclazide is unknown. Since the clinical use of this drug combination in diabetics suffering from cardiac arrhythmia is considerable, it is intended to study the possibility of the pharmacodynamic interaction initially in animal models which can be later studied in humans, if necessary to find out the clinical relevance. Thus in the present article, the effect of amiodarone on the pharmacodynamic activity of gliclazide is reported.

MATERIALS AND METHODS

Drugs and Chemicals

Gliclazide and Amiodarone are the gift samples from Micro Labs (Bangalore, India) and Aurobindo Pharma Ltd. (Hyderabad, India).

Alloxan monohydrate was purchased from SD fine-Chemicals Ltd., Mumbai, India. Glucose estimation kits of Excel diagnostics were purchased from local suppliers. All other reagents and chemicals used were of analytical grade.

Animals

Albino rats of either sex, weighing between 160 – 250g and of young age (6 to 8 weeks old) procured from Tina Bio Labs, Hyderabad (Registration number 177/99CPCSEA) were used in the study. Albino rabbits of either sex, weighing 1.38 – 1.68 kg and approximately 3 months old were also procured from the same vendor. Animals were acclimatized to standard laboratory conditions like ambient temperature of $25 \pm 2^\circ\text{C}$, (12:12) hour (h) light and dark cycle and a relative humidity ($50 \pm 5\%$) for at least a week period prior to initiation of the experiment. Animals were fed with a commercial pellet diet (Hindustan Lever, India) and drinking water was allowed ad libitum. Strict hygienic conditions were maintained throughout the study. A fast of at least 16 hours is maintained prior to each experiment and animals were withdrawn from food and water during the experiment. The experiments were conducted in accordance with the ethical guidelines provided by CPCSEA and after obtaining prior approval of the experimental protocol by our Institutional Animal Ethics Committee. Also the institution where the experiments were performed was approved by CPCSEA, Government of India, Regd. No. (1533/PO/a/11/CPCSEA).

Single and multiple dose pharmacodynamic study in normal/diabetic rats

A group of six rats were administered with 2 mg/kg body weight of gliclazide (a few drops of 0.1N NaOH used as solubilizing agent) orally [10]. After a washout period of one week, the same group was administered with amiodarone (72 mg/kg body weight) orally [11]. After a further washout of one week, the same group (N=6) was also administered with amiodarone (72 mg/kg body weight) 30 min prior to administration of 2mg/kg gliclazide orally. After completion of single dose pharmacodynamic interaction study, the same group of animals was used for carrying out multiple dose interaction study. The rats were orally administered with amiodarone 72 mg/kg body weight daily for the next eight days with regular feeding. On the ninth day, after 16 hrs fasting, they were dosed orally with a combination of 72 mg/kg body weight of amiodarone and 30 min later with 2 mg/kg body weight gliclazide in a similar manner to that of the previous experiment. The entire procedure was repeated in a group of six alloxan induced diabetic rats.

Induction of diabetes

Diabetes was induced in rats by intraperitoneal injection of an aqueous solution of alloxan monohydrate at an initial dose of 100mg/kg body weight. After 18 hrs of administration, the blood glucose levels were measured. For rats in which diabetes is not induced, an additional dose of 29 mg/kg administered intraperitoneally [12-13]. At 72 h, the blood samples from all

surviving rats were estimated for glucose levels. Rats with blood glucose levels of 200 mg/dL and above were considered as diabetic and used in the study. Care is exercised throughout the induction period to prevent occurrence of sudden hypoglycemic states by intermittent feeding with 10% dextrose solution and standard pellet diet.

Pharmacodynamic and pharmacokinetic study in rabbits

A group of six rabbits were orally administered with 5.6 mg/1.5 kg body weight [14] of gliclazide. The same group was administered with 100 mg/1.5 kg body weight [15] of amiodarone and a combination of amiodarone (100 mg/1.5 kg body weight) and gliclazide (5.6 mg/1.5 kg body weight). A washout period of one week is maintained between the treatments. After completion of single dose studies, the same rabbit group is subjected to multiple dose study. They were dosed daily with a single oral dose of 100 mg/kg body weight of amiodarone, orally for eight days with regular feeding, later after 16 hrs fasting on the ninth day, they were dosed with combined treatment of amiodarone (100 mg/1.5 kg body weight) and gliclazide (5.6 mg/1.5 kg body weight) with a gap of 30 minutes between them.

Collection of blood samples

Blood samples were collected from retro-orbital plexus from each rat and marginal ear vein from rabbits at 0 (pre-dose), 1, 2, 3, 4, 6, 8, 10 and 12 h time intervals after each treatment on first day in case of single dose interaction study and on ninth day in case of multiple dose interaction study. All the collected blood samples were centrifuged within 30 minutes of collection at 3000 rpm for 10 minutes and the serum separated were analyzed for blood glucose by Glucose oxidase/Peroxidase (GOD/POD) method [16] using commercially available glucose kits and absorbance was measured at 505 nm in uv-visible spectrophotometer.

Data and Statistical analysis

Data were presented as mean \pm standard error of mean (SEM). The statistical significance was determined by applying student's paired 't' test.

RESULTS

Gliclazide produced biphasic reduction of blood glucose levels with maximum activity attained at 2 h and 8 h in normal and diabetic rats (Table 1-2), and at 4 h in normal rabbits (Table 3). Therapeutic dose of amiodarone alone has minor effect on blood glucose levels in rats (both normal and diabetic) and normal rabbits, whereas in combination with gliclazide both as a single and multiple dose, produced a marked reduction of blood glucose levels. The reduction was more significant ($P < 0.05$) at multiple dose treatment with amiodarone than at single dose treatment (Table 1, 2, 3). Also a significant hypoglycemic effect is observed at the terminal elimination phase in combination treated rabbits following single and multiple dose amiodarone whereas negligible hypoglycemic activity at 24 h is observed following gliclazide alone.

Table 1: Mean percent blood glucose reduction after oral administration of gliclazide (G), amiodarone (A) and their combination in normal rats (N = 6).

Time (h)	Gliclazide (G)	Amiodarone (A)	G+A (Combination, Single Dose)	G+A (Combination, Multiple Dose)
1	25.75 \pm 1.12	1.41 \pm 0.92	32.50 \pm 1.28***	44.25 \pm 1.24***
2	43.36 \pm 1.09	3.29 \pm 1.30	49.72 \pm 0.978*	55.10 \pm 1.06***
3	33.06 \pm 1.50	3.00 \pm 0.95	39.31 \pm 1.41*	46.13 \pm 1.01***
4	24.09 \pm 1.36	5.84 \pm 1.20	33.52 \pm 1.27**	37.81 \pm 1.23***
6	33.42 \pm 1.26	6.48 \pm 1.16	40.27 \pm 1.47*	46.79 \pm 1.11**
8	45.48 \pm 0.93	3.71 \pm 1.07	50.56 \pm 1.21*	52.74 \pm 0.63**
10	28.11 \pm 1.54	4.44 \pm 0.90	34.26 \pm 1.22*	41.10 \pm 0.72***
12	23.71 \pm 1.38	4.95 \pm 1.24	30.10 \pm 1.10**	35.57 \pm 1.06**

G+ A: Gliclazide+Amiodarone; SEM: Standard error of the mean

Mean \pm SEM; *** Significant at $P < 0.001$; ** Significant at $P < 0.01$; *Significant at $P < 0.05$ compared to gliclazide control

Table 2: Mean percent blood glucose reduction after oral administration of gliclazide, amiodarone and their combination in diabetic rats (N = 6).

Time (h)	Gliclazide (G)	Amiodarone (A)	G+A (Combination, Single Dose)	G+A (Combination, Multiple Dose)
1	30.26 ± 1.51	2.24 ± 1.69	35.12 ± 1.44	38.58 ± 1.29**
2	46.63 ± 1.74	3.87 ± 1.82	48.98 ± 0.84	54.54 ± 1.99*
3	35.07 ± 1.44	4.57 ± 1.47	39.94 ± 1.49	40.27 ± 1.99
4	27.08 ± 1.36	5.99 ± 1.13	41.55 ± 0.84***	35.57 ± 0.97**
6	34.79 ± 1.49	6.65 ± 0.86	46.17 ± 0.97***	46.49 ± 0.58**
8	49.21 ± 1.14	6.01 ± 0.30	55.14 ± 1.58	60.08 ± 1.21**
10	33.39 ± 1.37	4.95 ± 0.48	46.17 ± 2.24**	43.02 ± 1.65**
12	26.65 ± 1.00	5.01 ± 0.97	39.53 ± 2.13**	37.75 ± 1.42**

Mean±SEM; *** Significant at P<0.001; ** Significant at P<0.01; *Significant at P< 0.05 compared to gliclazide control

G+ A: Gliclazide+Amiodarone

Table 3: Mean percent blood glucose reduction after oral administration of gliclazide, amiodarone and their combination in rabbits (N = 6).

Time(h)	Gliclazide (G)	Amiodarone (A)	G+A (Combination, Single Dose)	G+A (Combination, Multiple Dose)
1	4.17±0.53	1.00±0.12	13.55±1.04***	15.80±0.92***
2	14.28±1.74	1.73±0.44	13.02±0.90	14.45±0.46
3	27.77±1.89	1.60±0.36	24.35±1.81*	26.37±2.93
4	30.92±1.80	3.97±0.53	24.85± 0.46**	30.37±2.70***
6	29.82±2.24	5.28±0.72	25.83±0.60	31.23±2.62***
8	23.77±3.44	2.80±0.27	27.67±0.46***	32.350±2.51*
10	17.28±2.46	3.87±0.22	32.75±0.25**	38.38±1.21***
12	12.70±1.65	3.42±0.42	32.37±3.80**	34.92±2.23**
18	6.03±0.30	2.53±0.29	20.50±1.68***	24.78±2.50***
24	3.78±0.41	2.05±0.24	16.58±1.58***	18.75±1.34***

Mean±SEM; *** Significant at P<0.001; ** Significant at P<0.01; *Significant at P< 0.05 compared to gliclazide control

G+ A: Gliclazide+Amiodarone

DISCUSSION

Diabetic patients are likely to suffer with cardiovascular disorders and hence most often anti-arrhythmics are co-administered along with oral anti-diabetic drugs. Drug interactions are commonly observed in clinical practice and the mechanisms of such interactions are evaluated usually in animal models. We studied the influence of amiodarone on the pharmacodynamic activity of gliclazide in both normal and diseased condition. The normal rat model served to quickly identify the interaction and diabetic rat served to validate the occurrence of the interaction in the actually used clinical condition of drug and the rabbit model is another dissimilar species. Since small amount of blood was required for glucose analysis, the blood samples were collected by retro-orbital plexus as it was reported to be good method when small samples of blood are required [17]. Diabetes was induced with diabetogen, alloxan monohydrate, since it was more economical and easily available.

Gliclazide alone when administered produced hypoglycemia in normal rats, with peak activity at the 2 h and 8 h. The maximum reduction attained at the 2 h may be due to the stimulation of initial rapid release of insulin (phase I) by gliclazide, and to the ability of gliclazide to increase the sensitivity of pancreatic β -cell to glucose. Gliclazide does not have any effect on prolonged insulin release (phase II) [18]. Gliclazide also increases the sensitivity of peripheral tissues to insulin, which may be the reason for the reduction at the 8 h. Gliclazide is metabolized to several metabolites by hepatic cytochrome P450 3A4 and 2C9 iso enzymes, and is eliminated in urine. A part of gliclazide is eliminated through the biliary route, which involves entero hepatic circulation in rats [19]. The reabsorption of gliclazide eliminated through the biliary route may be responsible for a second peak in its hypoglycemic effect in normal and diabetic rats.

Amiodarone per se has negligible effect on the blood glucose levels indicating that it is devoid of blood glucose lowering activity. On the other hand amiodarone when administered in combination with gliclazide significantly enhanced the blood glucose lowering effect in

both normal and diabetic rats. This indicates that there exists an interaction when these two drugs when used together, but the interaction seems to be not of pharmacodynamic type. Literature indicates that amiodarone is metabolized by CYP3A4 and is known to be weak inhibitor of CYP2C9, CYP3A4 and CYP2D6 isozymes. Data from literature states that amiodarone resulted in increased amounts of inactive cytochrome P-450-Fe (II) metabolite complexes in rodents and hamsters when administered repeatedly [20-21]. Amiodarone appears to be similarly affecting the isozymes responsible for gliclazide hydroxylation (CYP2C9 and CYP3A4) [22] resulting in prolongation of its half-life and hypoglycaemic activity. Thus the increased hypoglycemic activity observed in rats/rabbits when administered in combination might be the result of inhibition of gliclazide metabolism by amiodarone thereby resulting in prolongation of gliclazide levels in the systemic circulation which in turn by acting on ATP sensitive K^+ channels, resulted in increased insulin release with subsequent reduction in blood glucose levels.

CONCLUSIONS

Interaction is observed between gliclazide and amiodarone in two dissimilar species, it is more likely to occur in humans also. The interaction is observed upon single dose and multiple dose administration. Although the combination was well tolerated and did not result in hypoglycemic shock in experimental animals, the combination warrants close monitoring of blood glucose levels when such drugs are co administered.

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