RESEARCH ARTICLE

Influence of calcium channel antagonist on the pharmacodynamics of a second-generation sulfonylurea in rats and rabbits

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Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose concentration known as hyperglycemia. 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. In the present study, the possible pharmacodynamic interaction was studied with amlodipine besylate and gliclazide in diabetic rats and healthy rabbits. The animals were divided into three groups. Gliclazide was studied at a dose of 1.44 mg/200 g and 5.6 mg/1.5 kg body weight in rats and rabbits respectively. Amlodipine besylate at a dose of 0.090 mg/200 g and 0.350 mg/1.5 kg body weight was used for the interaction study in rats and rabbits respectively. The drugs were administered orally and the blood samples were collected before and after administration of drug for a period of 16 h in rats and 24 h in rabbits. The serum samples were then subjected to glucose estimation by glucose peroxides method. The percentage reduction in blood glucose levels were calculated with respect to initial levels. Gliclazide showed a significant reduction of elevated and normal blood glucose levels. The extent of blood glucose reduction was comparatively reduced in the case of combination therapy of amlodipine besylate and gliclazide. The study also suggests the necessity to readjust the dose of gliclazide when co-administered with amlodipine besylate.

Key words: *Alloxan, amlodipine, drug interaction, dynamic interaction, gliclazide, hyperglycemia*

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, and proteins, and an increased risk of complications from vascular disease.[1-4] The management of diabetes mellitus involves utilization of various drugs to save life and alleviate symptoms; secondary aims are to prevent long-term diabetic complications and by eliminating various risk factors to increase longevity. During such a therapy, there is every possibility of occurrence of drug interactions, which may be serious and deleterious to the patients. Most drugs used in the current therapy have the capacity to influence many physiological systems.[5] Two drugs concomitantly administered will often affect some of the same systems. Diabetes mellitus may be categorized in to two major types, type 1 and type 2. Type 2 diabetes is the most common form of diabetes. Oral hypoglycemic agents like sulfonylureas, biguanides, alphaglucosidase inhibitors, meglitinides analogs, and thiazolidediones are useful in the treatment of type 2 diabetes mellitus.[1] Hypertension is the most common co-morbid condition present along with diabetes and involves various drug therapies of which calcium channel blockers are common.[6] Calcium channel blockers are reported to have influence on the blood glucose reducing effect of sulfonylureas.[7] Based on the above evidence, this study was designed to elaborate on the pharmacodynamic interactions that may exist between amlodipine besylate and gliclazide.

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MATERIALS AND METHODS

Drugs and chemicals

Gliclazide and amlodipine besylate were obtained from Aurobindo, Hyderabad and Sun Pharmaceuticals Ltd, Mumbai, India respectively. The glucose estimation kits were obtained from Excel diagnostics Pvt. Ltd, Hyderabad, India.

Equipments

The UV spectrophotometer (Elico), microcentrifuge (Remi), micropipettes (Tarsons), and microcentrifuge tubes (Tarsons) were used from the laboratory.

Animals

Adult wistar rats of either sex, weighing 150-260 g, and albino rabbits, weighing 1.3-1.5 kg, obtained from the animal house of Bapatla College of Pharmacy (1032/ac/07/CPCSEA); Bapatla, were maintained at a constant temperature of 26 ± 2°C and humidity 30-40% with 12 h light/dark cycle, throughout the experiments. The animals were fed with commercial rat, rabbit feed and sterile water was given *ad libitum*. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Bapatla College of Pharmacy, Bapatla, and was in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals.

Pharmacodynamic interaction in diabetic rats

Experimental induction of diabetes[8-10]

Experimental diabetes in rats was induced by injecting alloxan monohydrate intra-peritoneally at a dose of 150 mg/kg in ice-cold normal saline. After 72 h, samples were collected from rats by orbital puncture of all surviving rats and the serum was analyzed for glucose levels. Rats with blood glucose levels of 200 mg/dl and above were considered as diabetic and selected for the study.

Interaction studies on diabetic rats

The diabetic rats were randomly divided into three groups of six animals each. All the animals were subjected to fasting for 18 h prior to experimentation, and during the course of time the animals had free access to water. Group I served as control and received distilled water with few drops of 0.1 N NaOH. Group II received gliclazide 1.44 mg/200 g and Group III received amlodipine besylate 0.090 mg/200 g and gliclazide 1.44 mg/200 g. All the drugs were administered orally. The blood samples were collected before and after the administration of the drugs at 0, 1, 2, 3, 4, 6, 8, 10, 12, and 16 h by retro-orbital puncture method. The samples were centrifuged, and the separated serum was subjected to glucose estimation by glucose peroxidase method.[11] The Percent reduction at each time was calculated with respect to initial levels.

Single-dose interaction studies

In the single-dose interaction study, the animals of Group III were fasted for a period of 18 h with water *ad libitum* and received amlodipine besylate 0.090 mg/200 g followed by gliclazide 1.44 mg/200 g administration 30 min later. The blood samples were collected at predetermined intervals and the serum samples were analyzed for glucose by glucose peroxidase method.

Multiple-dose interaction studies

The animals of Group III were administered with the amlodipine besylate for the following seven consecutive days. On the seventh day after administration of drug, the animals were fasted for 18 h, and on the eighth day, the animals were administered with the last dose of amlodipine besylate followed by gliclazide administration. The blood samples were collected as discussed earlier and the serum samples were subjected for glucose estimation.

Interaction studies on healthy rabbits[12]

The healthy albino rabbits were randomly divided into three groups of five animals each. All the animals were subjected to fasting for 18 h prior to experimentation, and during the course of time, the animals had free access to water. Group I served as control and received distilled water with few drops of 0.1 N NaOH. Group II received gliclazide 5.6 mg/1.5 kg and Group III received amlodipine besylate 0.350 mg/1.5 kg and gliclazide 5.6 mg/1.5 kg. All the drugs were administered orally. The blood samples were collected before and after the administration of the drugs at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24 h from the marginal ear vein. The samples were centrifuged, and the separated serum was subjected to glucose estimation by glucose peroxidase method. In the single-dose interaction study, the animals of Group III were fasted for a period of 18 h with water *ad libitum* and received amlodipine besylate 0.350 mg/1.5 kg followed by gliclazide 5.6 mg/1.5 kg administration 30 min later. The blood samples were collected at predetermined intervals and the serum samples were analyzed for glucose by glucose peroxidase method. In case of multiple dose study, the animals of Group III were administered with the amlodipine besylate for the following seven consecutive days. On the seventh day after administration of drug, the animals were fasted for 18 h, and on the eighth day, the animals were administered with the last dose of amlodipine besylate followed by gliclazide administration. The blood samples were collected as discussed earlier and the serum samples were subjected for glucose estimation.

Statistical significance

The data are presented as mean ± SEM. The significance of the observed difference in the pharmacodynamic parameters of gliclazide and in combination with other drugs was assessed by Student's unpaired *t*-test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Gliclazide at a dose of 1.44 mg/200 g was studied in diabetic rats and the percent reduction is shown in Figure 1. In the case of diabetic rats, gliclazide showed a maximum reduction of 40.82% at second hour and 40.16% at eighth hour. A single-dose interaction was performed in combination of amlodipine besylate at a dose 0.090 mg/200 g and gliclazide 1.44 mg/200 g on diabetic rats and a maximum percentage reduction in blood glucose levels, exhibited by gliclazide alone was reduced by 5.19% ($P < 0.05$) and 10.16% ($P < 0.01$) at second and eighth hour. Further more, multiple-dose interaction study revealed that the maximum percentage reduction in blood glucose levels, exhibited by gliclazide was reduced by 8.63% ($P < 0.001$) and 12.92% ($P < 0.01$) at

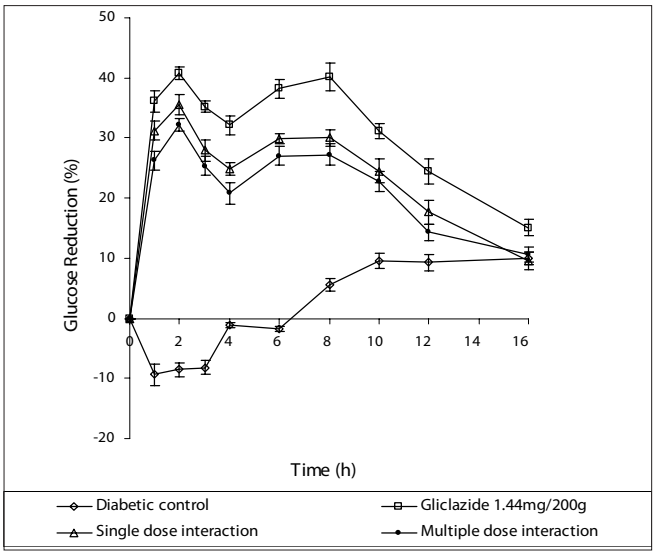

Figure 1: Influence of amlodipine besylate on the pharmacodynamics of gliclazide in diabetic rats. Percent blood glucose reduction of gliclazide 1.44 mg/200 g alone and in combination with amlodipine besylate 0.090 mg/200 g at single- and multiple-dose interaction studies in diabetic rats.

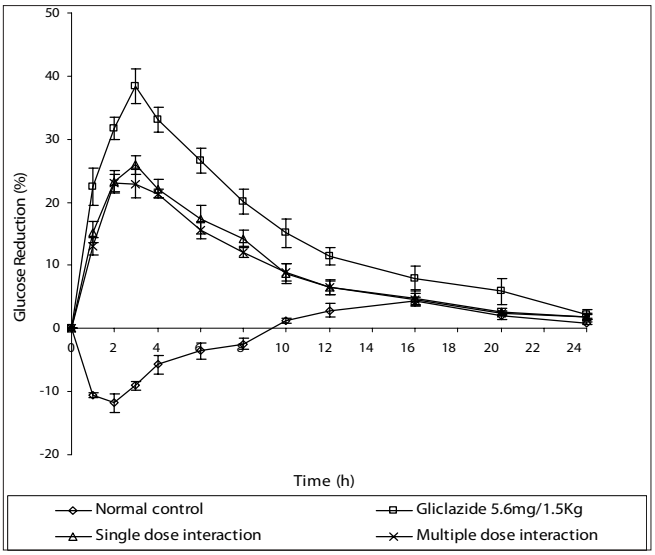

Figure 2: Influence of amlodipine besylate on the pharmacodynamics of gliclazide in healthy rabbits. Percent blood glucose reduction of gliclazide 5.6 mg/1.5 kg alone and in combination with amlodipine besylate 0.350 mg/1.5 kg at single and multiple dose interaction studies in rabbits.

second and eighth hour on co-administration with amlodipine besylate. Statistical treatment of the experimental results showed a significant drug interaction. The incidence of interactions is likely to vary from one species to another because of variations existing in absorption, distribution, metabolism and elimination characteristics and receptor functions. The prediction of drug interaction in human beings is more precise if the experimental finding demonstrates the existence of interactions in more than one species, so the drug interaction studies were also performed in healthy rabbits. The effect of gliclazide at a dose 5.6 mg/1.5 kg was studied in normal rabbits and the percent reduction was calculated and shown in Figure 2. The maximum blood glucose reduction of 38.42% was seen at third hour in the rabbits. Single-dose pharmacodynamic interaction with amlodipine besylate at a dose of 0.350 mg/1.5 kg exhibited a reduction of 12.5% at third hour. Further more, multiple-dose interaction exhibited a reduction of 15.49% on co-administration with amlodipine besylate. Statistical treatment showed significant ($P < 0.01$) pharmacodynamic interaction between amlodipine besylate and gliclazide.

DISCUSSION

Diabetic patients are most likely to suffer with hypertension and hence most often anti-hypertensives are co-administered along with oral anti-diabetic drugs. Frequently prescribed anti-hypertensives belong to the class of calcium channel blockers. Literature survey shows that calcium channel blockers have hyperglycemic effect which adds on an evidence for a probable pharmacodynamic interaction between oral anti-diabetic drugs and calcium channel blockers. The onset of action was observed at first hour and the duration of action was noticed up to 16 h in rats and rabbits. The maximum reduction was seen at second and eighth hour in rats and at third hour in rabbits. Gliclazide treatment showed a maximum reduction at second hour; this may be due to the stimulation of initial rapid release of insulin and by the property to increase the sensitivity of pancreatic beta cells to glucose.[13,14] Gliclazide also increases the sensitivity of peripheral tissues to insulin, and 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. The re-absorption of gliclazide eliminated through the biliary route might be responsible for a second peak in its hypoglycemic effect in rats .This might be the reason for the reduction at eighth hour.[15,16] The biphasic response was not observed in the case of rabbits, and it may be due to the absence of entero-hepatic cycling.[16] The onset and duration of action was not altered in case of single and multiple-dose interaction studies carried in diabetic rats and healthy rabbits. Single and multiple-dose interaction in diabetic rats and healthy rabbits confirmed the presence of potential pharmacodynamic interactions between gliclazide and amlodipine besylate. The hypoglycemic activity of gliclazide was found to be decreased in the presence of amlodipine besylate, this may be due to the calcium channel blockade effect of dihydropyridines, as calcium is responsible for exocytosis process and due to the hyperglycemic activity exerted by the amlodipine besylate. Pharmacodynamic interactions were observed in rodents as well as in non rodent species. Hence, the present investigation warrants further studies to find out the relevance of this interaction in human beings. Therefore, the dose of gliclazide must be readjusted, whenever it needs to be co-administered with amlodipine besylate.

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REFERENCES

1. Bastaki S. Diabetes mellitus and its treatment. Int J Diabetes Metab 2005;13:111-34.
2. Hardman JG, Limbird LE. Goodman and Gilman's The pharmacological basics of therapeutics. 9th ed. New York: McGraw Hill; 1996. p. 1493.
3. Svensson M, Eriksson JW, Dahlquist G. Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type 1 diabetes: A population-based study in northern Sweden. Diabetes Care 2004;27:955-62.
4. Saely CH, Aczel S, Marte T. Cardiovascular complications in type 2 diabetes mellitus depend on the coronary angiographic state rather than on the diabetes state. Diabetologia 2004;47:145-6.
5. David BT, Remington: The science and practice of pharmacy. 21st ed. Philadelphia: Lippincott Williams and Wilkins; 2005. p. 1889.
6. Mohan V. Handbook of diabetes mellitus. 2nd ed. Chennai: MV Diabetes Specialities Centre and Madras Diabetes Research Foundation; 2005. p. 99.
7. McEvoy GK. AHFS drug information. Bethesda: American Society of Health-system Pharmacists, Inc; 2004. p. 3038.
8. Swami AM, Shetty SR, Kumar SM, Rao NV. A study on drug-drug interaction of roxithromycin and antidiabetic drugs. Indian Drugs 2005;42:808-13.
9. Dhanabal SP. Antihyperglycemic activity of *Polygala arvensis* in alloxan diabetic rats. Indian Drugs 2004;41:690-5.
10. Setty SR. Influence of itraconazole on sulfonylureas-induced hypoglycemia in diabetic rats. Indian J Pharm Sci 2005;67:677-80.
11. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative glucose acceptor. Ann Clin Biochem 1969;6:24-7.
12. Satyanarayana S. Influence of quinidine, selegiline and amphotericin-B on the pharmacokinetics and pharmacodynamics of Tolbutamide in rabbits. Indian Drugs 1998;35:640-4.
13. Bressler R, Johnson DG. Pharmacological regulation of blood glucose levels in non-insulin dependent diabetes mellitus. Arch Intern Med 1997;157:836-48.
14. Chiasson JL. Acarbose for prevention of type 2 diabetes mellitus. Am J Med 1991;90:450.
15. Silva EM. Ultrasonographic abnormalities of the pancreas in IDDM and NIDDM patients. Diabetes Care 1993;16:1296-7.
16. Satyanarayana S, Eswar kumar K, Rajasekar J, Thomas L, Rajanna S, Rajanna B. Influence of aqueous extract of Fenugreek- seed powder on the Pharmacodynamics and Pharmacokinetics of Gliclazide in rats and rabbits. Therapy 2007;4:457-463.

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