A Study on Drug-Drug Interaction of Diltiazem with Nateglinide in Diabetic Animals

Raza Hasan*, Suresh D.K, Hamza Sheth, Md. Saifuddin Khalid and Mohiuddin M

Luqman College of Pharmacy, Old jewargi road, Gulbarga-585102, Karnataka, India

ABSTRACT

Aim of this investigation was to study the drug-drug interaction between antidiabetic drugs and antianginal drugs. Interaction of Nateglinide, the known Meglitinide antidiabetic drugs with Diltiazem (antianginal drug) was evaluated in normal healthy and STZ induced diabetic rats. The blood samples were collected from normal healthy and diabetic rats at different time interval upto 24 hrs and blood glucose was estimated. Diltiazem pre-treatment (15 mg/kg for seven days), has not significantly altered the onset of antidiabetic effect of Nateglinide in healthy and STZ induced diabetic rats but significantly increased the peak antidiabetic effect from 40.80±2.54 % reductions before treatment to 51.9±61.14% reduction after treatment at 6th hr and 46.16±1.25% reduction before treatment to 55.80±0.30% reduction after treatment at 6th hr in both healthy and in diabetic rats respectively. Duration of antidiabetic effect was enhanced from 08hrs to more than 18hrs in both groups. This study indicates that therapeutic drug monitoring is required, and the therapeutic dose of Diltiazem and Nateglinide, needs to be altered when used concomitantly.

Keywords: Diltiazem, Nateglinide, STZ (Streptozotocin), Antidiabetic activity.

INTRODUCTION

Drug interaction is a chemical or physiological reaction that can occur when two different drugs are taken together. It can occur when the effects of one drug are modified by the prior or concurrent administration of another drug. A drug interaction may result in beneficial or harmful effects. However, harmful effects are usually predominated. In considering the clinical relevance of pharmacokinetic drug-drug interactions mediated by drug-metabolizing enzymes, efficacy linked to dosage requirements and/or toxicity can be considered as appropriate endpoints. It may modify the diagnostic, preventive or therapeutic activity of either drug[1].

In multiple drug therapy, it is important to determine the incidence and frequency of occurrence of drug interactions, in hospitalized patients. Further, it is also useful to find out agents that are most likely to produce hazardous interactions[2]. A study which was conducted on drug-drug interactions in selected community pharmacies in which out of 1368 prescriptions evaluated over a span of 3 months, 613 interactions were found in 516 prescriptions, out of which 16.15% interactions were severe, 3.75% interactions were found where patient was receiving more than 8 drugs and 11.58% interactions had a significance level[3]. Almost 783,936 people in the United States die every year from conventional medicine mistakes[4].

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Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, glycosuria, Hyperlipidemia, negative nitrogen balance and sometimes ketonaemia[5]. Diabetes is always coinciding with serious complications and adverse effects. Microvascular and macrovascular disease account for most of the morbidity and mortality associated with diabetes. Nearly 80% of deaths in those with type 2 diabetes involve cardiovascular disease, Angina Pectoris or stroke[6]. Diabetic patients are more prone serious complications, like cardiovascular diseases, hypertension, arrhythmia, angina pectoris, and fungal infections etc which require long term treatment[7]. The total cost of diabetes to the US health care system in 2002 was estimated at $132 billion, the majority of this associated with the treatment of chronic diabetic complications[8]. In such cases multiple drug therapy is needed to prescribe. So, there is always a need for co - administration of calcium channel blockers like verapamil, nifedipine, amlodipine and diltiazem etc along with oral Antidiabetic agents like Nateglinide or Pioglitazone.

There are reports that Nateglinide is predominantly eliminated by metabolism via the cytochrome P-450 enzyme 3A4 and CYP2C9 [9]. Diltiazem is also metabolized by Cytochrome P-450 enzyme 3A4 and 2C9 and 2D6 and is known to inhibit Cytochrome P-450 enzyme system[10], hence there is a possibility of occurrence of pharmacokinetic type of drug interactions with concomitantly used drugs. Therefore the present study was carried out on healthy and diabetic rats to assess the influence of Diltiazem pretreatment on the antidiabetic effects of Nateglinide.

MATERIALS AND METHODS

Animals

Albino Wistar rats of either sex, weighing 150-200 g, were used as the test animal. The experimental animals were procured from Sri Mahavir Enterprises, Hyderabad. The rats were fed on a standard pellet diet (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum* and maintained at 25°C with 12 hr light / dark cycle. After laboratory acclimation for 7 days, the rats were starved for 48 h. Prior approval by institutional ethics committee (reg. no: 346/CPCSEA) was obtained for carrying out the experiments. The study was conducted in the Department of Pharmacology of Luqman College of Pharmacy, Gulbarga.

Drugs

Nateglinide was obtained from Dr Reddy's Lab.; Hyderabad. Diltiazem was obtained from Sun Pharma, Silvassa, Gujarat. Nateglinide (50 mg/kg, p.o.) and Diltiazem (15 mg/kg, p.o) suspensions were prepared using 2% w/v gum acacia as suspending agent to represent 125 mg/ml and 7.5 mg/ml respectively.

EXPERIMENTAL PROCEDURES

1. In healthy rats

The healthy rats were marked conveniently and distributed randomly into two groups of 6 animals each. All the animals were over night fasted with water *ad libitum*. The animals in group-1 received Diltiazem (15 mg/kg, p.o.) and the animals in the group-2 received Nateglinide (50 mg/kg, p.o) in acacia suspension. Blood samples were withdrawn at 0, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hours intervals and were analyzed for blood glucose concentration determination by GOD/POD method using semi auto Analyzer (BCA201) and expressed as mg/dl of blood[12].

In the next phase of the experiment, after the washing period of 10 days, the same animals of group-2 received Diltiazem 15 mg/kg, p.o. for seven days. On the 7th day, 6 hours after administration of Diltiazem, the animals were fasted for 14 hours. On the 8th day, Diltiazem was given as usual. One hour after the treatment, animals of group-2 received Nateglinide 50 mg/kg, p.o. Blood samples were collected thereafter at above mentioned intervals and glucose levels were estimated. The percentage blood glucose reductions at various time intervals were calculated and compiled in (Table 1).

2. In diabetic rats

Experimental induction of diabetes mellitus

The animals were induced into a diabetic state by intraperitonially injection of a freshly prepared solution of Streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) in 0.05 mM citrate buffer (pH 4.5) at a dose of 50 mg/kg body weight for a single day[11]. Blood samples were collected after 24 hrs and blood glucose levels were estimated. Albino rats which have shown more than 250 mg/dl blood glucose levels were considered as diabetic. The blood glucose levels were inspected for further four days. From this it was

Table 1: Effect of Diltiazem and Nateglinide on percentage decrease in blood glucose levels at different time intervals in healthy albino rats.

Percentage reduction in blood glucose concentration (mean ± SEM)			
Time in Hr	**Diltiazem (15 mg/kg. p.o.)**	**Nateglinide (50 mg/kg. p.o.)**	**Diltiazem (15 mg/kg. p.o.) + Nateglinide (50 mg/kg. p.o.)**
Fasting	–	–	--
0.5	1.78±0.44	6.84±1.02	8.17±0.52
1.0	2.73±1.64	19.70±1.93	24.23±0.95**
2.0	1.34±0.99	27.76±1.74	32.03±1.16*
4.0	-0.30±076	36.45±2.45	42.02±1.13***
6.0	0.41±0.90	40.80±2.54	51.96±1.14***
8.0	0.31±0.44	21.49±3.32	39.21±0.87***
12.0	2.23±0.40	10.64±3.72	32.98±3.21
18.0	-0.19±0.91	3.51±2.70	18.59±2.45**
24.0	-0.27±0.35	0.95±2.35	5.88±1.18

n=6 * Significant at p< 0.05; ** highly significant at p<0.01;
*** very highly significant *represent that comparison of Nateglinide with Nateglinide + Diltiazem interaction

confirmed that diabetes was induced in 48 hrs and stabilized within 7 days. These animals were used for further studies.

The same procedure as mentioned in the healthy rats should be carried out for further study. Blood samples were collected thereafter at above mentioned intervals and glucose levels were estimated. The percentage blood glucose reductions at various time intervals were calculated and compiled in (Table 2).

Statistical analysis

The data were analyzed by Student't' test. P values lower than 0.05 were considered as statistically significant.

RESULTS

As shown in (Table 1), treatment with Diltiazem alone did not alter the blood glucose levels in healthy rats. However, diltiazem pre-treatment (15 mg/kg for seven days), has not significantly altered the onset of hypoglycemia (i.e. 19.70±1.93 to 24.23±0.95, $p< 0.01$) at 1st hr but significantly enhanced the peak hypoglycemia (40.80±2.54 % before treatment to 51.9±61.14% after treatment, $p<0.001$) at 6th hr and duration of hypoglycemia was also significantly enhanced about 08 hrs to more than 18 hrs, (i.e. 21.49±3.32 to 18.59±2.45, $p<0.01$) induced by nateglinide.

Similarly, in (Table 2), treatment with Diltiazem alone did not alter the blood glucose levels in diabetic rats. However, diltiazem pre-treatment (15 mg/kg for seven days), has not significantly altered the onset of hypoglycemia (i.e. 19.70±0.58 to 21.00±1.15, $p<0.01$) at 1st hr but significantly enhanced the peak hypoglycemia (46.16±1.25 % before treatment to 55.80±0.30% after treatment, $p<0.01$) at 6th hr and duration of hypoglycemia was also significantly enhanced about 08 hours to more than 18 hours, (i.e. 31.33±0.89 to 16.22±1.24, $p<0.05$) induced by nateglinide.

DISCUSSION

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar either because the body does not produce enough insulin or because cells do not respond to the insulin that is produced, and it requires lifelong treatment. Angina pectoris and other cardio vascular diseases also require treatment for a prolonged period. If a patient suffers from diabetes mellitus as well as angina pectoris, he has to use antidiabetic drug such as Nateglinide and anti anginal agent like Diltiazem. In such cases, there is a possibility of occurrence of drug interactions. Our research study has shown that drug interactions occur when Diltiazem and Nateglinide are administered simultaneously at therapeutic doses.

For the assessment of the potentiation of antidiabetic effect, onset of action, (time taken for minimum of 15-20% reduction in blood glucose levels), peak effect and duration of anti diabetic effect (duration in which minimum of 15-20% reduction in blood glucose levels are maintained) were considered.

Table 2: Effect of Diltiazem and Nateglinide on percentage decrease in blood glucose levels at different time intervals in diabetic albino rats.

Percentage reduction in blood glucose concentration (mean ± SEM)			
Time in Hr	**Diltiazem (15 mg/kg. p.o.)**	**Nateglinide (50 mg/kg. p.o.)**	**Diltiazem (15 mg/kg. p.o.)+ Nateglinide (50 mg/kg. p.o.)**
Fasting	–	–	–
0.5	-0.514±0.51	10.72±0.42	5.82±0.42
1.0	0.04±0.46	19.70±0.58	21.00±1.15**
2.0	-0.36±0.65	33.64±0.82	36.94±0.79***
4.0	-0.15±0.86	42.00±1.07	44.91±0.85***
6.0	-0.70±0.28	46.16±1.25	55.80±0.30**
8.0	0.25±0.63	31.33±0.89	42.74±0.86***
12.0	0.49±0.58	11.14±0.94	29.99±1.07*
18.0	-0.88±0.49	8.28±1.17	16.22±1.24*
24.0	-0.40±0.45	1.96±0.66	3.00±0.32***

n=6 * Significant at $p< 0.05$; ** highly significant at $p<0.01$;
*** very highly significant *represent that comparison of Nateglinide with Nateglinide + Diltiazem interaction

In our study, diltiazem pre-treatment (15 mg/kg for seven days), has no significant effect on the onset of action of nateglinide, whereas peak effect and duration of antidiabetic effect were significantly enhanced as compared to Nateglinide (50 mg/kg. p.o.) plain treatment. This suggests that Diltiazem inhibits the metabolism of these antidiabetic drugs by inhibiting the enzymes responsible for their metabolism. There are reports that Nateglinide is mainly metabolized by CYP3A4 and CYP2C9[09]. Reports also indicate that Diltiazem is an inhibitor of CYP3A4 and CYP2C9[10]. It is revealed from the results that Diltiazem, in therapeutic dose enhanced the antidiabetic effect of Nateglinide. This may be due to inhibitory effect of Diltiazem on CYP3A4 and CYP2C9.

Our studies in healthy and diabetic rats suggested that drug interaction occurs between Diltiazem and Nateglinide when they used concomitantly in normal and pathophysiological conditions like diabetes mellitus.

The present study indicates clearly that during the concomitant administration of Nateglinide and Diltiazem at therapeutic doses, the dose and frequency of administration of Nateglinide need to be readjusted. Also blood glucose levels need to be monitored during treatment period as a precautionary measure, so as to avoid severe hypoglycaemia.

CONCLUSION

In the simultaneous treatment of diabetes mellitus and angina pectoris in a patient with nateglinide and diltiazem, therapeutic drug monitoring is required and the dose and frequency of administration of nateglinide needs to be adjusted. Diltiazem, by inhibiting cytochrome P450 enzyme system, potentiates the anti diabetes action of nateglinide .Hence the dose of nateglinide should be reduced.

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Address for Correspondence

Raza Hasan, Luqman College of Pharmacy, Old jewargi road, Gulbarga-585102, Karnataka, India

E-mail: raza_hasan21@yahoomail.co.in