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A Study of drug -drug interaction of Bosentan with Repaglinide

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ABSTRACT

About 5% of the diabetic patients suffer from insulin dependent diabetes mellitus and requires insulin to control the blood glucose levels whereas remaining 95% are suffering from non-insulin dependent diabetes mellitus, which is controlled by using oral hypoglycemic agents. The patient suffering from diabetes mellitus are more prone to develop Hypertension. In such case, anti-diabetic drug like Repaglinide and antihypertensive agents are needed to be administered simultaneously. In addition, also reports indicating that Bosentanare newer pulmonary antihypertensive agent inhibits human cytochrome p450 enzyme system. The most important isoenzyme that are inhibited or affected by antihypertensive drugs like Bosentan, are CYP3A4 and CYP2C9. Repaglinideare metabolized by CYP3A4, CYP2C8 and CYP2C9. Hence, there is a possibility of development of drug-drug interaction between these two types of therapeutic agents. Hence, in the present study Repaglinide is oral antidiabetic agents and Bosentanis an antihypertensive agent is being used to understand, evaluate and confirm the drug-drug interaction between them. In this project interaction between the above mentioned classes of drugs were assessed in normal glycemic rats anddiabetic rats. Bosentan has not significantly altered onset of action, but it reduced the peak hypoglycemia and the duration of hypoglycemia as induced by repaglinidein albino rats, rabbits and diabetic rats. From the study it is indicated that the isoenzymes of CYP-450 systems, that are responsible for the metabolism of Repaglinideis sensitive to Bosentan and hence therapeutic dose of it could inhibit the isoenzymes and thereby affect the hypoglycemia induced by Repaglinide. Therefore it seems there is a need to go for therapeutic drug monitoring so as to readjust the dose and frequency of administration of Repaglinide when it is used with Bosentan.

Keywords: *Bosentan; Repaglinide; Drug-drug interaction; hypoglycemic activity; Alloxan Monohydrate; Antidiabetic activity.*

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INTRODUCTION

Diabetes is a group of diseases marked by high levels of blood glucose, also called blood sugar, resulting from defects in insulin production, insulin action, or both.

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonaemia [1].

There are three main types of diabetes mellitus.

1. Type 1 diabetes mellitus or insulin dependent diabetes mellitus (IDDM).
2. Type 2 diabetes mellitus or non-insulin dependent diabetes mellitus(NIDDM).
3. Gestational diabetes–high blood sugar level during pregnancy [2].

Among diabetics approximately 95% of patients have type II diabetes mellitus, where as about 5% of patients have type I diabetes mellitus [3]. The global prevalence of type II diabetes is expected to double in the period 2000 – 2025 and may reach a level of almost 300 million people [4].

A drug interaction is a situation in which a substance affects the activity of a drug, i.e. the effects are increased or decreased, or they produce a new effect that neither produces on its own [5]. Drug interactions are often categorized as pharmacokinetic or pharmacodynamic in nature. It can be either beneficial or detrimental to patients. Generally it has been found that the detrimental effects of drug interactions are more as compared to its beneficiary effects. In such cases it is needed to alter the dose and frequency of administration of one or all drugs, which are to be administered simultaneously.

Diabetes is a polygenic disease characterized by abnormally high glucose levels in the blood; any of several metabolic disorders marked by excessive urination and persistent thirst [7]. Diabetes is always coinciding with serious complications and adverse effects.

Amongst many possible side effects and complications, Hypertension is more prone to develop in diabetic patients [8], so use of antihypertensive drugs become necessary to co-administer with antidiabetic drug therapy. Hence, there may be a great chance of occurring drug-drug interactions while using such multi drug therapy regimen together. There are clear cut indications that anti hypertensive agents interfere with cytochrome P450 enzyme system hence they are used concomitantly. The isoenzymeCYP3A4 and CYP2C9 are responsible for the metabolism of Repaglinide [9]. Bosentan – a dual Endothelin-receptor antagonist is an inducer of above mentioned isoenzyme [10].Hence, keeping the possibility of interact ions between Bosentan and Repaglinide in this view, the present study was planned.

MATERIAL AND METHODS

Various drugs used in the study

Various drugs used in the study are the generous compliments from the esteemed pharmaceuticals mentioned against each drug below. The same may be considered as their source of procurement throughout the study.

1. Bosentan
2. Repaglinide

Mode of administration:

All the drugs used in the study administered orally in clinical practice. Hence the same route is followed.

METHODOLOGY

The studies were carried out in the Department of Pharmacology in our institution which is duly licensed by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments in Animals). The study protocols were approved according to current regulations of CPCSEA by the Institution Animal Ethics Committee for studies in experimental animals.

ANIMALS:

All the animals (rats) were used for this experimental study and were procured from Laxmi Biotec, Ale Phata, Pune . All rats were housed under standard husbandry conditions in the institutional animal house and approved by IAEC [Registration number ARACOP/IAEC/20/4]. A total of 50 rats (either sex) were selected for the current study.

Preparation of the animals for the study:

All the animals were given only standard pelleted animal diet (Nutrivet Life Sciences) from one week prior to the study till the completion of the study. All the animals were fasted for 18 hrs prior to the study with water *ad libitum.*

Method for oral administration: [11]

Oral feeding administration was done by oral feeding needle (purchased from Space Labs, Nashik.

Method for blood sampling: [12, 13, 14, 18]

The rat was anesthetized by anesthetic ether in anesthetic chamber. Now put animal on operation table and tail is squeezed with Xylene to dilate the vein and cut the tip of tail and blood is collected in the epindroff tubes containing pinch of anticoagulant mixture (sodium fluoride and potassium oxalate in 1:3 ratio).

NORMAL ALBINO RATS

Four groups (n=6) of animals were selected (I,II,III,IV);
Group I: Received repaglinide(50 mg/kg)
Group II : Received Bosentan(10mg/kg)
Group III :Bosentan(10mg/kg) then after 30 minutes repaglinide
Group IV: were treated with Bosentan(50mg/kg) for 7 days with regular feeding and on 8th day one hrafter bosentan treatment the animals of group IV were administered with repaglinide (50mg/kg).
The blood samples were collected from tail vein from rats and blood glucose levels were analyzed so as to attain the influence of pre-treatment of bosentan on hypoglycemia induced by repaglinide. The same method was repeated with bosentan (10mg/kg) andrepaglinide (50mg/kg) on diabetic rats with same dose of these three drugs as used in healthy rats.

DIABETIC RATS

Induction of diabetes: [15, 16, 17]

Induction of diabetes Albino rats of either sex were used for the induction of diabetes. These animals were injected with a freshly prepared aqueous solution of alloxan monohydrate in two doses of 100 mg/kg and 50 mg/kg body weight intraperitoneally for two consecutive days. Then 10% dextrose was administered to combat the immediate hypoglycemia. Blood sugar was measured and rats showing fasting blood sugar levels above 250 mg/dL were selected for the study.
The diabetic rats of either sex were divided into four groups of six each. All the rats weighing between 165 to 250g were given different drugs orally, as follows :

Four groups (n=6) of animals were selected (I,II,III,IV);
Group I: Received repaglinide (50 mg/kg)
Group II : Received Bosentan(10mg/kg)
Group III :Bosentan(10mg/kg) then after 30 minutes repaglinide
Group IV: were treated with Bosentan(50mg/kg) for 7 days with regular feeding and on 8th day one hr after Bosentan treatment the animals of group IV were administered with repaglinide (50mg/kg).

Estimation of blood glucose:

Enzyme,GOD-POD endpoint colorimetry

The GOD/POD methodisone such evolved method by Trinderin [19]. I*n vitro* quantitative determination of glucose in serum/plasma or cerebrospinal fluid done by[20] .

Effect of Bosentan pre-treatment on the antidiabetic activity of Repaglinidein diabetic rats

The earlier experiments in this project have revealed that the Bosentan has got potential interaction with antidiabetic drugs like Repaglinide in healthy albino rats and rabbits.
However, it is not clear whether Bosentan has got any influence on antidiabetic drugs in the pathophysiological conditions like diabetes mellitus in various species of animals.
Hence, in the present study, we have planned to use diabetic rats as experimental animals to clarify this aspect. In the earlier part of this study it was observed that pre-treatment with Bosentan at dose (10mg/kg) has significant influence at the peak hypoglycemia induced by Repaglinide. Therefore the dose of Bosentan was selected for the study toverify the interactions in diabetic albino rats.

RESULTS

On set of hypoglycemia (time taken to reduce blood glucose level to the extent of 15%-20%), duration of hypoglycemia (time duration in which more than 15%-20% reduction in blood glucose level is maintained) and peak hypoglycemia were the parameters considered for the evaluation of influence on rosiglitazone and repaglinide induced hypoglycemia. In this study Bosentan pre-treatment,i.e.10mg/kg has not significantly altered the onset of hypoglycemia (24.72± 1.93% reduction before treatment to 21.12±1.21% reduction after treatment) at1st hr but peak hypoglycemia was reduced significantly(48.07±2.54%reduction before treatment to 42.74±1.30% reduction after treatment., P<0.0001) at 5thehr of Repaglinide, While duration of hypoglycemia was 11hours before treatment and reduced to 7 hours only after treatment (21.24±3.72% reduction before treatment to23.51±6.86% reduction after treatment, P<0.001). These findings are recorded in table No.1and 2 and graphically shown in figure No.1

Table No 1: Blood Glucose Levels with Repaglinide (50 mg/kg) in healthy Albino Rats before and after Bosentan (10 mg/kg) treatment

Sr. No.	Time in hours	Blood Glucose Levels (mg %) with Repaglinide MEAN±SEM	Blood Glucose Levels (mg %) with Repaglinide + Bosentan MEAN±SEM
1	0	93.61±3.61	98.11±2.61
2	30	89.26±3.41	86.96±2.80
3	1	77.59±3.72	71.15±1.18
4	2	67.03±3.08	52.14±0.99
5	4	61.58±2.49	41.05±1.04
6	6	57.52±1.65	59.44±2.11
7	8	74.35±1.53	75.48±2.22
8	12	85.24±2.23	81.96±1.92
9	18	89.30±2.47	91.24±1.47
10	24	92.87±2.42	97.39±2.34

Table No 2: Percentage Blood Glucose Levels with Repaglinide (50 mg/kg) in healthy Albino Rat before and after Bosentan(10 mg/kg) treatment.

Sr. No.	Time in hours	Blood Glucose Levels (mg %) with Repaglinide MEAN±SEM	Blood Glucose Levels (mg %) with Repaglinide + Bosentan MEAN±SEM
1	0	-	-
2	30	14.11±1.02	7.90±0.76
3	1	24.72±1.93	21.12±1.21*
4	2	28.76±1.74	25.03±1.21**
5	4	41.06±2.45	35.63±0.97***
6	6	48.07±2.54	42.74±1.30***
7	8	32.97±3.32	25.51±6.86**
8	12	-21.24±3.72	15.30±1.21*
9	18	-12.51±2.70	7.56±1.29***
10	24	-0.92±2.35	0.060±2.18***

*Significant at p< 0.01; ** Highly significant at p<0.001; *** Very highly significant at p<0.0001 * represent that comparison of Repaglinide with Repaglinide + Bosentan interaction

Effect of Bosentan pre-treatment on antidiabetic activity of Rosiglitazone and Repaglinide in diabetic rats:

Earlier experiments have revealed that Bosentan pre-treatment has enhanced the hypoglycemic effect of rosiglitazone and Repaglinide in both carnivorous and herbivorous species. However, inthe present experiment the influence of Bosentan pre-treatment on same drugs under patho-physiological condition i.e. experimentally induced diabetes in albino rats was studied.

In this study Bosentan pre-treatment,i.e.10mg/kg has not significantly altered the onset of hypoglycemia (27.21 ± 0.58% reduction before treatment to19.691.18% reduction after treatment) at 1sthrbut peak hypoglycemia was reduced significantly (48.2±01.25% reduction before treatment to39.70 1.61% reduction after treatment, P<0.0001) at 5hr of repaglinide. However duration of hypoglycemia was only 11hours before treatment and reduced to 7hours after treatment (24.86±0.94% reduction before treatment to 27.68 ±1.23% reduction after treatment, P<0.0001). The results are shown in table No.3 and 4.

Table No 3: Blood Glucose Levels with Repaglinide (50 mg/kg) in Diabetic Albino Rats before and after Bosentan (10 mg/kg) treatment

Sr. No.	Time in hours	Blood Glucose Levels (mg %) with Repaglinide MEAN±SEM	Blood Glucose Levels (mg %) with Repaglinide + Bosentan MEAN±SEM
1	0	263.47±5.63	265.94±9.83
2	30	235.25±4.00	239.72±4.66
3	1	196.18±3.44	205.14±5.38
4	2	164.83±3.64	173.40±3.84
5	4	153.13±3.91	143.53±2.16
6	6	142.39±3.95	177.23±3.40
7	8	159.56±5.02	197.4±3.08
8	12	201.99±2.51	230.3±3.67
9	18	240.95±3.00	250.51±4.32
10	24	257.67±5.57	261.84±4.81

Table No 4: Percentage Blood Glucose Levels with Repaglinide (50 mg/kg) in Diabetic Albino Rats before and after Bosentan(10 mg/kg) treatment

Sr. No.	Time in hours	Blood Glucose Levels (mg %) with Repaglinide MEAN±SEM	Blood Glucose Levels (mg %) with Repaglinide + Bosentan MEAN±SEM
1	0	-	-
2	30	11.37±0.42	6.57±0.92
3	1	27.07±0.58	19.69±1.18***
4	2	39.24±0.82	28.39±1.35***
5	4	43.69±1.07	32.65±0.59**
6	6	48.20±1.25	39.70±1.61***
7	8	39.33±0.89	27.68±1.23***
8	12	24.86±0.94	14.76±1.69
9	18	9.11±1.17	6.52±0.69**
10	24	2.19±0.66	1.67±0.30***

** Highly significant at p<0.01; *** Very highly significant at p<0.001 *represent that comparison of Repaglinide with Repaglinide + Bosentan interaction

DISCUSSION

The patient suffering from diabetes mellitus are more prone to develop Hypertension. In such cases anti-diabetic drugs like Repaglinide and Rosiglitazone and antihypertensive agents are needed to be administered simultaneously. There are reports that Rifampicin induces the metabolism of Rosiglitazone [24]. There is a possibility that drug-drug interaction may occur between the antihypertensive drug and the drugs metabolized by these enzymes. Repaglinide are metabolized by CYP3A4, CYP2C8 and CYP2C9 [25,26]

In our studies in rats suggested that drug-drug interaction occurs between Bosentan and oral antidiabetic agents when they are used concomitantly in healthy conditions. However, the interaction in the patho-physiological conditions like in diabetes was not clear. Hence, in the fourth phase of our study the diabetic rats (Alloxan induced diabetic rats) were used, Repaeglinide were given to diabetic animals and the onset of hypoglycemia duration of hypoglycemia and peak antidiabetic effect was determined. To the same animals. Bosentan (10mg/kg) pretreatment for one week as usual and again oral antidiabetic agents (Repaglinide) were given, With Repaglinide has not significantly altered the onset of hypoglycemia (27.21±0. 58% reduction to 19.69±1.18% reduction) at1sthr but significantly decreased the peak hypoglycemia i.e. from 48.20 ..25% reductions to 39.70% reductions, P<0.0001 at 5thhr and duration of hypoglycemia are reduced i.e. from 11 hrs to less than 7 hrs (24.860. 94% reduction before treatment to 27.681. 23% reduction after treatment, P<0.0001) induced by Repaglinide.

CONCLUSION

Bosentan single-dose treatment has not influenced the blood glucose levels in healthy albino rats and diabetic rats. These findings are indicating that Bosentan does not possess hypoglycemic activity therefore it may be inferred that drug-drug interaction with Repaglinide is a pharmacokinetic type.

Bosentan pre-treatment for one week has not significantly influenced the onset with Repaglinide but peak hypoglycemia and the duration of hypoglycemia were reduced. However, Bosentan (10mg/kg) has a significantly reduced effect on the peak hypoglycemia, duration of hypoglycemia and the onset of hypoglycemia has no any significant change by Repaglinide in albino rats.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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