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Influence of Bosentan on Antidiabetic Effect of Pioglitazone and Nateglinide in Experimental Animals

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ABSTRACT

The present study was carried out to evaluate the drug-drug interaction between antidiabetic drugs and antihypertensive drug. Interaction of Pioglitazone, the known Thiazolidinedione antidiabetic drug and Nateglinide, the known Meglitinide class antidiabetic drug with Bosentan, an antihypertensive drug was evaluated in healthy and Streptozotocin (STZ) induced diabetic rats. The blood samples were collected from rats at different time interval upto 24 h and blood glucose was estimated. Bosentan (10 mg kg^{-1}, p.o.) pretreatment has not significantly altered the onset of antidiabetic effect of Pioglitazone (0.3 mg kg^{-1}, p.o.) but significantly decreased the peak antidiabetic effect in healthy and Streptozotocin induced diabetic rats (44.74±2.56% reduction before treatment to 34.59±0.25% reduction after treatment), while duration of antidiabetic effect was reduced from 16 h to less than 12 h in both groups. Similarly pretreatment with Bosentan (10 mg kg^{-1}, p.o.) has significantly no effect on the onset of antidiabetic effect of Nateglinide (50 mg kg^{-1}, p.o.) but it significantly reduced the peak antidiabetic effect in healthy and Streptozotocin induced diabetic rats (46.15±1.25% reduction before treatment to 37.57±1.61% reduction after treatment), while duration of antidiabetic effect was reduced from 12 h to less than 8 h in both groups. This study indicates that Therapeutic Drug Monitoring (TDM) has to be required to readjust the therapeutic doses of Bosentan and antidiabetic drugs like Pioglitazone and Nateglinide when they used concomitantly.

Key words: Influence of bosentan, antidiabetic effect of pioglitazone, nateglinide, streptozotocin (STZ), drug interaction

INTRODUCTION

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. Drug interaction may either beneficial or detrimental to our body but generally it has been found that the detrimental effects of drug interactions are more as compared to its beneficiary effects. It may modify the diagnostic, preventive or therapeutic activity of either drug (Sultanpur *et al*., 2011).

In poly-pharmacy, it is important to determine the incidence and frequency of occurrence of drug interactions, which serious implications, in hospitalized patients. In addition, it is also important to find out agents that are most likely to produce harmful interactions (Sunilkumar *et al*., 1998). It is known that the incidence of adverse drug reaction to drugs rise from

4.2% when five or fewer drugs are used to 45% when twenty or more drugs are used. According to report that, the drug interaction may be fourth to sixth leading cause for death in United States (Anitha *et al*., 2008).

Diabetes mellitus-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. Diabetic patients may also be affected with many other diseases like hypertension, cardiovascular diseases, peptic ulcer, fungal infections etc which require prolong treatment. There are reports that hypertension is likely more prone to develop with patients suffering from diabetes (Ejuoghanran *et al*., 2009). In such cases multiple drug therapy is needed to prescribe. So, agents like Bosentan, Amlodipine and Losartan etc and Antidiabetic agents like Pioglitazone, Nateglinide and Rosiglitazone etc are administered concomitantly.

There are reports that Bosentan is known to get metabolized through Cytochrome P-450 enzyme system and it is an inducer of this system (Kimberley and Bshouty, 2009), hence there is a possibility of occurrence of pharmacokinetic type of drug interactions with concomitantly used drugs. Pioglitazone and Nateglinide are metabolized by Cytochrome P-450 enzyme system (Sahi *et al*., 2003). Therefore, the present study was carried out on healthy and diabetic rats to assess the influence of Bosentan pretreatment on the antidiabetic effects of Pioglitazone and Nateglinide.

MATERIALS AND METHODS

Animals: The present study was conducted on healthy and diabetic rats of albino wistar strain of either sex, weight range 150-200 g. The animals were procured from Sri Mahavir Enterprises, Hyderabad. They were housed under standard conditions (temperature of 25±2°C and 50±2% relative humidity with 12 h light/dark cycle) and provided with water *ad libitum*. Prior approval by institutional ethics committee (reg.no: 346/CPCSEA) was obtained for conduction of experiments. The study was conducted in the Department of Pharmacology of Luqman College of Pharmacy, Gulbarga.

Drugs: Pioglitazone was obtained from MeproMax Lifesciences, Dehradun, Nateglinide was obtained from Dr. Reddy's Laboratories, Hyderabad and Bosentan was obtained from Cipla Ltd, Madgaon; Goa. Pioglitazone (0.3 mg kg^{-1}, p.o.) Nateglinide (50 mg kg^{-1}, p.o.) and Bosentan (10 mg kg^{-1}, p.o.) suspensions were prepared using 2% w/v gum acacia as suspending agent.

Experimental procedure

In healthy rats: The healthy rats were marked conveniently and distributed randomly into three groups of 6 animals each. All the animals were over night fasted with water *ad libitum*. The animals in group-1 received Bosentan (10 mg kg^{-1}, p.o.), the animals in the group-2 received Pioglitazone (0.3 mg kg^{-1}, p.o) and animals in group-3 received Nateglinide (50 mg kg^{-1}, p.o.) in 2% w/v acacia suspension.

Blood samples were collected at 0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 18.0 and 24.0 h through tail vein method. Blood glucose levels were estimated by GOD/POD method (Kumar *et al*., 2009) and expressed as mg dL^{-1} of blood.

In the next phase of the experiment, after the washing period of 10 days, the same animals of group-2 and 3 received Bosentan 10 mg kg^{-1}, p.o. for seven days. On the 7th day, 6 h after administration of Bosentan, the animals were fasted for 14 h. On the 8th day, Bosentan was given as usual to the animals of both groups. One hour after the treatment, animals of group-2 received

Pioglitazone 0.3 mg kg^{-1}, p.o. and animals of group-3 received Nateglinide 50 mg kg^{-1}, 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.

In diabetic rats

Experimental induction of diabetes mellitus: Diabetes was induced in the rats by administering STZ (50 mg kg^{-1}) intraperitonially into the 18 h fasted rats (Parthasarathy *et al.*, 2009). Blood samples were collected after 24 h and blood glucose levels were estimated. Albino rats which have shown more than 250 mg dL^{-1} blood glucose levels were considered as diabetic. The blood glucose levels were monitored for further four days. From this it was confirmed that diabetes was induced in 48 h and stabilized within 7 days. These animals were used for further studies.

For the next phases study, the same procedure was carried out as mentioned in the healthy rats above. 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

It is evident from Table 1 that treatment with Bosentan alone did not alter the blood glucose levels in healthy rats. However, Bosentan pretreatment 10 mg kg^{-1}, p.o. has no significant effect on the onset of antidiabetic effect of Pioglitazone and Nateglinide, but it significantly reduced peak antidiabetic effect of Pioglitazone from 48.74±2.68% before treatment to 40.86±0.86% after treatment at 6th h and of Nateglinide from 49.80±2.54% before treatment to 41.27±1.26% after treatment at 5th h. Duration of antidiabetic effect of Pioglitazone was decreased from 16 h to less than 10 h whereas duration of Nateglinide was decreased from 11 h to less than 7 h.

It is evident from Table 2 that treatment with Bosentan alone did not alter the blood glucose levels in diabetic rats. However, Bosentan pretreatment 10 mg kg^{-1}, p.o. has no significant effect

Table 1: Percentage decrease in blood glucose levels at different time intervals in healthy albino rats

Time (h)	Bosentan (10 mg kg^{-1}. p.o.)	Pioglitazon (0.3 mg kg^{-1}. p.o.)	Nateglinide (50 mg kg^{-1}, p.o.)	Bosentan (10 mg kg^{-1}. p.o.) +Pioglitazone (0.3 mg kg^{-1}. p.o.)	Bosentan (10 mg kg^{-1}. p.o.) +Nateglinide (50 mg kg^{-1}. p.o.)
Fasting	--	--	--	--	--
0.5	0.54±0.62	5.89±0.44	12.84±1.02	6.14±0.76	06.48±0.74
1.0	2.66±0.35	13.84±1.28	24.70±1.93	11.93±1.21**	21.30±1.27*
2.0	1.45±0.67	23.67±2.08	30.76±1.74	19.57±1.21***	26.88±1.41**
4.0	0.14±0.87	31.37±1.59	42.45±2.45	27.41±0.97***	32.38±0.83***
6.0	0.42±0.67	39.65±2.66	49.80±2.54	34.86±1.30***	41.27±1.26***
8	0.50±0.77	48.74±2.68	30.49±3.32	40.86±0.86**	23.66±6.88**
12	1.73±0.55	37.32±2.59	-23.64±3.72	24.48±1.21**	13.65±1.11*
18	0.86±0.91	25.58±2.08	-14.51±2.70	12.91±1.29*	6.54±1.79***
24	0.30±0.75	13.20±2.91	-0.95±2.35	11.70±2.18	-0.010±2.48***

* Significant at $p<0.05$, ** Highly significant at $p<0.01$, *** Very highly significant. Values are Mean±SEM (n = 6)

Table 2: Percentage decrease in blood glucose levels at different time intervals in diabetic albino rats

Time (h)	Bosentan (10 mg kg^{-1}. p.o.)	Pioglitazone (0.3 mg kg^{-1}. p.o.)	Nateglinide (50 mg kg^{-1}, p.o.)	Bosentan (10 mg kg^{-1}. p.o.) +Pioglitazone (0.3 mg kg^{-1}. p.o.)	Bosentan (10 mg kg^{-1}. p.o.) +Nateglinide (50 mg kg^{-1}. p.o.)
Fasting	--	--	--	--	--
0.5	0.40±0.42	7.49±0.57	10.72±0.42	6.63±0.55	5.73±0.92
1.0	1.46±0.95	16.02±1.12	25.70±0.58	14.73±0.88***	18.17±1.18***
2.0	0.35±0.57	27.69±2.39	37.64±0.82	26.31±0.52***	26.40±1.35***
4.0	0.17±0.57	33.42±2.75	42.00±1.07	36.89±0.86*	30.66±0.59**
6.0	0.49±0.57	39.84±2.60	46.16±1.25	40.39±0.50**	37.57±1.61***
8.0	0.90±0.40	44.50±2.56	39.33±0.89	34.59.±0.25**	26.03±1.23***
12.0	1.39±0.53	36.24±2.41	23.14±0.94	30.44±1.13	13.40±1.69
18.0	0.56±0.91	25.46±2.40	8.28±1.17	24.49±2.39***	5.66±0.69**
24.0	0.20±0.48	11.91±1.27	2.96±0.66	11.50±1.22**	1.49±0.30***

*Significant at p<0.05: **Highly significant at p<0.01: ***Very highly significant. Values are Mean±SEM (n = 6)

on the onset of antidiabetic effect of Pioglitazone and Nateglinide but it significantly reduced peak antidiabetic effect of Pioglitazone from 44.50±2.56% before treatment to 34.59.±0.25% after treatment at 6th h and of Nateglinide from 46.16±1.25% before treatment to 37.57±1.61% after treatment at 5th h. Duration of antidiabetic effect of Pioglitazone was decreased to less than 16 h whereas duration of Nateglinide was decreased from 11 h to less than 7 h.

DISCUSSION

Diabetes is defined as a disorder exhibiting hyperglycemia caused by deficient insulin action, which is determined by both the capacity to secrete insulin from pancreatic β-cells and insulin action in peripheral insulin-sensitive tissue such as muscle and liver, requiring lifelong treatment. As per the research statistics, Hypertension is more prone to occur with diabetes. Hypertension also requires treatment for a prolonged period. If a patient is suffers from diabetes mellitus as well as hypertension, he has to use antidiabetic drugs such as Pioglitazone and Nateglinide and antihypertensive agent like Bosentan. In such instances, there is a possibility of occurrence of drug interactions. Our pilot study has indicated that drug interactions occur when Bosentan and Pioglitazone or Bosentan and Nateglinide are administered concomitantly at therapeutic doses. However, the therapeutic dose was found to influence the antidiabetic effect significantly.

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

Since Bosentan (10 mg kg^{-1}) *per se* did not influence the blood glucose levels and thus the possibility of occurrence of pharmacokinetic interaction can be ruled out. In our study, pretreatment with Bosentan (10 mg kg^{-1}) has not significant effect on the onset of action of Pioglitazone and Nateglinide, whereas peak effect and duration of antidiabetic effect were significantly decreased as compared to Pioglitazone (0.3 mg kg^{-1}. p.o.) and Nateglinide (50 mg kg^{-1},. p.o.) plain treatment. This suggests that Bosentan enhances the metabolism of these antidiabetic drugs by inducing the enzymes responsible for their metabolism. There are reports that Pioglitazone and Nateglinide are mainly metabolized by CYP3A4, CYP2C8 and CYP2C9 (Sahi *et al*., 2003). Reports also indicate that Bosentan is an inducer of CYP3A4 and CYP2C8 (Dingemanse and Giersbergen, 2004). It is

evident from the results that the therapeutic dose of Bosentan reduced the antidiabetic effect of Pioglitazone and Nateglinide. This may be due to induction effect of Bosentan on CYP3A4 and CYP2C8 (Serasli *et al*., 2010).

Present studies in healthy and diabetic rats suggested that drug interaction occurs between Bosentan and Pioglitazone and Bosentan and Nateglinide when they used concomitantly in pathophysiological conditions like diabetes mellitus.

In this present study, it indicates clearly that during the concomitant administration of Bosentan with Pioglitazone and Nateglinide at therapeutic doses, the dose and frequency of administration of Pioglitazone and Nateglinide need to be readjusted. Simultaneously blood glucose levels are monitored during treatment period as precautionary measure so as to avoid severe hypoglycaemia.

CONCLUSION

The present study concluded that, during simultaneous treatment of diabetes mellitus with hypertension, Bosentan do interact with Pioglitazone and Nateglinide at therapeutic doses. Therefore it is necessary to adopt therapeutic drug monitoring so as to readjust dose and frequency of administration of these drugs, when they are used concomitantly to avoid the patients from severe hypoglycaemia.

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