

## INTERACTIVE POTENTIAL OF CLARITHROMYCIN IN RATS ADMINISTERED WITH GLICLAZIDE IN NORMAL AND DIABETIC CONDITIONS

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Article Received on: 12/07/11 Revised on: 23/08/11 Approved for publication: 16/09/11

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### ABSTRACT

To investigate the interaction between clarithromycin and gliclazide, the present study is designed in various animal models. Albino rats and rabbits were selected for the current study. The animals were suitably grouped. In the first part of the experiment, per se effect with clarithromycin was carried out. In the next phase, the animals were treated with gliclazide and hypoglycemic/antidiabetic activity was performed. In the last phase, the animals of the second part were pretreated with clarithromycin for 7 days and on the 8<sup>th</sup> day, one hour after clarithromycin administration; the animals were treated with gliclazide. The blood samples were collected from the animals (retro-orbital sinus of rats and marginal ear veins of rabbits) up to 24 h before and after clarithromycin administration and blood glucose levels were analyzed by GOD-POD. Onset, peak effect and duration of hypoglycemia/antidiabetic activity were considered as parameters of the study. Clarithromycin increased the peak effect and duration of hypoglycemia induced by gliclazide in all the animal models. These findings suggested that clarithromycin retarded the metabolism of gliclazide. It was reported that clarithromycin is an inhibitor of CYP 3A4 and further gliclazide is metabolized by CYP 2C9 and CYP 3A4. Hence, increased hypoglycemic/antidiabetic activity may be attributed to clarithromycin induced inhibition of CYP enzyme. Therefore, it may be suggested that during concomitant administration of clarithromycin and gliclazide, the dose and frequency of administration of gliclazide has to be readjusted as a precautionary measure so as to avoid the possibility of hypoglycemia.

**KEYWORDS:** Antidiabetic activity, Clarithromycin, CYP enzymes, Gliclazide.

### INTRODUCTION

Multiple drug therapy is very common nowadays in clinical practice to treat chronic diseases like diabetic mellitus or to manage either single or simultaneously occurring different diseases. In this condition, there are every possibility of occurrence of drug interactions and require clinical observations. Drug interactions may be pharmacodynamic or pharmacokinetic type and much of the reported drug interactions are due to either CYP enzyme induction or inhibition. In order to establish the nature/mechanism of drug interactions, it is mandatory to conduct animal study in preclinical set up<sup>1</sup>.

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia with overall altered biochemistry and resulted in many complications such as nephropathy, retinopathy, neuropathy, sexual impotence and low grade immunity leading to infections<sup>2, 3</sup>. It is estimated that 143 million people worldwide suffer from diabetes<sup>4</sup> and are effectively treated with sulfonylureas like gliclazide<sup>5-7</sup>. Diabetic patients were susceptible to bacterial infections as they are immunocompromised and require prompt treatment by antibacterials such as clarithromycin<sup>8-10</sup>.

When a patient is suffering with diabetes mellitus associated with bacterial infections, it may be required to treat such patients by both antidiabetic drugs like gliclazide and antibacterials like clarithromycin. In the literature, it was indicated that, gliclazide is metabolized by both CYP 2C9 and CYP 3A4<sup>5,6</sup> and clarithromycin is an inhibitor of CYP 3A4<sup>11-13</sup>. Even there are reports that macrolide antibiotics like erythromycin and clarithromycin enhance the hypoglycemic effects of repaglinide<sup>14</sup>. However, there are no reports of drug interactions between clarithromycin and gliclazide and hence, it was planned to study the possible interaction between them in various animal models.

### MATERIALS AND METHODS

#### Animals

The present study was conducted on albino rats of either sex, aged 6-7 weeks; weight range 200-280 G and normal albino rabbits, aged 3-4 months and weighing 1.5-2.5 kg were recruited for the study. The animals were procured from Sri Venkateshwara Enterprises, Bengaluru. They were housed under standard conditions (temperature- 28 ± 2°C, relative humidity- 60 ± 2% and 12 h light /

dark cycle) with water *ad libitum*. Prior approval by institutional ethics committee (reg. no: 157/99/CPCSEA) was obtained for conduction of experiments on animals. The study was conducted in SCS College of Pharmacy, Harapanahalli.

#### Chemicals and drugs

Gliclazide and clarithromycin gift samples were procured from Micro Labs, Bengaluru and Alembic pharmaceuticals ltd, Baddi, Himachal Pradesh respectively. Alloxan monohydrate was purchased from Sigma Chemicals. Glucose test kit (Enzymatic, GOD-POD Method) was purchased from CPC diagnostic Ltd. Mumbai, India. All other chemicals were procured from the local market.

#### Induction of diabetes

Diabetes was induced in albino rats by administration of aqueous alloxan monohydrate at a dose of 100 mg/kg body wt. intraperitoneally. Blood glucose was measured after 18h of alloxanisation. Another 50mg/kg was given through IP route; diabetes was induced in rats and it was further confirmed. Rats showing fasting blood glucose levels above 200mg/dl were selected for the study<sup>15</sup>.

Normal rats and diabetic rats of either sex, weight range 200-280 G were divided in to 3 groups of 6 animals each and normal rabbits weighing 1.5-2.5 kg divided in to 2 groups of 5 rabbits each were recruited for the study. In the first phase of the study, all the animals were treated with clarithromycin per se and the effect of it on the blood glucose levels were monitored. In the second phase, the animals were treated with gliclazide (2.8 mg/kg, p.o in rats & 1.8 mg/kg p.o in rabbits) and the hypoglycemic/antidiabetic activity was carried out. In the last phase, the animals of the second phase were treated with clarithromycin (45 mg/kg twice a day in rats and rabbits) consecutively for 7 days and on the 8<sup>th</sup> day, one hour after the administration of clarithromycin; gliclazide was administered to the respective groups. Then the blood samples were collected at predetermined periods and the blood glucose levels were estimated by previously reported GOD-POD method<sup>16-17</sup>. 1

#### Statistical analysis

The data were analyzed by Student Paired 't' test. P values less than 0.05 has been considered as statistically significant.

**RESULTS AND DISCUSSION**

It was evident from the table 1 that clarithromycin per se did not influence the blood glucose levels in rats and rabbits. Gliclazide produced biphasic hypoglycemic activity with maximum reduction of blood glucose levels of  $45.37 \pm 1.64$  at 2<sup>nd</sup> and  $46.17 \pm 1.23$  8<sup>th</sup> h in normal rats and antihyperglycemic activity with maximum reduction of blood glucose levels of  $45.60 \pm 1.19$  and  $41.58 \pm 1.58$  after 2<sup>nd</sup> and 8<sup>th</sup> h in diabetic rats respectively. Pretreatment with clarithromycin increased the peak effect to  $55.35 \pm 1.66$  &  $57.74 \pm 1.41$  and  $54.27 \pm 1.46$  &  $45.39 \pm 1.08$  in normal/diabetic rats at 2<sup>nd</sup> and 8<sup>th</sup> h. The duration of hypoglycemia was shifted from 17 h to 23 h in normal and diabetic rats. In rabbits, clarithromycin enhanced the peak hypoglycemia from  $39.91 \pm 2.91$  to  $50.06 \pm 0.74$  at 4<sup>th</sup>. Duration of hypoglycemia was shifted from 16 h to 22 h. Drug interactions are commonly reported in clinical practice and the preliminary study must be established using various animal models. We studied the influence of clarithromycin on the hypoglycemia produced by gliclazide in normal/diabetic rats and rabbits. The normal rat model served to quickly identify the interaction and diabetic rats served to validate the same response in pathophysiological conditions such as diabetes mellitus. The rabbits were used as it is mandatory to establish any drug interaction in two dissimilar species.

In the current investigation, the gliclazide produced biphasic response in rat models due to its entero-hepatic circulation<sup>18</sup>. Clarithromycin per se did not influence the blood glucose levels and hence the possibility of additive effect with gliclazide in the subsequent studies was ruled out. However, pretreatment with clarithromycin for 7 consecutive days potentiated the peak and duration of hypoglycemia induced by gliclazide in rats and rabbits. In the literature, it was indicated that gliclazide is metabolized by hepatic P450 CYP 2C9 and 3A4<sup>8</sup> and clarithromycin is an inhibitor of CYP P450 CYP 3A4 isoenzyme<sup>12</sup>. In our investigation, it was observed that onset was not altered significantly and this clearly indicates that clarithromycin does not affect the absorption phase of gliclazide. However, pretreatment with clarithromycin enhanced the hypoglycemia induced by gliclazide in various animal models. These findings suggested that clarithromycin inhibited the metabolism of gliclazide. Our studies in normal rats, diabetic rats and normal rabbits indicated that clarithromycin inhibits the enzymes responsible for the metabolism of gliclazide. Hence, during concomitant administration of clarithromycin and gliclazide, the dose and frequency of administration of gliclazide should be modified. Even therapeutic drug monitoring may be essential to avoid the possibility of severe hypoglycemia. However, evaluation of various pharmacokinetic parameters of gliclazide before and after clarithromycin treatment may be considered as the scope of future study.

**CONCLUSION**

It may be concluded that during simultaneous treatment with clarithromycin and gliclazide, it is suggested that, the dose and frequency of administration of gliclazide may be essential.

**ACKNOWLEDGEMENT**

The authors are thankful to Principal, S.C.S College of Pharmacy and the Management, T.M.A.E Society, Harapanahalli for providing all the facilities to carry out this research work. Thanks are also due to Alembic pharmaceuticals ltd (Baddi, H.P) for providing clarithromycin and Micro labs, (Bengaluru) for providing gliclazide as gift samples.

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TABLE I. PERCENTAGE BLOOD GLUCOSE REDUCTION IN NORMAL ALBINO RATS AND DIABETIC ALBINO RATS

Sl. no	Time in h	Percentage blood glucose reduction in normal albino rats			Percentage blood glucose reduction in diabetic albino rats	
		Clarithromycin Per se 45 mg/kg (Mean±SEM)	Gliclazide 2.8mg/kg (Mean±SEM)	Gliclazide (2.8 mg/kg)+ clarithromycin (45 mg/kg) (Mean±SEM)	Gliclazide 2.8 mg/kg (Mean±SEM)	Gliclazide(2.8 mg/kg)+ Clarithromycin (45 mg/kg) (Mean±SEM)
1.	Fasting	---	---	---	---	---
2.	1.0	-1.55±2.13	24.63±2.01	29.43±1.38	20.37±1.61	17.26±1.12
3.	2.0	1.34±2.28	45.37±1.64	55.35±1.66**	45.60±1.19	54.27±1.46***
4.	4.0	-0.43±2.93	37.46±2.22	49.57±1.36***	32.76±1.69	39.08±2.00*
5.	8.0	3.22±1.37	46.17±1.23	57.74±1.41***	41.58±1.58	45.39±1.08
6.	12.0	-1.61±2.67	24.54±1.58	35.22±2.32**	27.29±2.57	38.89±2.51**
7.	18.0	-1.46±3.30	14.47±1.06	19.13±1.62*	19.32±1.74	26.99±3.172
8.	24.0	1.88±3.22	5.28±1.09	15.32±1.52***	5.71±1.16	18.79±2.30***

N=6,\*Significant at p<0.05;\*\*Highly significant at p<0.01;\*\*\* very highly significant at p<0.001

TABLE II. PERCENTAGE BLOOD GLUCOSE REDUCTION IN NORMAL ALBINO RABBITS

N=5,\*Significant at p<0.05;\*\*highly significant at p<0.01; \*\*\* very highly significant at p<0.001

Sl.no	Time in h	Percentage blood glucose reduction in normal albino rabbits		
		Clarithromycin perse 45 mg/kg (Mean±SEM)	Gliclazide 1.8 mg/kg (Mean±SEM)	Clarithromycin (45 mg/kg)+ Gliclazide (1.8 mg/kg) 1.8 mg/kg (Mean±SEM)
1.	Fasting	---	---	---
2.	1.0	0.80±3.74	18.17±2.37	19.72±1.47
3.	2.0	-0.91±1.90	30.90±1.22	42.62±1.16**
4.	4.0	-0.61±4.50	39.91±2.19	50.06±0.74*
5.	8.0	1.40±5.96	33.57±0.87	42.02±1.73**
6.	12.0	-0.37±6.24	26.32±1.33	35.52±0.96**
7.	18.0	0.29±3.77	20.84±1.67	27.29±1.52*
8.	24.0	1.17±2.85	8.77±3.88	19.84±0.66*

Source of support: Nil, Conflict of interest: None Declared