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THE EFFECT OF PANTOPRAZOLE ON ORAL GLUCOSE INDUCED GLYCAEMIC CHANGES IN NORMAL ALBINO RATS

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ABSTRACT

Objective: To determine the effect of Pantoprazole on oral glucose induced glycaemic changes and on basal blood glucose levels in normal albino rats. Methodology: Normal adult albino rats of either sex weighing between 150 – 200g were randomly divided into 2 groups(n=6); Control(distilled water-1ml/rat) and Test(Pantoprazole-6.5mg/kg BW) and the respective drug given orally for 3 days. They were fasted overnight at water before the 3rd day. On the 3rd day, 2 hours after the last drug dose , Oral glucose tolerance test(OGTT) was performed with glucose 0.6g/kg body weight(BW). Results: The average capillary blood glucose(CBG) of the Test(Pantoprazole) group was lower compared to the Control group at 60, 120 and 150 minutes progressively with maximum hypoglycaemia (CBG 22.9% ↓) at 150 minutes after glucose administration. Conclusion: Oral Pantoprazole given for 3 days has hypoglycaemic effect following oral glucose administration (which is progressive over time since glucose administration) by direct and indirect glucose induced insulin secretagogue effect.

KEY WORDS : **Pantoprazole; oral glucose tolerance ; hypoglycaemia; Diabetes mellitus; glycaemic control**

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INTRODUCTION

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate.[1] The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The "diabetes epidemic" will continue even if levels of obesity remain constant.[2] The major driver of the diabetes epidemic is type 2 diabetes, which accounts for more than 90 per cent of all diabetes cases.[1]

Patients with type 2 diabetes have an increased risk of developing both microvascular and macrovascular disease and associated complications[3] and there is an association between the degree and duration of hyperglycemia and increased risk of microvascular complications, sensory neuropathy, myocardial infarction, stroke, macrovascular mortality and all cause mortality. The relative risk for myocardial infarction seems to increase with any increase in glycaemia above the normal range, whereas the risk for microvascular disease is thought to occur only with more extreme concentrations of glycaemia.[4] Individuals with very high concentrations of glycaemia would be most likely to benefit from reduction of glycaemia, but the data suggest that any improvement in glycaemic control across the diabetic range is likely to reduce the risk of diabetic complications.[3]

There is a high prevalence of upper gastrointestinal(GI) diseases in diabetes mellitus (DM), including gastro oesophageal reflux disease (GERD) and Acid peptic disease (APD). Moreover, diabetes complications and poor glycaemic control measured by HbA1c were independent risk factors for upper GI symptoms.[5] Individuals seropositive for *Helicobacter pylori* experienced a greater rate of incident diabetes.[6] Additionally, the risk of gastric cancer is found to increase dramatically among subjects who have both high HbA1c levels and an *Helicobacter pylori* infection.[7]

At present the treatment of DM includes Insulin , biguanides, α-glucosidase inhibitors, dipeptidyl peptidase IV inhibitors, sulfonylureas and thiazolidinediones, GLP-1 receptor agonists, Amylin agonists, medical nutrition therapy and life style modifications which lower glycaemia by various mechanisms.[8] Few of these adequately address the underlying problem of insulin resistance and have limited efficacy, limited tolerability and significant mechanism-based side effects. A particular problem is that many patients who respond initially become refractory to treatment over time . The reasons due to which the present antidiabetic agents become less effective and progressively fail to maintain glycaemic levels at optimal health status levels may include progressive loss of β cell mass in type 2 diabetes despite a normal capacity for β-cell replication and neogenesis observed in both obese and lean type 2 diabetic humans and in diabetic rodent models of genetic and experimental diabetes with an increase in β-cell apoptosis. Most medications being used to treat type 2 diabetes cannot prevent β-cell death or re-establish β-cell mass. Some agents with the potential to inhibit β-cell apoptosis and/or increase β-cell mass have been identified in preclinical studies Ex. DPP-IV inhibitors, GIP etc. Thus, newer approaches with particular emphasis on finding more drugs whose mechanisms are dependent on physiological responses like glucose-mediated insulin secretagogues,[4] β-cell regenerating and protecting drugs etc. are essential.

Endogenous hypergastrinemia induces hyperglycemia and potentiates insulin secretion by trophic activity on pancreas during regeneration process.[9,10,2] Gastrin, partially in synergism with glucose or

aminoacids, has shown insulin stimulatory capacities in normal animals and man.[11] Gastrin stimulates β-cell regeneration and survival of increased β-cell mass and improves glucose tolerance in rats.[12]

Gastrin can stimulate a rapid and short-lived release of insulin. In physiologic concentrations, gastrin potentiates the glucose-stimulated insulin secretion and has minimal effect on basal insulin secretion.[13] A small release of gastrin during oral glucose ingestion may to a limited extent contribute to the nonglycaemic insulin secretion. During protein ingestion, gastrin stimulates insulin secretion significantly by two mechanisms:

(a) by a direct effect on the acutely releasable pool of insulin in and possibly (b) by an indirect effect mediated through activation of other gastrointestinal hormones Ex.secretin released by acid in duodenum and jejunum. [9, 14]

The dose-response studies of the effect on basal insulin secretion showed that the gastrin doses required for a significant insulin response resulted in unphysiologic concentrations of gastrin in serum. In contrast the glucose-stimulated insulin secretion was significantly potentiated by small doses of gastrin (within physiologic limits). The effect was direct since the increase in the glucose-mediated insulin release was observed in the 1st min and though gastrin at initial oral glucose therapy caused hyperglycemia, later on its insulin secretagogue effect was more evident especially following a protein rich environment.[13]

Proton pump inhibitors (PPIs) Ex. Pantoprazole are inhibitors of acid secretion invivo and they act by irreversibly inhibiting the H+K+ ATPase of parietal cells with a half life of 1.5 hours but acid suppression action lasting at least 18hours.[15] Proton pump inhibitors(PPIs), used to treat hyperacidity, indigestion, gastroesophageal reflux disease (in addition to prokinetic agents) and gastric ulcers are gastrin agonists and can lead to consistent endogenous hypergastrinemia.[16,17,18] Thus, the hypothesis that proton pump inhibitors can be used to treat type 2 diabetes. The postulated mechanism for this effect is PPI causing endogenous hypergastrinemia which enhances pancreatic β cell regeneration and function, and stimulates glucose and amino acid induced insulin secretion.[16,17]

Type 2 DM is a progressive disease with worsening glycaemia over time. Long term consequences translate into enormous human suffering and economic costs. Current day management has failed to achieve and maintain glycaemic levels most likely to provide optimal health care status for people with DM .[19] Also, with *H. pylori* playing a potential etiological role in the development of type 2 diabetes, treatments such as antibiotics and proton pump inhibitor combinations for treatment of *H. pylori* should be explored as targets of intervention in high-risk communities.[6]

Data on the relationship between PPI therapy and glycaemic control is scanty in the literature.[16,17] However, even with the potential adverse effects of long term use of PPIs including: vitamin B_{12} deficiency, iron deficiency, hypomagnesemia, increased susceptibility to pneumonia, enteric infections, colon cancer, drug interactions and birth defects raising mild concerns, the benefits of proton pump inhibitor use outweigh its risks in most patients.[20] Hence, this study is undertaken to determine the effect of Pantoprazole on oral glucose induced glycaemic changes and its effect on basal blood glucose levels in normal albino rats.

MATERIALS AND METHODS

Chemicals used:

1. Glucose 0.6g/kg body weight dissolved in water – given orally

2. Pantoprazole (Tablet Pantocid procured from pharmacy and dissolved in distilled water) – 6.5mg/kg body weight - given orally
3. Distilled water

Animals

Adult albino rats of either sex weighing between 150 – 200 grams were randomly selected from central animal facility, JSS Medical College, Mysore excluding diseased and pregnant rats. Animals were housed in groups of 3 at an ambient temperature of 25 +/- 1^0C with ad libitum access to food and water. The study protocol was approved by Institutional Animal Ethics Committee.

Method of collection of data

In this study, 12 albino rats (Two groups containing six rats each) were used. Animals were randomly divided into 2 groups of 6 rats each; Group – 1 (CONTROL): Distilled water - 1 ml/rat; Group-2 (TEST): Pantoprazole dissolved in distilled water – 6.5mg/kg body weight/day for 3 days. All the rats were orally fed with the respective drug using gavage tube. The capillary blood glucose(CBG) levels were measured using ACCUCHEK glucometer during the oral glucose tolerance test(OGTT).

OGTT:[21]

The oral glucose tolerance test is a measure of the glucose induced insulin secretion mediated glycemic control alteration. This study used OGTT for normal rats with some modifications to the standard method (Du vigneaud and Karr, 1925) to assess the effect of pantoprazole on glucose induced glycaemic control alteration.

Both the groups of rats – 1 and 2 were subjected to OGTT. The rats of the control group (1) were given distilled water and the test (2) were given pantoprazole for 3 days. All the rats were fasted overnight at water before the 3rd day. On the third day, 2 hours after the last dose of the respective drug, OGTT was performed. 30 minutes before the OGTT, the CBG of all the rats were measured. 30 minutes after this, all the rats were given glucose (0.6g/kg body weight) dissolved in water orally using gavage tube. Following this, the serum glucose of blood sample from tail vein (obtained by tail snipping) was estimated at 0, 60, 120 and 150 minutes.

Statistical analysis

The data is presented by calculating the mean and SEM of the outcome parameters. One way Analysis of Variance (ANOVA) and independent samples T-test were applied to see the difference between two groups when required. Tests of significance were carried out at 5% level.

RESULTS

The average CBG for the test group is significantly higher than the control group at -30mins and at 0 hr of OGTT. However, the average CBG of the test group at 60 minutes is significantly lower than the control group and progressively reduces at 120 and 150 minutes of OGTT ($P<0.001$). There was a 16.2% , 17.2% and 22.9% lowering of CBG at 60, 120, 150 mins after onset of glucose challenge respectively in the pantoprazole test group as compared to the control group (Table 1, Fig. 1). The difference in CBG values at all the time intervals between test & control respectively after 60 min(T) and 60 min(C) show hypoglycaemia of T compared to C, the maximal difference of CBG levels being at 150 min(T) compared with 60min(C) with a lowering of 26.7mg/dl. This is persistent and progressive at all times except at 0 and -30 min. of Test group compared to all the time intervals of the Control group when there is hyperglycaemia in the Test group.(Table 2., Fig. 2).

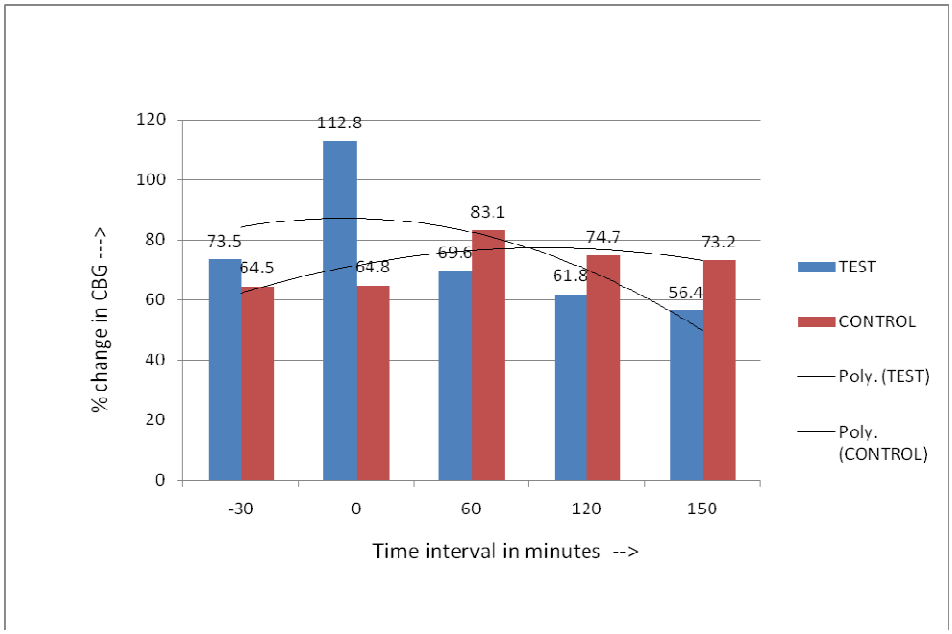
Table 1

Table depicting CBG values of test and control group expressed as mean +/- SEM

Sl. No.	Time since administration of glucose in minutes	Mean CBG± SEM		Comparison of CBG of T and C groups	% change of CBG of T over C
		Control group (C) (n=6)	Test group(T) (n=6)		
1.	-30	64. 5±2.605	73.5±2.766	T > C	12.2
2.	0	64.8±1.739	112.8±3.934	T > C	42.5
3.	60	83.1±2.687	69.6±5.210	T< C	16.2
4.	120	74.7±3.797	61.8±5.126	T<C	17.2
5.	150	73.2±3.468	56.4±5.964	T<C	22.9

Figure 1

Depicting the % change in CBG levels of test and control groups at different time intervals



The above bar graph represents the comparison of CBG levels of Test and Control at different time intervals from the time of glucose administration starting at -30 minutes and ending at 150 minutes. Significant difference (P<0.001) was found at all time intervals. T < C from 60 minutes onwards progressively.

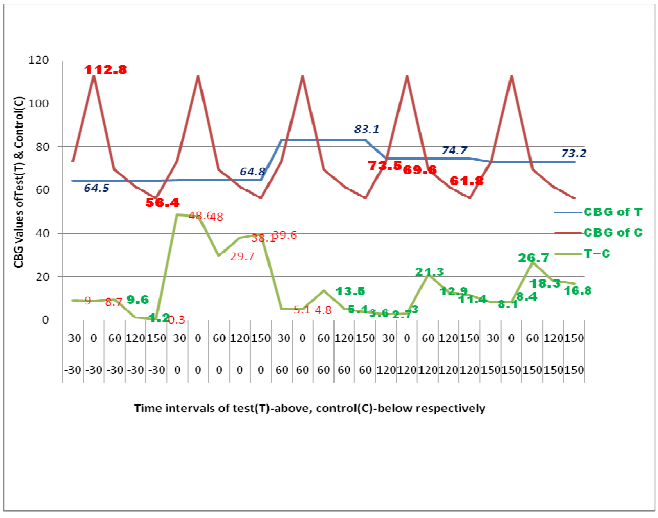
Table 2

Difference in CBG values at various time intervals since glucose administration of test & control compared.

Sl.no	Time interval of Test & Control respectively		Difference in CBG Values (mg/dl)
1.	T	C	
2.	-30 min	-30 min	9(T>C)
3.	-30 min	0 min	8.7(T>C
4.	-30 min	60 min	9.6(**T<C**)
5.	-30 min	120 min	1.2(**T<C**)
6.	-30 min	150 min	0.3(T>C)
7.	0 min	-30 min	48.6(T>C)
8.	0 min	0 min	48(T>C)
9.	0 min	60 min	29.7 T>C)
10.	0 min	120 min	38.1(T>C)
11.	0 min	150 min	39.6(T>C)
12	60 min	-30 min	5.1 (T>C)
13.	60 min	0 min	4.8(T>C)
14.	60 min	60 min	13.5 **(T<C)**
15.	60 min	120 min	5.1(**T<C**)
16.	60 min	150 min	3.6(**T<C**)
17.	120 min	-30 min	2.7(**T<C**)
18.	120 min	0 min	3(**T<C**)
19.	120 min	60 min	21.3(**T<C**)
20.	120 min	120 min	12.9(**T<C**)
21.	120 min	150 min	11.4(**T<C**)
22.	150 min	-30 min	8.1(**T<C**)
23.	150 min	0 min	8.4(**T<C**)
24.	150 min	60 min	26.7(T<C)#
25.	150 min	120 min	18.3(**T<C**)
26.	150 min	150 min	16.8(**T<C**)

Figure 2

The relationship of CBG values compared at different time intervals of Test and Control and the difference between CBG values of Test and Control.



The green line represents the difference in CBG levels of Test(T) and Control(C) depicted by blue and red lines respectively. The values on the green line depicted in green fonts represent those values where T < C at a particular time interval since glucose administration of T and C.

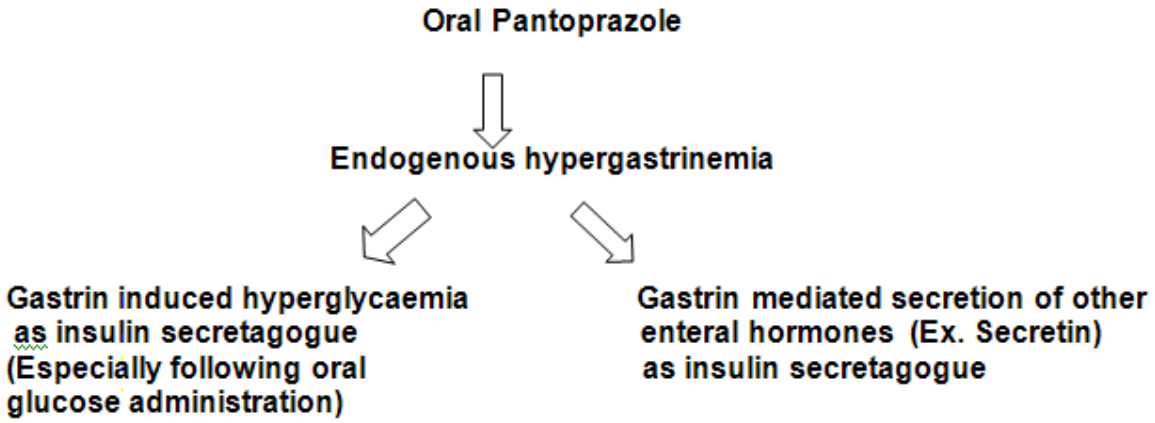
DISCUSSION

Pantoprazole given orally over a period of 3 consecutive days exhibits significant hypoglycaemic activity observed by OGTT in normal rats. Though initial response immediately after glucose challenge is that of hyperglycemia (-30mins and 0 hour), optimal and significant hypoglycaemia is seen at 1 hour after the glucose challenge which persists and progresses throughout the duration of the glucose challenge (i.e CBG at 150 mins. < 120 mins < 60mins of OGTT). This shows that pantoprazole has hypoglycaemic activity in normal rats at a dose being used for Acid peptic disease. The initial hyperglycaemia can be explained by endogenous hypergastrinemia induced increase in blood sugar levels which acts as a stimulus for the onset of glucose induced insulin secretagogue action. In the above study, PPIs (Ex. Pantoprazole) were administered for 3 days before the conduct of OGTT. Since PPIs (Ex. Pantoprazole) are known to induce endogenous hypergastrinemia which persists throughout most parts of the day when administered for 3 consecutive days, the results of this study indicate that PPI induced endogenous hypergastrinemia has hypoglycaemic activity when glucose challenge is given which is similar to the effect of exogenous gastrin administration . Pantoprazole causes hypoglycaemia when used for 3 consecutive days (short term) by the mechanism of endogenous hypergastrinemia which causes stimulation of glucose induced insulin secretion directly and by stimulating secretion of other enteric hormones (Ex. Secretin) which have insulin secretagogue effect.

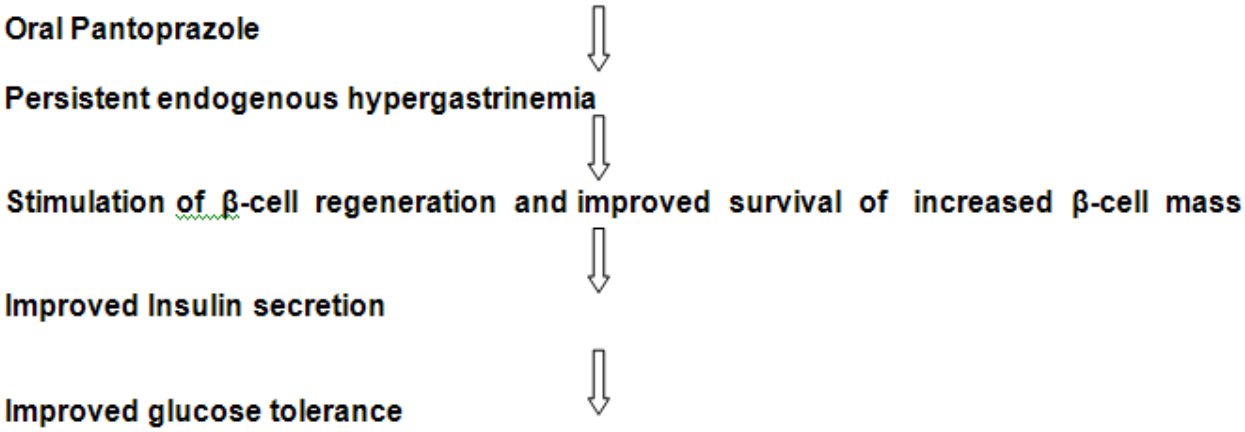
Since the safety profile of long term oral PPI use has been established and oral PPI use for long durations (1 to 3 months) has been approved for various GI disorders, its long term use as an additional drug in DM patients already on other oral hypoglycaemics (OHGs) to achieve better glycaemic control has a lot of potential. Further, since its effect is evident with just 3 days of therapy, PPIs (Ex. Pantoprazole) can be used on a short term basis in addition to other OHGs only when intensification of glycaemic control is needed as and when required thus minimizing any potential adverse effects of long term use of PPIs (Ex. Pantoprazole) and dose dependent adverse effects of the OHGs. Also, its better effect on glucose induced insulin secretion mediated by endogenous hypergastrinaemia can be clinically applied by using them in patients with uncontrolled postprandial hyperglycemia as the highest gastrin levels are induced by protein and glucose ingestion (i.e. postprandial). The efficacy of hypoglycaemic effect of PPIs when used alone and in combination with other antidiabetic drugs following long term use needs to be explored further including the benefit it offers by effective control of upper GI diseases which are frequently seen in association with uncontrolled DM and for the eradication of *H. Pylori* in patients with impaired glucose tolerance (IGT), thus preventing overt DM from emerging in IGT patients.

Since the enhanced β-cell regeneration and survival effect of gastrin takes longer duration to take effect, our study cannot comment on this except for proposing a possible mechanism. We plan to study the effect of PPIs following administration for longer duration/continued use to see the grade and type of effect they have on glycaemic control in both normal and diabetic rats and human beings. Longer duration of PPI use may be able to demonstrate better hypoglycemic effect including at 0 hour (immediately after glucose challenge) by the above mentioned effects on β- cells and other mechanisms which may come into play on longer use.

PROPOSED MECHANISM OF ACTION OF PANTOPRAZOLE ON SHORT TERM USE (3 CONSECUTIVE DAYS)



PROPOSED MECHANISM OF ACTION OF PANTOPRAZOLE ON LONG TERM USE (14 DAYS -3 MONTHS)



CONCLUSIONS

Pantoprazole has significant hypoglycaemic activity on glucose induced glycaemic levels following short term therapy. The blood sugar lowering effect is evident at 1 hour and progresses with further reduction throughout the period of glucose challenge by gastrin mediated enhancement of glucose induced insulin secretion. PPIs may be very useful medications for intensive glycaemic control in uncontrolled type 2 DM especially postprandial glycaemic levels and may thus have a dose sparing effect on other OHGs when used in addition to them. This study provides modest conclusions about glycaemic control improvement caused by short term use of Pantoprazole and only long term clinical studies will be able to establish whether the PPIs can produce sustained improvement in β-cell function and survival, thus improving glycaemic control when given on and off or with prolonged use in human beings with type 2 DM .

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