EFFECT OF VERAPAMIL ON PHARMACODYNAMICS OF PIOGLITAZONE IN NORMAL AND DIABETIC RATS

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Abstract:
Pharmacodynamic studies were carried out by measuring the plasma concentration of Pioglitazone. Prior to drug interaction studies a single dose of Pioglitazone was administered in rats and plasma concentrations were obtained by plotting a dose relationship curve, Cmax and Tmax were calculated. This increase in AUC suggests the possibility of drug-drug interaction between the two. Effect of verapamil on hypoglycemic activity of Pioglitazone was done by measuring the blood glucose level. Pioglitazone was administered in the dose of 10mg/kg. A fixed dose of Pioglitazone (10mg/kg) and verapamil (10mg/kg) were given to study the effect on blood glucose level and the percentage of reduction in blood glucose level was comparatively higher than when given Pioglitazone alone.

Keywords: Pioglitazone, Pharmacodynamic Interactions, Antidiabetic, Alloxan, Verapamil.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

Diabetes mellitus is one of the main threats to human health in the 21st century. The World Health Organization (WHO) estimated that there were 135 million diabetics in 1995 and this number would increase to 300 million by the year 2025. India leads the world today with the largest number of diabetics in any given country. Due to increasing obesity, sedentariness and dietary habits in both Western and developing countries, the prevalence of type 2 DM is growing at an exponential rate.

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action.

Symptoms of hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Long-
Term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes.

**Pathogenesis and Pathophysiology:**
Glucose is a simple sugar found in food. Glucose is an essential nutrient that provides energy for the proper functioning of the body cells. Carbohydrates are broken down in the small intestine and the glucose in digested food is then absorbed by the intestinal cells into the bloodstream, and is carried by the bloodstream to all the cells in the body where it is utilized. However, glucose cannot enter the cells alone and needs insulin to aid in its transport into the cells. Without insulin, the cells become starved of glucose energy despite the presence of abundant glucose in the bloodstream. The abundant, unutilized glucose is wastefully excreted in the urine. Insulin is a hormone that is produced by specialized cells (beta cells) of the pancreas. The pancreas is a deep-seated organ in the abdomen located behind the stomach. In addition to helping glucose enter the cells, insulin is also important in tightly regulating the level of glucose in the blood. After a meal, the blood glucose level rises. In response to the increased glucose level, the pancreas normally releases more insulin into the bloodstream to help glucose enter the cells and lower blood glucose levels after a meal. When the blood glucose levels are lowered, the insulin release from the pancreas is turned down. It is important to note that even in the fasting state there is a low steady release of insulin than fluctuates a bit and helps to maintain a steady blood sugar level during fasting.

**Materials & Methods:**

**Drugs & Chemicals**
- Pioglitazone (Aurobindo, Hyderabad)
  - Verapamil
  - Alloxan Monohydrate (Sigma, Aldrich, Bangalore)
- Diethyl ether
  - Glucose kits (local suppliers)

**Equipments:**
- Colorometry (Elico)
- Biofuge (Hearus instrument-Germany)
- Micropipettes (Tarsons)
- Microcentrifuge tubes (Tarsons)
- Heparinized capillaries
- Ultra sonicator

**Experimental Protocol:**

Inbred Wistar albino rats weighing 160 to 220 g were divided into 8 groups containing 6 rats each. The first set of 4 groups’ of animals was used for Normal studies and second set of 4 groups’ of animals was used for Diabetic studies.
Normal rats:

The Normal rats were subdivided into four groups as follows; group 1\textsuperscript{st} (control) given vehicle (1% W/V CMC); group 2\textsuperscript{nd} rats given Pioglitazone (10 mg kg\textsuperscript{-1}, orally in 1% W/V CMC); The treatment was given for seven days. To the 3\textsuperscript{rd} group of normal rats verapamil (10 mg/kg,p.o)was administered. To the 4\textsuperscript{th} group of normal rats verapamil was administered for 7days and 8\textsuperscript{th} day Pioglitazone after 1hr verapamil were administered. During this period the animals had free access to food and water. After 18 h fast on seventh day they were again given the combined treatment with verapamil and Pioglitazone 1hr later verapamil were administered. In all the groups, the blood sample was collected after 1 h drug treatment.

Diabetic rats:

The diabetic rats were subdivided into three groups as follows; group 5\textsuperscript{th} (diabetic control) given vehicle (1% W/V CMC); group 6\textsuperscript{th} diabetic rats given Pioglitazone (10 mg kg\textsuperscript{-1}, orally in 1% W/V CMC); The treatment was given for seven days. To the 7\textsuperscript{th} group of animals verapamil (10mg/kg,p.o.,)was administered. To the 8\textsuperscript{th} group of diabetic rats verapamil was administered for 7days and 8th day Pioglitazone 1hr later verapamil was administered. During this period the animals had free access to food and water. After 18 h fast on seventh day the combination of drugs were given. In all the groups, the blood sample was collected after 1 h drug treatment.

RESULTS & DISCUSSION:

Oral Glucose Tolerance Test (OGTT)
OGTT was carried out to confirm the induction of type 2 diabetes. Rats, after 12 hr of fasting were given orally, a glucose challenge of 2g/Kg body weight. Blood glucose was determined by the above mentioned method at 0, 30, 60,120 and 180 min after glucose challenge. A plot of Blood glucose level versus time obtained was analyzed for impairment in glucose tolerance, to confirm the induction/extent of diabetes.

CONCLUSION:
Diabetes mellitus is metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defect in insulin secretion, insulin action, or both cause by pancreatic β-cell destruction without enough insulin, body tissues, in particular, liver, muscle and adipose tissues fail to take and utilize glucose from the blood circulation. This results in elevated blood glucose levels, a condition known as hyperglycemia. If blood glucose levels remain high over a long period of time, this can result in long term damage of organs such as the kidneys, eyes, nerves, heart, and blood vessels. Complications in some of these organs can lead to death. Diabetes is a chronic metabolic disorder and needs prolonged treatment for maintenance of normal blood glucose levels.
Table 1: the plasma glucose (mg/dl) values done on the 7th day after the treatment by OGTT.

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>0hr</th>
<th>30min</th>
<th>1hr</th>
<th>2hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>70±2.1</td>
<td>73±2.2</td>
<td>70±1.9</td>
<td>71±1.1</td>
<td>72±2.9</td>
</tr>
<tr>
<td>DIABETIC</td>
<td>298±2.5</td>
<td>291±3.1</td>
<td>317±2.6</td>
<td>286±1.5</td>
<td>260±2.5</td>
</tr>
<tr>
<td>PIOGLITAZONE</td>
<td>274±1.3</td>
<td>289±2.3</td>
<td>298±1.6</td>
<td>265±2.1</td>
<td>240±2.5</td>
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<tr>
<td>VERAPAMIL</td>
<td>262±1.2</td>
<td>270±2.9</td>
<td>281±2.9</td>
<td>246±2.9</td>
<td>228±1.7</td>
</tr>
<tr>
<td>VERAPAMIL + PIOGLITAZONE</td>
<td>276±1.9</td>
<td>286±2.4</td>
<td>294±3.4</td>
<td>270±3.0</td>
<td>260±2.2</td>
</tr>
</tbody>
</table>

Values are significant as the P values are <0.0001, were determined by using one way ANOVA test with DUNNET’S multiple comparison test

Fig 1: OGTT ON 7TH DAY

Table 2: the plasma glucose (mg/dl) values done on the 14th day after the treatment

<table>
<thead>
<tr>
<th>CONDITION</th>
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<th>30min</th>
<th>1hr</th>
<th>2hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>71±1.87</td>
<td>72±4.1</td>
<td>70±1.2</td>
<td>71±1.11</td>
<td>72±1.01</td>
</tr>
<tr>
<td>DIABETIC</td>
<td>270±2.76</td>
<td>280±2.22</td>
<td>292±2.1</td>
<td>282±2.59</td>
<td>269±2.0</td>
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<tr>
<td>PIOGLITAZONE</td>
<td>268±1.9</td>
<td>270±3.7</td>
<td>286±1.89</td>
<td>241±1.84</td>
<td>212±1.4</td>
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<tr>
<td>VERAPAMIL</td>
<td>246±3.2</td>
<td>260±1.98</td>
<td>270±3</td>
<td>232±1.91</td>
<td>226±1.8</td>
</tr>
<tr>
<td>VERAPAMIL + PIOGLITAZONE</td>
<td>226±3.2</td>
<td>240±1.98</td>
<td>260±3</td>
<td>252±1.91</td>
<td>236±1.8</td>
</tr>
</tbody>
</table>

Values were significant as P values are <0.0001, by using one way ANOVA test with DUNNET’S multiple comparison test by using Prism.

Fig 2: OGTT ON 14TH DAY.
Table 3: the plasma glucose (mg/dl) values done on 21st day after the treatment.

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>0hr</th>
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<th>1hr</th>
<th>2hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>70±1.8</td>
<td>72±4.1</td>
<td>70±1.2</td>
<td>71±1.11</td>
<td>72±1.01</td>
</tr>
<tr>
<td>DIABETIC</td>
<td>210±2.76</td>
<td>220±3.22</td>
<td>232±2.1</td>
<td>202±2.59</td>
<td>198±2.09</td>
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<tr>
<td>PIOGLITAZONE</td>
<td>228±1.9</td>
<td>230±3.67</td>
<td>236±1.89</td>
<td>198±1.84</td>
<td>188±1.47 ***</td>
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<tr>
<td>VERAPAMIL</td>
<td>216±3.2</td>
<td>221±1.98</td>
<td>231±3.1</td>
<td>195±1.91</td>
<td>176±1.83</td>
</tr>
<tr>
<td>VERAPAMIL + PIOGLITAZONE</td>
<td>206±3.2</td>
<td>210±1.98</td>
<td>216±3.1</td>
<td>176±1.91</td>
<td>166±1.83</td>
</tr>
</tbody>
</table>

Values were significant as P values are<0.0001, determined by using one way ANOVA test with DUNNET’S multiple comparison test.

The drugs at dose levels 10mg/kg were administered orally for 21 days to Alloxan induced diabetic rats. They significantly reduced the serum glucose, total cholesterol and triglyceride levels. Thus the present study was planned to investigate the influence of verapamil on the Pharmacodynamic of Pioglitazone in rats in vivo, in both normal and Alloxan diabetic condition.

There was a very significant influence on the percentage reduction in diabetic rats under multiple dose treatment but no significant influence in normal rats.

From the observations of study performed it is confirmed that verapamil exerted significant Antidiabetic activity. These could be used support treatment for diabetes mellitus

**BIBLIOGRAPHY:**