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Original Research Article

Evaluation of Different Calcium Channel Blockers on Blood Glucose Level in Albino Rabbits

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Abstract:

Background: Calcium channel blockers (CCBs) are a class of drugs and natural substances that disrupt the movement of calcium (Ca^{++}) through calcium channels. The most widespread clinical use of calcium channel blockers is to decrease blood pressure in patients with hypertension, with particular efficacy in treating elderly patients. The present study was done to evaluate the effect of different CCB on blood glucose level in normal albino rabbits.

Methods: The present study was conducted on healthy albino rabbits of either sex weighing 1.5-2.0 Kg. The animals were made available in the animal house of Department of Pharmacology & Therapeutics of a tertiary care teaching hospital of Uttar Pradesh. Blood glucose level estimation was done by using glucose oxidase-peroxidase method in all the four groups viz; Normal saline, Verapamil, Diltiazem and Nifedipine group at predetermined intervals (0, 1, 2 and 6 hrs). Changes in blood glucose level in all groups were compared.

Results: Verapamil caused hyperglycemia of significant level ($p<0.001$) at 1 and 2 hours. Increased in blood glucose level by Diltiazem and Nifedipine was also significant ($p<0.05$) at 1 and 2 hrs but the changes observed at 6 hours were insignificant in all the groups. ($p>0.05$)

Conclusion: This study concluded that all three calcium channel blockers cause significant increase in blood glucose level in normal albino rabbits. Therefore it is advisable to prescribe anti-diabetic drugs and calcium channel blockers carefully with utmost caution and necessary dose adjustment in individuals suffering from diabetes.

Keywords: Diabetes, Insulin, Verapamil, Diltiazem, Nifedipine

Introduction

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar either because the body does not produce enough insulin or because cells do not respond to the insulin that is produced [1]. The World Health Organization recognize three main forms of diabetes mellitus: type 1, type 2 and gestational diabetes (occurring during pregnancy), which have different causes and population distributions. Ultimately all forms are due to the beta cells of the pancreas being unable to produce sufficient insulin to prevent hyperglycemia [2,3].

Diabetes can cause many complications. Acute complications (diabetic ketoacidosis or nonketotic hyperosmolar coma) may occur if the disease is not adequately controlled. Serious long-term complications include cardiovascular disease, chronic renal failure, retinopathy, neuropathy and microvascular damage, which may cause impotence and poor healing [4].

Ca^{++} plays a pivotal role in the physiology and biochemistry of organisms and the cell. It plays an important role in signal transduction pathways where it acts as a second messenger, in neurotransmitter release from neurons, contraction of all muscle cell types and fertilization [5]. Many enzymes require calcium ions as a cofactor, those of the blood-clotting cascade being notable

examples. Extracellular calcium is also important for maintaining the potential difference across excitable cell membranes as well as proper bone formation [6].

Calcium channel blockers (CCBs) are a class of drugs and natural substances that disrupt the movement of calcium (Ca^{2+}) through calcium channels. CCBs have effects on many excitable cells of the body such as cardiac muscle, smooth muscles of blood vessels and neurons. The most widespread clinical use of calcium channel blockers is to decrease blood pressure in patients with hypertension, with particular efficacy in treating elderly patients [7]. Also, calcium channel blockers frequently are used to control heart rate, prevent cerebral vasospasm and reduce chest pain due to angina pectoris.

Long acting dihydropyrides CCBs reduce cardiovascular morbidity and mortality to an extent equivalent to that of diuretics & β blockers. The effect was greater among diabetics [8]. Thus CCBs are often use as 1st line hypertensive therapy. CCBs also affect entry of calcium into β cell which in turn affect release of insulin from pancreatic β cells [9]. There are some evidences that calcium channel blockers causes hyperglycemia [10] but studies related to different types of CCBs' on blood glucose level are lacking. The present study was done to evaluate the effect of different CCB on blood glucose level in normal albino rabbits.

Material and Methods

The present study was conducted on healthy albino rabbits of either sex weighing 1.5-2.0 Kg. The animals were made available in the animal house of Department of Pharmacology & Therapeutics of a tertiary care teaching hospital of Uttar Pradesh. The rabbits were housed in iron cages and maintained under standard conditions (12 hrs light and dark cycle, at room temperature 25± 3ºC and 35-60% humidity) fed on standard pellet diet and provided water ad libitum.

Drugs used

Calcium channels blockers (Verapamil, Diltiazem, Nifedipine,) were used. Drugs were purchased from local market in tablet form. The tablets were grinded to fine powder and then dissolved in 2 ml of distilled water for each experiment as per calculated drug dose ratio and give orally by feeding cannula.

Estimation of blood glucose level

It was done by using glucose oxidase-peroxidase method. Due to specificity of enzymes the enzymatic method is the most accurate. Span diagnostic reagent kit (code no. is 93DP100-74) was used for estimation of blood glucose level.

Equipments

UV spectrophotometer, micropipettes, centrifuse, microcentrifuse tubes were used.

Specimen

Blood samples (0.5 ml) were collected at predetermined intervals (0, 1, 2 & 6 hrs) from marginal vein of ear in fluoride vials, under aseptic conditions. Blood sample was collected in fluoride vials because addition of sodium fluoride to anticoagulant potassium oxalate in proportion of 1:3 prevents any loss of glucose due to inhibition of glycolysis. Blood samples were then centrifuged at 3000 rpm for 10 minutes. The clear supernatant serum was taken for estimation of blood glucose level. Absorbance was read at 505 nm against a reagent blank

Dose calculation

Surface area ratio of 1.5 Kg rabbits and 70 kg man is 0.07. (As per table from Paget and Barnes, 1964) [11]. If 70 kg man needs N gm dose of drug then according to surface area ratio, 1.5 Kg rabbit needs 0.07X N gm dose for same pharmacological effect.

Doses of drug used in study

Dose of Verapamil, Diltiazem and Nifedipine used was 16.0, 7.4 and 2.0 mg/kg/day respectively.

For this study rabbits were divided into four groups of six rabbits in each group. Rabbits were fasted overnight during the study but free access of water was allowed during this period. Glucose tolerance test (GTT) was done in all the groups by giving 3.5 gm/kg/PO glucose dissolved in distilled water (5ml) [12]. All drugs and glucose were given orally through nasogastric tube, those not soluble in water were rendered emulsified with the help of gum acacia. Blood sample were taken at 0 hr, 1 hr, 2 hr and 6 hr in all the groups.

Group 1: Rabbits of this group were given normal saline for 7 days and on 7th day glucose was given to conduct glucose tolerance test. This group served as control.

Group 2: Rabbits of this group were given Verapamil for 7 days and on 7th day GTT was done. This group shows effect of Verapamil on blood glucose level.

Group 3: Rabbits of this group were given Diltiazem for 7 days and on 7th day was done. This group shows effect of Diltiazem on blood glucose level.

Group 4: Rabbits of this group were given Nifedipine for 7 days and on 7th day GTT was done. This group shows effect of Nifedipine on blood glucose level.

Statistical Analysis

Changes in blood glucose level by various drugs were determined in mg%. Data are expressed as the Mean±SD. Statistical analysis was carried out using unpaired Student's't'- test. $P < 0.05$ was considered to be statistically significant.

Results

The mean blood glucose levels at '0' hr (fasting) was 88.17 mg%. It became 219.17, 179.5 & 89.83 mg% after 1, 2 & 6 hours respectively following administration of Verapamil and glucose (Group II). These values were compared statistically with normal control group (Group I). These observations indicate that Verapamil caused hyperglycemia of significant level ($P<0.001$) at 1 & 2 hours but the changes observed at 6 hours were insignificant ($P>0.05$). (Table 1 and figure 1)

The mean of blood glucose levels at '0' hr (fasting) was 87.17 mg%. It became 135.00, 116.33 & 91.67 mg% after 1, 2 & 6 hours respectively following administration of Diltiazem and glucose (Group III). These values were compared statistically with normal control group (group I) These observations indicate that increased in blood glucose level by Diltiazem was significant ($P<0.05$) at 1 & 2 hrs but insignificant at 6 hours. (Table 1 and figure 1)

The mean blood glucose levels at '0' hr (fasting) was 86.83 mg%. It became 136.83, 116 & 88.5 mg% after 1, 2 & 6 hours respectively following administration of Nifedipine and glucose (Group IV). These values were compared statistically with normal control group (Group I). These observations indicate that increased in blood glucose levels by Nifedipine was significant ($P<0.05$) at 1 & 2 hrs but insignificant at 6 hours. (Table 1 and figure 1).

Table 1: Blood glucose levels in fasted rabbits and after glucose powder following administration of Verapamil, Diltiazem, Nifedipine and Normal Saline

Groups	Treated with	Mean blood glucose level in mg% (Mean±SD)			
		Fasting	After Glucose		
		0 hr	1 hr	2 hr	6 hr
I	Normal Saline	88.17±5.53	126.17±4.75	106.17±4.67	86.67±7.91
II	Verapamil	88.50±6.06	219.17±9.13*	179.50±9.81*	89.83±7.17
III	Diltiazem	87.17±5.70	135.00±6.96*	116.33±7.63*	91.67±7.94
IV	Nifedipine	86.83±7.83	136.83±8.04*	116.00±9.91*	88.50±6.35

***Significant**

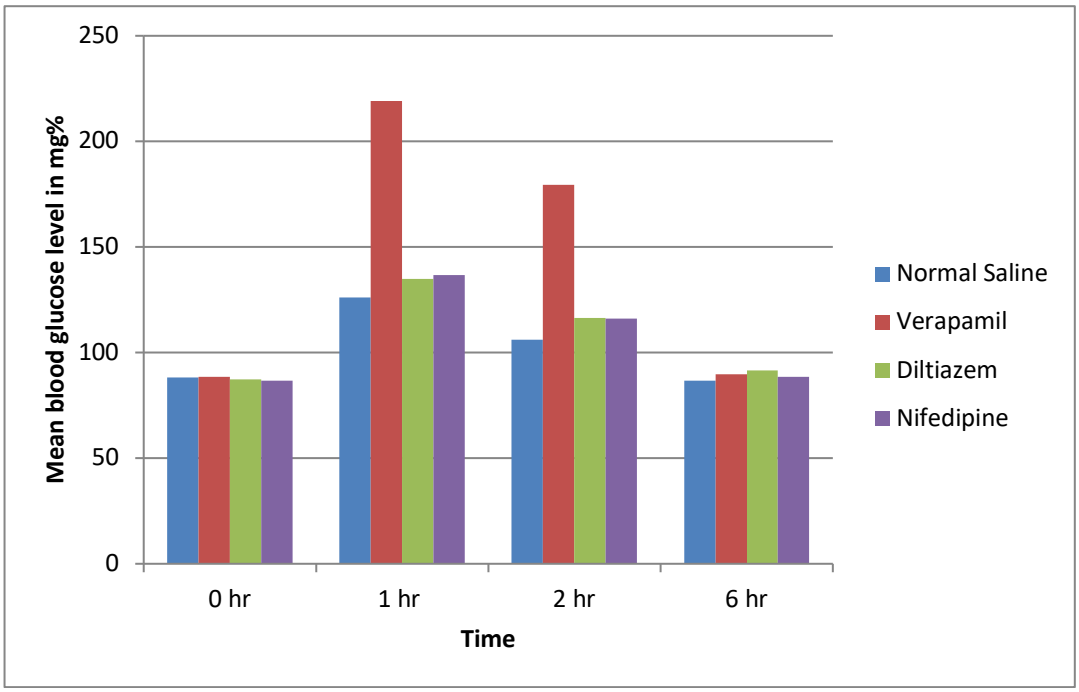

Figure 1: Comparative effects of calcium channel blockers on blood glucose level at 0, 1, 2 and 6 hr in albino rabbits

Discussion

When glucose is metabolized in β cells, glucose-6 phosphate is produced by glycolysis, which also produces ATP. K^+ channels that are sensitive to ATP are inhibited by the ATP that is produced. When these K^+ channels are inhibited, the β cell membrane depolarizes, which opens voltage-dependent calcium channels and allows calcium to enter. Calcium enters cells at a higher concentration when it flows in. By means of exocytosis, increased intracellular calcium concentrations trigger the release of insulin from storage granules [13]. Thus, by blocking voltage-sensitive calcium channels, calcium channel blockers obstruct the flow of calcium into β cells. As a result, exocytosis eventually results in decreased insulin secretion.

In present study Verapamil treated rabbit showed significant change in blood glucose level 147.65%, 102.82% at 1hr and 2hr respectively from fasting level 0 hr. Diltiazem also showed significant change in blood glucose level 54.87%, 33.15% at 1hr and 2hr respectively from fasting level 0 hr. Nifedipine also showed significant change in blood glucose level 57.58% and 33.59% at 1hr, 2hr respectively from fasting level 0 hr. When the change in blood glucose level in each group was compared with normal saline treated group individually, the change was significant in all the groups at 1 hr and 2hr. So it was evident that calcium channel blockers (Verapamil, Diltiazem and Nifedipine) per se increased the blood glucose level in normal conditions in experimental rabbits. This rise was found maximum in Verapamil. This may be probably by inhibiting insulin release or activity of GLUT-1 receptors. The inhibition of GLUT-1 may be one of the contributing factors to the hyperglycemia observed with CCBs [14]. Insulin is first secreted by glucose-mediated means after being transported into the β cell by the GLUT-2 transporter.

Krempf et al showed that calcium antagonist Nifedipine interferes with calcium influx into pancreatic beta cells and inhibit insulin release in non-insulin-dependent diabetics [15]. Giugliano et al also studied impairment of insulin secretion in men by Nifedipine. This study showed that Nifedipine treatment result in reduction in both of insulin secretion and glucose tolerance [16]. Segresta et al also observed that calcium antagonist decreased the insulin release in vitro by glucose and sulphonylurea's compounds in healthy volunteers. Intracellular Ca^{++} can act on pancreatic beta cells either as universal coupling agents during cell activation or as a prerequisite for the contractile protein activity involved in insulin secretion [17]. Another study had also shown that calcium channels blockers decrease glucose lowering effects of sulfonylurea's by increase in hepatic metabolism and renal excretion or inhibiting insulin secretion [18].

Louters et al investigated the effects of the calcium channel blocker Verapamil on the glucose uptake activity of GLUT-1 in L-929 fibroblast cells with 2-deoxyglucose. Verapamil had a dose-dependent inhibitory effect on both basal and stress-activated transport activity of GLUT-1. Basal

activity was inhibited up to 50% by 300 μM Verapamil, while 150 μM Verapamil completely inhibited the activation induced by the stress of glucose deprivation. This study reveals the unique finding that verapamil has inhibitory effects on the transport activity of GLUT-1 independent of its effects on calcium concentrations [14].

Most diabetic patients are likely to suffer from hypertension hence most often anti-hypertensive are co-administered along with oral anti-diabetic drugs. Frequently prescribed anti-hypertensive belongs to the class of calcium channel blockers [7]. Literature shows that calcium channel blockers alter blood glucose levels which add on an evidence for a probable pharmacodynamic interaction between oral antidiabetic drugs and calcium channel blockers.

Conclusion

All group of calcium channel blockers cause significant increase in blood glucose level in normal albino rabbits. Therefore, there is a chance that using calcium channel blockers in combination with oral antidiabetic medications could reduce the hypoglycemic impact of the medication in a diabetic patient. Therefore it is advisable to prescribe these two drugs carefully with utmost caution and necessary dose adjustment in individuals suffering from diabetes.

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