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Original Article

COMPARISON OF THE POSSIBLE CARDIAC EFFECT OF SITAGLIPTIN, METFORMIN AND GLIMEPIRIDE ON INSULIN INDUCED HYPOGLYCEMIA IN RABBITS

EKHLAS H HUSSEIN[1] & NABEEL AJALI[2]

[1]*BSc, Pharmacy, Department of Pharmacy, Al-Sader Teaching Hospital Basrah, Iraq*

[2]*(MBCHB, PhD) Department of Pharmacology, College of Medicine, University of Basrah, Iraq*

ABSTRACT

Background

Hypoglycemia is a common in diabetic patients, especially during strict blood glucose control with insulin and oral hypoglycemic drugs. Acute or recurrent hypoglycemia may cause sudden death during diabetes mellitus treatment, the cause of which is not well known and was attributed to cardiovascular complications.

Objectives

To measure the effect of insulin on troponin T, C-Reactive Protein (CRP), and malondialdehyde (MDA) and the ECG changes, and to compare the possible additive effect of sitagliptin, metformin and glimepiride on these parameters

Methods

Fifty rabbits (twenty five males) were randomly divided into five groups (10 each); 5 male and 5 female

Group 1: (control group), Group 2:(insulin), Group 3: (Metformin + Insulin), Group 4: (Glimepiride + insulin), Groups 5: (Sitagliptin + Insulin). Animals were anesthetized with ketamine for ECG recording at day 3and were then sacrificed. Blood was collected for measurement of troponin T in both the serum and heart tissue, serum CRP, serum potassium and heart tissue Malondialdehyde.

Results

Insulin significantly increased serum CRP (0.15 ± 0.09 mg/l) and decreased in blood glucose level. Metformin + insulin increased in serum CRP (0.15 ± 0.103 mg/l), decreased in serum troponin T (124.62 ± 23.12 pg/ml), heart tissue MDA (0.52± 0.61 ng/ml), and decreased blood glucose level. Glimepiride + Insulin decreased in cardiac troponin T (200.60 ± 20.31 pg/ml) and blood glucose level. Sitagliptin + Insulin decrease in blood glucose level, $p < 0.05$, insignificant reduction in in serum and cardiac troponin T and heart tissue MDA. Insignificant increase in serum CRP and heart rate.

Conclusions

Metformin has both antioxidant activity and cardio-protective effect. While glimepiride has also cardio-protective effect. Insulin in combination or with oral hypoglycemic drugs cause a statistically significant decrease of blood glucose level with no therapeutic difference between various drugs.

KEYWORDS: Hypoglycemia, Insulin, Sitagliptin, Metformin, Glimepiride

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INTRODUCTION

Hypoglycemia is a common event in diabetic patients and most frequently occurred during intensive glucose lowering agent. Acute or recurrent hypoglycemic can cause cardiovascular complications during diabetic treatment like thrombosis and inflammation [1], abnormal cardiac repolarization, arrhythmia and atrial fibrillation [2]. About 90% of patients who take insulin have experienced incidents of hypoglycemia, the occurrence of which z differs among studies, in general occasions of symptomatic hypoglycemia in patients with Type1 Diabetes (T1 D) are estimated two occasions per week and one occasion of sever hypoglycemia once per year. A predictable 2–4% of deaths in those patients have been ascribed to hypoglycemia [3], [4]. During hypoglycemia especially among old diabetic patients with coronary artery disease, myocardial ischemia may occur. In addition to decrease myocardial perfusion, hypoglycemia can create changes in the conduction system of the heart, and these changes including Prolongation of the QT interval [5], [6] and prolongation repolarization [7] Cardiac arrhythmias may consequence during hypoglycemia and may lead to the complication of sudden cardiac death [8]. The effect of insulin induce hypoglycemia on potassium, troponin T, C-Reactive Protein, MDA as well as the effect of insulin on heart rate, PR interval and QRS duration will be examinated. The possible additive effect of sitagliptin, metformin and glimepiride on these parameters will also be measured.

MATERIALS AND METHODS

Animal groups

The study was conducted in the Department of Pharmacology, College of Medicine, University of Basrah, during the period from October 2015 until September 2016. Animals were divided randomly into five groups ten rabbits in each group (5 males and 5 females), Six of animals died during experiment and two of animals had blood hemolysis, and they were excluded from the study. Blood glucose level was determined before oral dose at 0 hour and one hour after insulin dose and at 3hour for each treatment by (Counter ®) glucometer.

Group 1 (Control Group): Rabbits in this group were treated with (0.4 ml/kg) distilled water orally initially and after one hour (0.5 ml/kg) subcutaneously for three days and sacrificed at the third day of the study.

Group 2 (Insulin Group): Rabbits in this group were treated with (0.4 ml/kg) distilled water orally and after one hour regular insulin (0.5Uint/kg/day) subcutaneously for three days and sacrificed at the third day of the study.

Group 3 (Metformin+ Insulin Group): Rabbits in this group were treated with Metformin (100mg/kg/day, orally) and after one hour regular insulin (0.5 unit/kg/day) subcutaneously for three days and sacrificed at the third day of the study.

Group 4 (Glimepiride + Insulin Group): Rabbits in this group were treated with Glimepiride (0.1mg/kg/day, orally) and after one hour regular insulin (0.5 unit/kg/day) subcutaneously for three days and sacrificed at the third day of the study.

Group 5 (Sitagliptin + Insulin Group): Rabbits in this group were treated with Sitagliptin (10mg/kg/day, orally) and after one hour regular insulin (0.5 unit/kg/day) subcutaneously for three days and sacrificed at the third day of the study.

ECG (Electrocardiograph)

ECG was recorded using (Contec™ electrocardiograph) with five leads; right and left anterior arm (R & L), right and left leg (N & F) and V5: left anterior axillary line. The animals were anaesthetized with ketamine in a dose of (30mg/kg, intramuscular).

Blood Sampling

At the third day of experiment, the rabbits were anesthetized by Ketamine in dose (30mg/kg, i.m.) after ECG recording blood samples were collected directly from heart via cardiac puncture before sacrificing. Blood samples 5 – 10 ml were collected into gel tube and left 20 minutes to clot. The serum was separated by centrifugation at 3000 rpm for 20 minutes. The supernatants collected carefully, and samples stored in aliquot at -20 c for analysis. Kits (MyBio Source, Spectrum) were used for the determination serum and cardiac troponin T, cardiac MDA, serum c-reactive protein and potassium. Estimation was done by ELISA method.

Tissue Handling

The animals were sacrificed by inhalation of ether, the heart from each rabbit was immediately removed and rinsed in phosphate-buffered saline PH=7. Part of heart was homogenized for MDA level and cardiac troponin T measurement.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) of samples. Analysis was made by using SPSS (statistical package for social sciences) version 15. Independent samples t-test was used to compare between different groups. Paired t-test was used to test the significant of difference among the same group. Statistical significant was accepted at a level of $P < 0.05$.

RESULTS

Effect on Troponint

The serum troponin T was significantly decrease in the group treated with (metformin +insulin) in comparison to the control group, $P < 0.05$. Also the other drugs in each groups reduced serum troponin T but this reduction was not statistically significant (Table 1). The level of heart tissue troponin T was significantly decrease in the group treated with (Glimepiride + Insulin) in comparison to control group and the group treated with (Metformin + Insulin) (Table 1)

Table 1: Effect of Different Treatments on Serum and Cardiac Troponin T Level (Mean ± Standard Deviation)

Group (No.)	Serum Troponin T (pg/ml)	Cardiac Troponin T (pg/ml)
Control (8)	155.75± 21.25	231.44 ± 36.90
Insulin (8)	135.91 ± 37.76	205.01 ± 20.51
Metformin +Insulin (9)	124.62 ± 23.12 *	230.82 ± 11.38
Glimepiride +Insulin (9)	140.59 ± 28.23	200.60 ± 20.31 *¥
Sitagliptin +Insulin (8)	144.15 ± 27.05	219.94 ± 21.71

*= Significant decrease from control group, ¥= significant decrease from Metformin + Insulin group, P< 0.05

Effect on Cardiac MDA

The level of heart tissue MDA in the group treated with Metformin + Insulin was significantly decrease in comparison to the control group and insulin alone group. Although other drugs have also decrease in the level of heart

tissue MDA but this was not statistically significant (Table 2).

Table 2: The Effect of different Treatment on Cardiac MDA Level (NG/Ml) (Mean ± Standard Deviation)

Group (No.)	Cardiac MDA
Control (8)	1.35 ± 0.90
Insulin (8)	1.26 ± 0.74
Metformin +Insulin (9)	0.52 ± 0.61*π
Glimepiride +Insulin (9)	0.70 ± 0.62
Sitagliptin + Insulin (8)	0.79 ± 0.74

*= significant decrease from Control, π= significant decrease from Insulin, P< 0.05

Effect on Serum C-Reactive Protein (CRP)

The level of CRP was significantly increase in the group treated with Insulin alone and (Metformin + Insulin) in comparison to the control group, P < 0.05 (Table-3). Although Glimepiride group and Sitagliptin group also caused increase in the serum CRP in comparison to Control group, however the difference was not statistically significant.

Table 3: The Effect of different Treatment on Serum C- Reactive Protein (MG/l) (Mean ± Standard Deviation)

Group (No.)	Serum c- Reactive Protein (Mg/L)
Control (8)	0.05 ± 0.08
Insulin (8)	0.15 ± 0.09 *
Metformin + Insulin (9)	0.15 ± 0.10 *
Glimepiride + Insulin (9)	0.11 ± 0.12
Sitagliptin + Insulin (8)	0.15 ± 0.12

*= significant increase from Control, P< 0.05

Effect on Blood Glucose Level

Blood glucose levels decreased significantly in the insulin treated group from 83.00 ± 9.243 mg/dl at base line to 37.00 ± 9.813 mg/dl at 3hrs of experiment in first day of the study. In second day, blood glucose level was significantly decrease from 84.13 ± 6.175 mg/dl at base line to 36.75 ± 5.064 mg/dl after 3 hrs. And at third day of experiment, blood glucose level was significantly decrease from 73.88 ± 10.575 mg/dl at base line to 29.38 ± 7.367 mg/dl after 3 hrs. Similar changes in blood glucose levels were also occurred in the other insulin containing groups (Table 4).

Table 4: The Effect of Different Treatment on Blood Glucose Level (Mg/Dl) (Mean ± Standard Deviation)

	Blood Glucose (MG/Dl) at Day 1		Blood Glucose (MG/Dl) at Day 2		Blood Glucose (MG/DL) at Day 3	
Group (No.)	0 hrs	3hrs	0 hrs	3 hrs	0 hrs	3 hrs
Control (8)	89 ±12.49	76 ± 12.98	70 ± 9.06	72± 8.26	77± 11.11	82 ± 12.05
Insulin (8)	83 ± 9.24	37 ± 9.81*$	84 ± 6.17 *	36± 5.06 *$	73± 10.58	29 ± 7.37 *$
Metformin +Insulin (9)	81 ± 7.26	39 ± 9.50*€	77±10.33	29 ±7.42*€π	80± 10.82	27± 8.46 *€
Glimepiride+ Insulin (9)	91 ± 13.34	40 ± 8.81*¢	71±17.17	34± 12.77*¢	69 ± 13.65	29± 9.98 *¢
Sitagliptin +Insulin (8)	80 ± 13.70	31±4.24*¥£	87±7.45*€¢	31± 6.57 *¥	79± 6.40	27± 6.63 *¥

*= significant from control, $ = significant decrease from Insulin at 0 hrs., €= significant decrease from Metformin at 0 hrs., π= significant from Insulin at 3 hrs., ¢ = significant decrease from Glimepiride at 0 hrs. and ¥= significant decrease from Sitagliptin at 0 hrs., P< 0.05

Effect on Electrocardiography (ECG)

All the treatment in this study lead to increase in the heart rate comparison to control group which was not a statistically significant. Also all the treatment tend to shorten the PR intervals but was not statistically significant with no

effect detected on QRS duration.

Table 5: The Effect of different Treatment on PR Interval Millisecond, QRS Duration and Heart Rate

Group (No.)	Hear t Rate (Beat/Minute)	PR Interval Millisecond	QRS Duration Millisecond
Control (8)	235.38 ± 48.67	141.25 ± 74.55	43.25 ± 4.89
Insulin (8)	238.25 ± 29.79	102.25 ± 42.05	43.33 ± 4.08
Metformin +Insulin (9)	248.63 ± 27.39	94.25 ± 38.35	46.75 ± 7.49
Glimepiride+ Insulin (9)	242.44 ± 18.62	115.22 ± 77.89	49.33 ± 13.15
Sitagliptin +Insulin (8)	238.63 ± 28.19	82.00 ± 36.15	42.00 ± 2.67

Table 6: Number of Rabbits Death in Each Group

Group	Number of Deaths
Control	2
Insulin	2
Metformin + Insulin	1
Glimepiride + Insulin	1
Sitagliptin + Insulin	2

DISCUSSIONS

In this study metformin when given in a combination with insulin produced a significant reduction in serum troponin T. This is probably due to the direct effect of metformin which may indicated cardiac protection. The elevation of serum troponin T in the control group may be due to the tachycardia and stress during animal handling and injecting [9]. The elevation of troponin T does not occur only in myocardial necrosis, it can be increased for five days after open heart surgery [10]. Furthermore, strenuous exercise which reported as one of multi causes of troponin T elevation, however the mechanism of elevation is unclear. Interestingly, this elevation is transient and decrease or return to normal level within 24 hours[11]. Troponin T is also elevated in acute pulmonary embolism [10] and renal failure [12]. Metformin probably has cardio-protective effect, which is similar to the result of previous experimental and clinical studies in diabetes mellitus [13]. The result of the current study demonstrated that metformin has cardio-protection effect even in non-diabetic rabbits.

Effect of Studied Treatments on Cardiac Tissue Troponin T

In the current study glimepiride when given in a combination with insulin, it show a statistically significant reduction in cardiac troponin T. The more plausible explanation for glimepiride effect is due to its action that leads to inhibition of potassium ATP (K+-ATPase) channels, which induced depolarization of the membrane of pancreatic β-cells and influx of calcium which resulted in insulin release in the blood. Glimepiride has more specificity to the pancreas tissue than the myocardium. Glimepiride does not block cardiac mitochondria K+-ATPase channel that plays central role in ischemic precondition protection [14], it is considered as an one of the most potent mechanisms to protect cardiac myocyte against ischemic myocardial injury [15].

Effect of Studied Treatments on Malondialdehyde (MDA)

Metformin in this study showed anti-oxidant activity by producing a statistically significant reduction in the cardiac tissue MDA level. There are two possible explanations for metformin antioxidant activity first one may be due to its ability to decrease intracellular ROS which play an important role in both tissue and organ damage and increase in the inflammatory responses [16]. Second explanation belong to its actions in the activation of the adenosine monophosphate-activated protein kinase (AMPK), which mediates many of its protective effect on cardiovascular system

[17]. The anti-oxidant properties of metformin were also reported in two different studies[18], [19]. Although, previous studies demonstrated antioxidant effect of both sitagliptin and glimepiride [20]–[22] this was not detected in our study, which may be due to the short treatment period (three days).

Effect of the Studied Treatments on Serum C - Reactive Protein (CRP)

In this study, insulin alone or in combination with metformin produced a statistically significant increase in serum CRP.

In a study by Wright, 2008 showed that after acute hypoglycemia there was an up -regulation of CRP[23]. Other study demonstrated that acute hypoglycemia in non-diabetic or diabetic patients may induce up -regulation and release of vasoactive substances, it was suggested that hypoglycemia induced metabolic stress lead to inflammation, and microangiopathy by its putative effects on vascular blood vessels [24].

The Effect of different Treatments on Electrocardiograph

All the treatment in this study leads to increment in the heart rate which was not a statistically significant. Also all the treatment tend to shorten the PR intervals but was not statistically significant with no effect detected on QRS duration. Continuous ECG monitoring may be better than single recording time in detecting any transient disturbances of cardiac rhythm. Mortality that occur in various groups.

Effect of the Studied Treatments on Blood Glucose Level

The different treatments of all groups in our study were showed a statistical significant reduction in the blood glucose levels. Although, there was no a statistical significant difference found between groups in the blood glucose levels, the group treated with (sitagliptin + insulin) had higher reduction during three days of the study. Otherwise the all groups have comparable therapeutic effect.

CONCLUSIONS

The combination of metformin or glimepiride with insulin offer better glucose control and lower incidence of cardiac adverse effects.

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