ALISKERIN: A DIRECT RENIN INHIBITOR IMPROVES PANCREATIC FUNCTIONAL B CELL MASS IN TYPE 2 DIABETIC MICE

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ABSTRACT

Objective: To evaluate the effects of Aliskerin on functional pancreatic β cell mass in type 2 diabetic mice.
Study Design: Analytical experimental study.
Place and Duration of Study: The study was conducted at the animal house of National Institute of Health, Islamabad. Duration of the study was of twelve weeks after initial acclimatization of one week.
Material and Methods: Twenty four BALB/c mice, both male and female, of 35 to 40 grams were used for this study. Animals were randomly divided into four groups. Two served as control, one was normal and the other was diabetic control. The remaining two were used as interventional groups and received either pioglitazone or aliskerin for four weeks after induction of diabetes. Fasting blood glucose levels with fasting insulin levels were estimated. Insulin resistance was determined by calculating homa IR and pancreatic morphology was assessed by evaluating pancreatic beta cell mass.
Results: After treatment, pioglitazone reduced all the biochemical parameters significantly when compared with diabetic control and negative correlation between glucose and insulin was changed into positive correlation (r value, 0.92) with significant *p*-value (0.015), while aliskerin caused a significant rise (*p*-value 0.009) in functional pancreatic β cell mass.
Conclusion: Aliskerin has a significant anti-diabetic role as far as pancreatic morphology is concerned.

Keywords: Diabetes mellitus, Homeostatic model assessment for insulin resistance, Peroxisome proliferator activated receptor gamma, Renin angiotensin system.

INTRODUCTION

Hypertension and type 2 diabetes mellitus (DM) have taken the shape of global epidemic[1]. It is estimated that the total number of people with diabetes will rise to 366 million by 2030. Raised blood glucose level is the first stage in the development of future diabetes[2]. Compensatory mechanisms in body attempts to regulate this hyperglycaemia with increased secretion of insulin from pancreatic islets leading to chronic hyperinsulinemia. Chronic hyperinsulinemia develops a perplexing situation in the body as not all tissues develop equal insulin resistance[3]. For example, ability of kidneys to enhance sodium reabsorption in the presence of insulin is not hampered. As a consequence insulin-resistant /hyperinsulinemic, non-diabetic individuals are even at a higher risk for development of salt and water retention. Likewise, effects of insulin will remain the same on sympathetic nervous system, which will result in increased sodium and water retention as well as vasoconstriction. Therefore hyperinsulinemia in response to insulin resistance, can increase the probability to develop essential hypertension[4]. On the other hand, hypertension affects approximately 70% of patients with diabetes and is approximately twice as common in persons with diabetes as in those without it. Hypertension and obesity are known to contribute, directly or indirectly, to the devel-opment of long-term complications of type 2 dia-betes mellitus (type 2 DM)[5]. As a consequence use of anti-hypertensive agents which have some insulin sensitizing activity can possibly delay development of diabetes in those hypertensive patients who are genetically predisposed to develop diabetes mellitus.

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Received: 27 Feb 2017; revised received: 17 Jul 2017; accepted: 07 Sep 2017

Multiple animal studies show the possible relationship between renin angiotensin system (RAS) and development of insulin resistance[6]. As a result blockade of RAS cascade can potentially improve insulin sensitivity. Emerging evidence already indicates that angiotensin converting enzyme (ACE) inhibitors not only improve the pancreatic morphology but also slow the course of diabetic nephropathy[7,8].

Cascade of RAS starts with the secretion of renin from the juxtaglomerular cells in the kidneys. This is the rate limiting step of RAS[9]. So far the only recognized substrate for renin in the body is angiotensinogen. Use of renin inhibitors to block RAS at its origin has been a temptation for a long time as this leads to blockade of synthesis of Angiotensin I (AngI) to Angiotensin II (Ang II) with no activation of Angiotensin type I receptors, and no interference with bradykinin metabolism[10]. Aliskiren is the first orally active nonpeptide, low molecular weight renin inhibitor. Aliskiren has a beneficial effect on pancreatic injury and glucose intolerance, making it a promising agent for the inhibition of progression of diabetes in hypertensive individuals[11]. Hypotensive dose of aliskiren enfeebles insulin resistance, reduces pancreatic oxidative stress and remodelling, and improves skeletal muscle glucose transport in animal studies[12].

Peroxisome proliferator activated receptor (PPAR) are nuclear hormone receptors with ligand binding transcription factors, with a documented influence in the metabolism of fats and lipids on activation[13]. They also determine sensitivity of insulin in peripheral tissues like adipose tissue and skeletal muscles. Thiazolidinediones are agonists of these receptors and on activation, improve glycemic control in type 2 DM. Pioglitazone, one of the proto type thiazolidi-nedione, increases insulin sensitivity by acting on peripheral tissues involved in glucose hemostasis[14]. They are losing their therapeutic efficacy with associated increased risk of cardiovascular complications due to fluid retention and adipogenesis. This study was designed to explore the properties of aliskerin, a direct renin inhibitor, its effect on insulin resistance and to compare its anti-diabetic role with pioglitazone. The purpose of the study was to evaluate the effect of aliskerin in improving pancreatic morphology, so whenever it is given in combination with pioglitazone, the dose of later could be reduced to counteract the associated side effects.

MATERIAL AND METHODS

The study was performed in Army Medical College in collaboration with National Institute of Health, Islamabad. Study design was analytical and experimental. Laboratory facilities at the Department of Chemical Pathology, Army Medical College Rawalpindi, were used for estimation of biochemical parameters. Animal experimentation guidelines (NIH Publication No. 85-23, revised 1996) were followed.

Study approval was sought from Ethics committee of Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College, Rawalpindi. Total duration of study was 12 weeks with initial 01 week of acclimatization. Streptozocin was purchased from Sigma Chemicals USA. Pioglitazone was generously gifted by Werrick Pharmaceuticals Pakistan while aliskerin was procured from Novartis Pharmaceuticals. Standard laboratory conditions were maintained in animal house of National Institute of Health, Islamabad.

Thirty two BALB/c mice of both genders weighing about 35 to 40 grams were used for the study. Nonprobability convenience method was used for animal selection and then divided randomly through lottery method into four groups. For this study, mouse model resembling type 2 diabetes was made by giving streptozocin injection with prior administration of high fat diet consisting of 58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal for two weeks[15]. After two weeks, mice were weighed again and there was non-significant rise in body weight. Though our requirement for study was 18 diabetic mice but we took extra 08 to cater for

any mortality. This initial 2 weeks dietary manipulation was followed by five injections of low dose of streptozocin (35 mg/ kg) intra-peritoneally[16] on five consecutive days in all mice except for one group that was labeled as normal control later on.

In addition to high fat diet and low dose streptozocin, we also started glucose (7mg/kg) in drinking water after three days of first injection of strpetozocin which was given initially to treat hypoglycemia due to release of insulin from destruction of pancreatic β cell but later on we continued it for six weeks along with high fat diet to support the development of type 2 diabetes. Contrary to the available literature plasma glucose level which was supposed to increase one week after the last injection of strepozocin, animals in our study showed normal glucose levels even after three weeks of the last injection of streptozocin. Weekly estimation of glucose levels was undertaken with the help of gluco-meter for monitoring the development of type 2 DM. Glucose levels started raising as compared to normal values after four weeks of last injection and finally after six weeks of fifth injection of streptozocin, mice with fasting blood glucose levels more than 250 mg/dl were considered diabetic[17]. These diabetic mice were separated for the study, caged and three groups were formed with six mice in each group. Male and female mice were kept in separate cages to avoid pregnancy. From here onwards these mice started receiving normal pallet diet and normal water for the rest of the study. These hyper-

Table-I: Baseline and pretreatment biochemical parameters of all groups.

Para-meters	Normal control (Group-1) (Mean ± SD)			Diabetic control (Group-2) (Mean ± SD)			Diabetic with Pioglitazne (Group-3) (Mean ± SD)			Diabetic with Aliskerin (Group IV) (Mean ± SD)		
	Baseline	Pretreatment	*p*-value	Baseline	Pretreatment	*p*-value	Baseline	Pretreatment	*p*-value	Baseline	Pretreatment	*p*-value
Glucose (mg/dl)	114.83 ± 6.67	108.33 ± 9	0.23	116.33 ± 8.4	298.33 ± 25.12	<0.001	114.67 ± 6.24	299 ± 21.31	<0.001	114.33 ± 7.763	300.83 ± 24.75	<0.001
Insulin (µIU/ ml)	0.16 ± 0.02	0.15 ± 0.02	0.73	0.16 ± 0.04	0.17 ± 0.03	0.697	0.16 ± 0.04	0.17 ± 0.03	0. 55	0.145 ± 0.03	0.172 ± 0.03	0.158
Homa IR	0.04 ± 0.005	0.04 ± 0.008	0.6	0.05 ± 0.02	0.12 ± 0.014	0.001	0.04 ± 0.015	0.12 ± 0.02	<0.001	0.042 ± 0.01	0.127 ± 0.01	<0.001
Homa β (%)	108.04 ± 6.07	122.26 ± 12.33	0.08	105.03 ± 11.15	26.18 ± 7.9	<0.001	109.3 ± 15.7	25.98 ± 7.9	<0.001	101.73 ± 8.21	26.65 ± 7.83	0.001

p-value is significant <0.05*, *p*-value is highly significant <0.01** and calculated with baseline levels. All value are expressed as means ± SD (Standard deviation)

glycaemic, hyperinsulinemic mice were similar in features observed in patients of type 2 DM.

Group-2 was taken as diabetic control and did not receive any drug throughout the study. Pioglitazone[18] (15.4 mg/kg) was given to Group III for four weeks through oral gavage after suspending it in vegetable oil. Group IV received Aliskiren[19,20] (25 mg/kg) admixed in drinking water for 4 weeks after induction of type 2 diabetes. Vegetable oil as vehicle was also given to the rest of the three groups. Biochemical parameters were assessed thrice during this study. Initially blood through tail vein was collected for baseline blood samples in an un-anaesthetized state. After diabetic model was prepared, blood sample was again collected for all parameters through tail vein. Finally terminal cardiac sampling was done after four weeks of drug intervention.

Fasting Blood Glucose Levels

Fasting blood glucose levels were assessed after six hours of fast. Auto analyzer was used by applying GOD-PAP[21] enzymatic method.

Fasting Plasma Insulin Levels

Samples were collected after six hours fast for analysis of fasting insulin levels, and stored at -80°C. Enzyme-linked immunosorbent assay (ELISA) technique was applied using Rat insulin ELISA kit[22].

Insulin Resistance

Fasting plasma glucose and insulin concentration were used to evaluate insulin resistance (HOMA-IR)[23] with the help of formula: Fasting blood glucose (mg/dl) × fasting insulin (µIU/ml) /405.

Beta Cell Mass (Percentage)

Percentage of functional beta cell mass (HOMA-β) 24 was approximated by using equation:

360×Insulin / Glucose -63.

Statistical Analysis

SPSS version 22 was used to calculate mean ± standard error of means. Paired sample "t" test was used to determine the level of significance between baseline, pretreatment and post treatment levels. For multiple comparisons between groups, one way ANOVA followed by Post hoc Tukey Test was applied. Correlation between fasting glucose and insulin levels was determined by Pearson correlation.

RESULTS

All groups were compared three times during this study. Baseline parameters of four groups show non-significant statistical difference with each other. At this point, there was strong positive Pearson correlation between glucose and insulin with positive *r*-value (*r*-value 0.851, *p*-value 0.032). When diabetic model was developed, we compared the baseline parameters of each group with its pretreatment levels by applying paired sample "t" test, it was found that there was non-significant difference in group-1 which was normal control while highly significant difference in other three diabetic groups as shown in table-I. By applying multiple comparison between groups, there was highly significant difference between the three diabetic groups and group-1 (normal control) regarding all parameters (*p*-value < 0.001), except for insulin levels which showed non-significant difference

Table-II: Post treatment biochemical parameters of all groups.

Parameters	Normal Control (group-1) ± S.E.M	Diabetic Control (group-2) ± S.E.M	Diabetic with Pioglitazone (group-3) ± S.E.M	Diabetic with Aliskerin (group-4) ± S.E.M
Glucose (mg/dl)	108.33 ± 1.84 (1)	301.33 ± 8.39 (0.36)	182.67 ± 5.54 (<0.001**)	263.67 ± 6.56 (0.044*)
Insulin (µIU/ ml)	0.15 ± 0.005 (0.9)	0.16 ± 0.007 (0.4)	0.14 ± 0.006 (0.04*)	0.185 ± 0.012 (0.537)
Homa IR	0.04 ± 0.002 (1)	0.12 ± 0.003 (0.4)	0.06 ± 0.005 (<0.001**)	0.122 ± 0.008 (0.688)
Homa β (%)	122.01 ± 1.64 (0.97)	24.24 ± 1.94 (0.36)	41.68 ± 0.83 (0.006**)	35.28 ± 0.803 (0.031*)

p-value is significant <0.05*, *p*-value is highly significant <0.01** and calculated with pretreatment levels. All value are expressed as means ± S.E.M

with group-1 (normal control). At this stage, correlation between glucose and insulin became negative with significant *p*-value in all diabetic groups (*r*-value -0.905, *p*-value 0.013). After administering pioglitazone and aliskerin to group-3 & 4 (diabetic groups) for four weeks table-II. Also Pearson correlation between glucose and insulin in this group changed from strong negative to strong positive (*r*-value 0.92, *p*-value 0.02).

Aliskiren treated group-4 showed that after treatment it lowered the fasting glucose levels

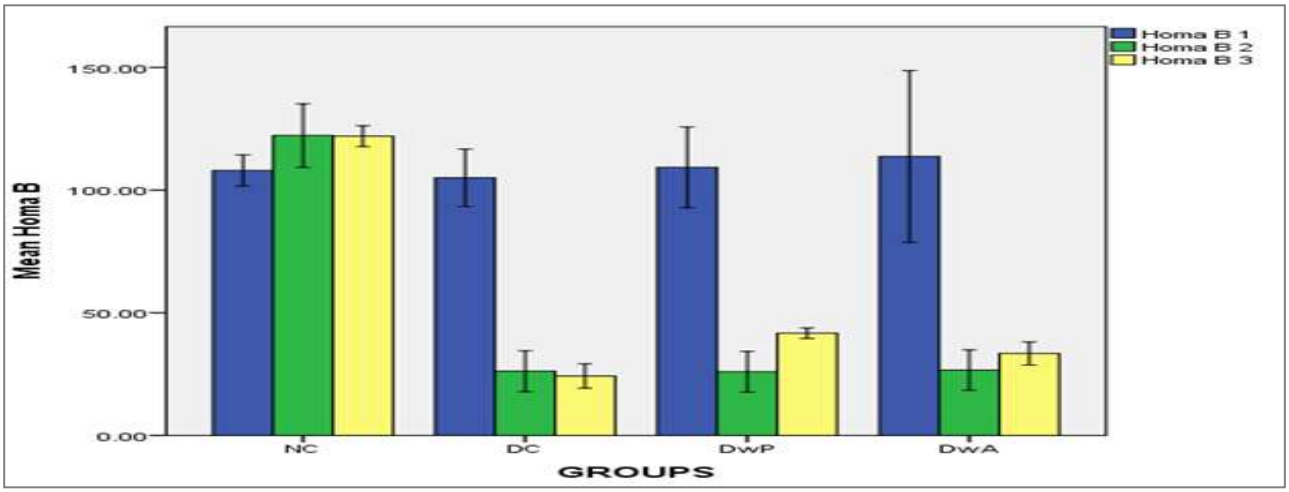

Figure-1: Mean Homa B values in all groups. (Homa B 1: Baseline, Homa B 2: Pretreatment, Homa B 3: Post treatment values) NC: Normal control group, DC: Diabetic control group, DwP: Diabetic group with Pioglitazone, DwA: Diabetic group with Aliskerin.

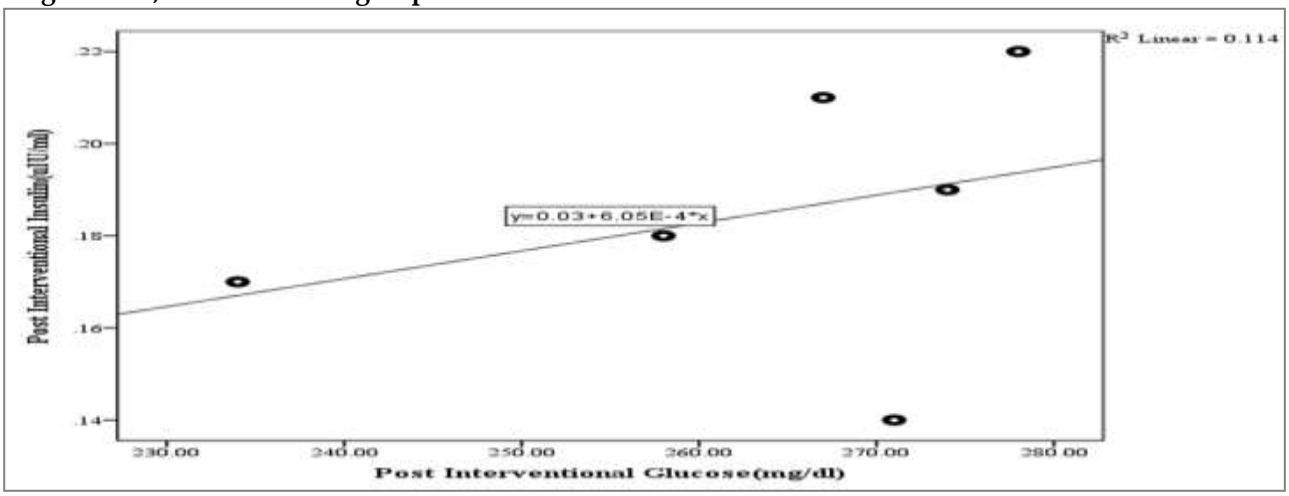

Figure-2: Scatter diagram for Pearson correlation between glucose and insulin levels in Aliskiren treated group (group-4).

respectively, the parameters were compared again with pretreatment levels for each group. It was found that in group-3 with the treatment of pioglitazone, all parameters had changed significantly with *p*-values <0.001 for fasting glucose and Homa IR while 0.006 and 0.04 for homa β and insulin respectively as shown in significantly but not to the extent to Pioglitazone. On the other hand Aliskiren increased fasting plasma insulin content even more than pioglitazone after treatment, but could not reduce resistance as much as pioglitazone did. Aliskerin also increased the functional pancreatic beta cell mass significantly from its pretreatment

levels (*p*-value, 0.031). Pearson correlation in aliskerin treated group-4 revealed a change from strong negative to non-significantly positive correlation (*r*-value 0.34, *p*-value 0.5) as shown in fig-2. Values of insulin and glucose were directly proportional as indicated by upward fit line in fig-1. This means that there was proportional rise in secretion of insulin with unit rise in glucose. The *r*-value was significantly negative in diabetic control group (group-1) and aliskerin treated group (group-4), prior to intervention and the conversion to positive r value supports the anti-diabetic role of aliskerin.

After four weeks of intervention with pioglitazone and aliskerin, multiple comparison between all groups was done by applying ANOVA, which showed that there was highly significant difference with regard to reduction in fasting plasma glucose levels with pioglitazone (*p*-value<0.001) being superior on comparison with aliskerin treated group, but more insulin secretion with aliskerin. Functional pancreatic β cell mass also increased in group-4 with significant *p*-value (*p*-value 0.07) when compared with group-3. This indicated the anti-diabetic properties of aliskerin.

DISCUSSION

Cardiometabolic syndrome comprises two closely integerated components that is hyperinsulinemia and hypertension[25]. Genetically predisposed individuals have a frequent co-occurrence of both. Raised insulin levels through effects on tissues, vasculature and sympethetic nervous system can predispose to hypertension. There is consensus for the past few years that overstimuled RAS is linked to the development of diabetes besides being playing a central role in the pathogenesis of essential hypertension. Administration of aliskiren, a direct renin inhibitor, not only prevents but also reverses the development of hyperglycaemia, hyper-insulinaemia and insulin resistance, suggesting that renin associated mechanisms play a pivotal role in the development of metabolic syndrome in mice fed with a high fructose diet[26]. Keeping this in view we gave aliskiren (25mg/kg) to one of the diabetic group. At this dose Aliskerin showed the potential contribution to reduce oxidative stress[19], with increase in pancreatic islet insulin content and beta cell mass and decrease in pancreatic islet fibrosis in mice[20]. Aliskiren treated diabetic group (group-4), showed improvement in fasting glucose levels significantly with *p*-value of 0.044 when pre and post treatment levels were compared which was in congruence with the results of Chu-Lin Chou and his fellows[26]. Dong and his colleagues[27] had also demonstrated beneficial role of aliskiren on fasting glucose levels which was again in the support of our study. Moreover, we found that administration of aliskiren, a direct renin inhibitor, also had significant positive effect on functioning of pancreatic β cells with *p*-value of 0.03 which could be atributed to the beneficial effects of aliskiren on pancreatic injury and attenuation of fibrosis as demonstrated by work done by Dong & his team who showed that the beneficial effects of aliskiren on insulin content and pancreatic β cell mass were associated with the attenuation of pancreatic oxidative stress. In our study, effects of Aliskiren on insulin content and Homa IR were non significant, though it had slightly increased insulin levles and reduced peripheral reistance but that was not significant and it corresponds with the work done by Cheng and his fellows[28]. Pearson corelation after treatment in this group showed conversion of significant negative correlation prior to intervention (*p*-value 0.003 and *r*-value -0.955) to non significant positive correlation (*p*-value 0.513 and *r*-value 0.337). When this group (Aliskerin treated) was compared with group-3 (Pioglitazone treated group), it was found that difference was immensely significant regarding all the four parameters which could be explained by the fact that though aliskiren, might be efficacious in reducing blood glucose levels and improving pancreatic function in type 2 diabetics but not to the extent of pioglitazone. However, further study on the effect of longer periods of aliskiren treatment on glucose tolerance is required to

define the significance of direct renin inhibition in type 2 diabetes.

CONCLUSION

In conclusion, this study showed that aliskerin has anti-diabetic role as far as pancreatic morphology is concerned. In hypertensive type 2 diabetics a combination of these two drugs may help in reducing the dose of pioglitazone and consequently the cardiovascular adverse effects of pioglitazone. Evaluation of such effect in type 2 DM is required in further studies.

ACKNOWLEDGEMENT

Department of Chemical Pathology, Army Medical College for the laboratory facilities and Dr. Hussain of NIH for animal house facilities.

LIMITATION OF STUDY

Combination group of aliskerin and pioglitazone could not be made due to study limitations. It can be done to evaluate their effects in a single animal. Other biochemical parameters can be considered with the administration of Aliskerin including complete lipid profile alone and in combination with Pioglitazone.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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