ROLE OF HIGH DOSE ASPIRIN IN MODULATING HYPERLIPIDEMIA AND HYPERGLYCEMIA

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ABSTRACT

This study was designed to evaluate the antidiabetic and antihyperlipidemic effects of high dose aspirin in rabbits. Healthy rabbits were made diabetic by injecting 150mg/kg body weight of Alloxan, in normal saline solution. intravenously in the marginal ear vein for seven days. The rabbits with serum glucose levels greater than 200mg/dl were considered as diabetic and used in this study. High dose aspirin (120mg/kg) p.o. showed maximum decrease in serum triglyceride level (42.07%), serum total cholesterol level (19.36%), and serum LDL level (6.18%). Glibenclamide showed decrease in fasting blood glucose level 56.31% as compared to high dose aspirin (23.91%). In the light of these results, it is suggested that such study should be carried out in human beings to make use of agents having S inhibitory activity of kappa Kinase Beta (IKKß) pathway, which will provide a novel approach for treatment of diabetes- associated hyperlipidaemia.

Key words: Alloxan, Antihyperlipidemic, Diabetes, IKKß, Salicylates

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INTRODUCTION

In 2000, according to World Health Organization, at least 171 million people worldwide suffer from diabetes that is 2.8% of the total population. Diabetes mellitus especially type 2 diabetes is more common in developed countries but increased prevalence is expected to occur in Asia and Africa by the end of 2030 [1]. TYPE 2 diabetic patients are at high risk for the development of hyperlipidemia especially in term of raised triglyceride levels and lowered HDL level. Hyperlipidemia is one of the major risk factors in the development of atherosclerotic heart diseases [2]. Insulin resistance is a primary factor in the development of type 2 diabetes, and recent studies have implicated fatty acid activation of serine/threonine kinase cascade (IKKβ) in the pathogenesis of insulin resistance [3]. This study focuses to find alternative pathways for the treatment of diabetes and hyperlipedima associated with obese diabetics by improving the acceptability of insulin on its target sites.)

Alloxan was used to induce diabetes in rabbits. Alloxan destroys beta cells of islets of langerhans in pancreas, insulin production stops and blood glucose level rises to significant level [4]. The normal value of blood glucose level in rabbits ranges 78-155mg/dl [5].Sulfonylurea (glibenclamide) was standard antidiabetic drug in this study. IKKβ plays an important role in mediating insulin resistance in obesity associated with dyslipidemia. High dose salicylates (120mg/kg aspirin) inhibit IKKβ activity, reverse hyperglycaemia, hyperinsulinemia, and dyslipidemia in obese rodents by sensitizing insulin signalling [6].

The study was designed to evaluate antidiabetic and antihyperlipidemic effects of high dose aspirin in diabetic rabbits by improving the insulin signalling and to develop innovative approach for the treatment of diabetes and hyperlipidemia.

MATERIALS AND METHODS

Materials:

Glibenclamide powder manufactured by Merck Germany, Alloxan manufactured by GNC and analytical kits manufactured by Randox U.K. were purchased from local market. Aspirin (analytical grade powder) was obtained from consolidated chemical laboratories (Pvt.) Ltd. Lahore, Pakistan.

Animals:

Albino rabbits of either sex with weight range 0.9-1.4 kg, purchased from the local market, and were employed in this study. The rabbits were kept in the animal house of University College of Pharmacy at temperature 20˚-25˚C. The animals were fed fresh greed fodder twice daily and fresh water was available ad libitum.

Experimental design:

Forty rabbits were used in this study. On zero day, fasting blood glucose levels of all the animals were determined. Blood samples of all the animals were also collected, for the determination of serum total cholesterol, HDL, LDL and triglycerides levels to establish the pre-treatment values.

Then diabetes was induced in all animals by injecting 150mg/kg body weight of Alloxan in normal saline solution intravenously in the marginal vein of ear for seven days Rabbits having glucose level more than 200 mg/ dl are considered to be diabetic and again blood samples were collected to find the lipid profile and glucose levels before starting the various therapies. Now the rabbits were divided randomly into 4 groups each group with 10 rabbits. Specific therapy was assigned to each group and was given once daily for 15days. The therapies assigned to different groups are as follows: Group A, diabetic control, received placebo orally. Group B received Glibenclamide (10mg/kg per oral) .Group C was given Aspirin in low dose (50mg/kg per oral) Group D received Aspirin in high dose (120mg/kg) orally.

Collection of blood samples:

The marginal vein was punctured with sterilized butterfly needle and 2-3 ml of blood was collected in non-heparinized blood collecting tubes. The tubes were centrifuged at 3000 rpm for 20 minutes to separate serum. All samples were stored at -20˚C pending analysis.

Analytical methods:

Serum glucose level was estimated by glucometer. Serum cholesterol was determined by enzymatic end point kit method manufactured by Randox UK. LDL-cholesterol was measured in serum by LDL-cholesterol precipitating reagent in conjunction with serum cholesterol enzymatic kit manufactured by Randox UK Kit. Serum HDL level Serum triglyceride levels were determined with Randox UK Kit.

Statistical analysis:

One-way analysis of variance (ANOVA) was applied to calculate statistical comparisons between groups by using SPSS computer package and P values of 0.05 and below were considered as significant.

RESULTS

Zero day values in all tables showed fasting blood glucose, total cholesterol, LDL, HDL, triglyceride levels after the diabetes had been induced.

Effect on fasting blood glucose levels

The decrease in fasting blood glucose level was maximum with glibenclamide 56.31% as compared to low dose aspirin 14.49% and high dose aspirin 23.91% at the end of study as described in Table 1.

Table-1 Fasting blood glucose levels (mg/dl) of diabetic rabbits

Days	Group-A (Diabetic control)	Group-B	Group-C	Group-D
0	276.79±12.50	284.01±7.07	275.91±3.72	276.68± 3.10
3	279.72±6.67	262.91±6.61*	270.89±2.38	270.37± 3.98*
6	278.26±5.53	231.75±12.13*	265.79±2.45	259.98±4.49*
9	275.93.2±5.77	192.0±13.07*	257.45±2.33*	241.01±2.06*
12	273.58±9.17	157.74±10.96*	245.04±2.82*	227.83±2.28*
15	270.58±9.17 ↓ (2.24%)	124.07±4.69* ↓ (56.31%)	235.91±1.30* ↓ (14.49%)	210.51±1.89* ↓ (23.91%)

Each figure represents mean± standard deviation of ten rabbits.
* $p<0.05$ when compared with zero day values.
↓ Percent decrease in comparison to zero day figures.

Effect on serum triglyceride levels

Table 2 shows significant decrease in serum triglyceride level with high dose aspirin 42.07% while decrease with low dose aspirin was 28.63% and with glibenclamide reduction in serum triglyceride level was only 3.26%.

Table-2 Serum triglyceride levels (mg/dl) of diabetic rabbits

Days	Group-A (Diabetic control)	Group-B	Group-C	Group-D
0	159.0.79±1.46	159.11±1.33	161.21±4.22	164.21± 3.80
3	158.98±1.47	158.44±0.84	156.79±5.68	159.50± 3.57
6	158.40±1.18	157.69±0.86	150.68±6.46	147.84±5.28
9	158.06±1.11	156.71±1.13	143.2±7.18*	134.23±4.14*
12	157.82±1.04	155.32±0.97*	128.35±4.82*	118.52±4.54*
15	157.70±1.17 ↓ (81%)	153.92±0.84* ↓ (3.26%)	115.05±3.44* ↓ (28.63%)	95.12±2.51* ↓ (42.07%)

Each figure represents mean± standard deviation of ten rabbits.
* Ssp<0.05 when compared with zero day values.
↓ Percent decrease in comparison to zero day figures.

Effect on serum total cholesterol levels

Maximum decrease in serum total cholesterol level was observed with high dose aspirin 19.36%. Glibenclamide and low dose aspirin showed 6.87% and 13.91% decrease respectively as shown in Table 3.

Table-3 Serum total cholesterol levels (mg/dl) of diabetic rabbits

Days	Group-A (Diabetic control)	Group-B	Group-C	Group-D
0	107.95±2.96	108.22±1.44	106.67±2.05	107.90± 3.60
3	107.51±2.98	107.52±1.82	105.60±1.71	106.61± 3.17
6	107.16±2.87	106.68±1.18	104.04±1.92	103.58±2.43
9	106.69±2.67	105.40±1.38	100.35±1.13*	99.95±2.16*
12	106.51±2.80	103.36±1.57	96.86±1.13*	95.67±2.74*
15	105.90±2.52 ↓ (1.87%)	100.82±1.66* ↓ (6.83%)	91.78±1.56* ↓ (13.91%)	87.00±1.27* ↓ (19.36%)

Each figure represents mean± standard deviation of ten rabbits.
*p<0.05 when compared with zero day values.
↓Percent decrease in comparison to zero day figures

Effect on serum HDL levels

Table 4 shows that decrease in serum HDL level was minimum with high dose aspirin 4.86% while glibenclamide lowered 9.48% and low dose aspirin decreased 10.56% serum HDL level.

Table-4 Serum HDL levels (mg/dl) of diabetic rabbits

Days	Group-A (Diabetic control)	Group-B	Group-C	Group-D
0	42.44±0.80	41.32±1.02	42.20±0.63	41.28± 0.72
3	42.35±0.89	41.17±0.70	41.95±0.61	40.98± 0.63
6	42.10±1.00	40.16±1.10*	41.56±0.75	40.78±0.60
9	42.05±0.87	39.43±0.75*	40.51±1.16	40.09±0.92*
12	42.01±0.85	38.30±0.84*	39.06±1.19*	39.10±0.72*
15	41.89±0.91 ↓ (1.29%)	37.25±0.64* ↓ (9.48%)	37.74±0.90* ↓ (10.56%)	39.27±0.50* ↓ (4.86%)

Each figure represents mean± standard deviation of ten rabbits.
*$p<0.05$ when compared with zero day values.
↓ Percent decrease in comparison to zero day figures.

Effect on serum LDL levels

The significant decrease in serum LDL level was recorded with high dose aspirin 6.18% only. The decrease in serum LDL level with low dose aspirin and glibenclamide was insignificant i.e. 2.31% and 2.76% respectively as shown in Table5.

Table-5 Serum LDL levels (mg/dl) of diabetic rabbits

Days	Group-A (Diabetic control)	Group-B	Group-C	Group-D
0	94.08±097	94.87±1.64	94.14±1.25	94.14± 1.25
3	94.01±0.82	94.70±1.44	94.03±1.43	94.03± 1.43
6	93.97±1.25	94.70±1.44	93.40±1.54	93.51±1.04
9	93.74±1.08	93.62±1.49	92.54±1.39	92.70±0.89
12	93.47±0.83	93.20±1.45	92.52±1.55	91.96±0.68
15	93.15±0.78 ↓ 0.98%)	92.25±1.32 ↓ (2.76%)	92.03±1.72 ↓ (2.31%)	88.32±4.06* ↓ (6.18%)

Each figure represents mean± standard deviation of ten rabbits.
*p<0.05 when compared with zero day values.
↓ Percent decrease in comparison to zero day figures.

DISCUSSION

Hyperlipidaemia along with diabetes is one of major risk factors in the development of atherosclerotic heart diseases [2]. In this study, diabetes was induced with Alloxan administered intravenously, which raised blood glucose, total cholesterol, LDL, and triglyceride levels than normal range [7]. Recent animal studies by Ripudaman S. Hundal et al., 2002 have shown that high dose aspirin protects against insulin resistance due to increased plasma fatty acid concentrations induced by infusing intralipid along with heparin to activate lipoprotein lipase. High dose aspirin results in significant reduction in fasting blood glucose and serum triglyceride levels and such reductions enhanced insulin sensitivity. IKKβ play an important role in mediating insulin resistance in obesity-associated hyperlipidaemia. High dose salicylates (120mg/kg aspirin) inhibit IKKβ activity, reversed hyperglycaemia, hyperinsulinemia, and hyperlipidemia by sensitizing insulin signalling [6]. The results of this study are in full agreement with the findings of Boden.G et al [8] and Yuan et al [6]. In view of potential toxicities associated with

high dose aspirin, its use for treatment of type 2 diabetes could not be recommended. This study is consistent with hypothesis that a serine kinase cascade is involved in the pathogenesis of insulin resistance in type 2 diabetes and suggests that IKKβ pathway may represent a new approach for treating complicated cases of disease.

CONCLUSION

It is determined that inhibition of IKKβ pathway may provide a novel approach in drug design to treat hyperlipidaemia in obese diabetics.

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