

ORIGINAL ARTICLE

Irbesartan; An Antihypertensive Drug Reduces LDL-Cholesterol in Diet Induced Hyperlipidemic RabbitsSabeen Shakir¹, Akbar Waheed², Noreen Hafeez³**ABSTRACT**

Objective: This study on animal model was designed to explore the LDL-Cholesterol lowering effect of an antihypertensive drug Irbesartan.

Study Design: Randomised controlled experimental study.

Place and Duration of Study: The study was conducted in the animal house of National Institute of Health (NIH), Islamabad. Biochemical analysis of rabbit's serum was carried out in the department of chemical pathology, Army Medical College, Rawalpindi from February 2013 to June 2013.

Materials and Methods: Eighteen rabbits were divided into three groups (group A, group B and group C) of six rabbits each. Group one was labelled as normal control. The other two groups (B and C) were made hyperlipidemic by feeding them with high cholesterol diet. Of these, group B was taken as hyperlipidemic control and group C as treatment group. Serum LDL levels were estimated at three different occasions i.e. baseline, after giving high cholesterol diet to hyperlipidemic groups and after 30 days of giving irbesartan to treatment group.

Results: Serum analysis for the estimation of LDL-Cholesterol of all the groups was done and their means were calculated and compared with the base line values using SPSS Version 20. Irbesartan treated group showed a marked reduction in serum LDL cholesterol in comparison with the hyperlipidemic control group.

Conclusion: It is concluded that Irbesartan an antihypertensive agent, has also the ability to markedly reduce raised serum LDL cholesterol levels.

Key Words: *Hyperlipidemia, Irbesartan, LDL-Cholesterol.*

Introduction

Cardiovascular diseases (CVD) have become a major problem nowadays and are responsible for 76 percent of deaths and disabilities due to myocardial infarction, atherosclerosis and stroke.¹ The risk of cardiovascular events is strongly associated with high blood pressure as well as high blood cholesterol levels.² There are different groups of drugs used to lower blood pressure.³ Likewise there is separate class of drugs to reduce elevated blood cholesterol levels having its own mechanism of action.⁴ Therefore patients with cardiovascular diseases require drugs from both these classes i.e.

antihypertensives as well as antihyperlipidemics to manage two major determinants of CVD i.e. hypertension and hyperlipidemia.⁵

Lipids are the essential biomolecules, divided into five different classes i.e. chylomicrons, VLDL-Cholesterol, IDL-Cholesterol, LDL-Cholesterol and HDL-Cholesterol. Among these, low density lipoproteins (LDL-Cholesterol) in normal range is necessary for innumerable normal healthy body functions.⁶ However the elevated levels of LDL molecules loaded with cholesterol, get accumulated on the walls of the arteries and cause various fatal and nonfatal cardiovascular abnormalities.⁷

Irbesartan, an antihypertensive drug, is a novel, orally active, noncompetitive angiotensin receptor antagonist, specific for angiotensin 1 receptor subtype.⁸ The drug has a stronger capability to lessen the risk of combined major cardiovascular events (stroke, atherosclerosis, heart failure, angina, myocardial infarction, peripheral arterial disease and death).⁹

Irbesartan is metabolized in the liver by glucuronide conjugation and oxidation.¹⁰ A big fraction of irbesartan i.e. 80 percent remains unchanged and possess pharmacological activity whether given

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orally or intravenously.¹¹ Only 6 percent of circulating drug gets converted into inactive glucuronide conjugate. The rest of the metabolites are pharmacologically inactive and are excreted via urine or bile.¹²

Angiotensin II is a potent vasoconstrictor in vascular smooth muscles.¹³ It synthesizes in a sequential step of conversion of angiotensinogen in the presence of renin to angiotensin I and then to angiotensin II. This angiotensin II then acts on angiotensin 1 (AT1) receptor subtype, stimulates it and causes vasoconstriction. It also promotes synthesis and secretion of aldosterone by stimulating the adrenal cortex thereby decreasing sodium excretion and increasing potassium excretion.¹⁴ Irbesartan inhibits the action of angiotensin II and promotes vasodilatation, by selectively binding to AT1 receptor subtype and blocking it noncompetitively.¹⁵ Irbesartan also antagonizes the effects of aldosterone. Irbesartan has been reported to be a peroxisome proliferator activated receptor (PPAR) alpha activating agent. PPAR alpha is a nuclear transcription receptor, which regulates the expression of genes involved in fatty acid oxidation and energy homeostasis.¹⁶

Irbesartan has a good safety profile with least or no adverse reactions.¹⁷ Unlike angiotensin converting enzyme (ACE) inhibitors, the only complication seen with the use of angiotensin receptor blockers (ARB's) is mild angioedema but it is extremely rare.¹⁸

It is obvious from a large number of clinical trials that there is 30 percent decline in the risk of development of CVD by pharmacologically lowering serum LDL cholesterol.¹⁹ Therefore, in addition to controlling high blood pressure in patients with cardiovascular disease, it becomes necessary to improve plasma lipid biochemistry.²⁰ This study explores the antihyperlipidemic property of irbesartan to cope with the two major risk factors of cardiovascular diseases.

Materials and Methods

This randomized controlled study was conducted in the animal house of National Institute of Health (NIH), Islamabad. Biochemical analysis of rabbit's serum was carried out in the department of chemical pathology, Army Medical College, Rawalpindi, from February 2013 to June 2013, after approval from the Ethics committee of Centre for Research in

Experimental and Applied Medicine (CREAM), Army Medical College.

Eighteen healthy adult domestic breed rabbits (*Oryctolagus Cuniculus*) having a weight of 1.5 to 2.0 kg were selected. They were of mixed breed both males and non-pregnant females. Animals under 1.5 years of age were not included in the experimental study. Standard laboratory conditions were maintained in animal house of National Institute of Health and rabbits were provided with controlled environment assuring twelve hours day and night cycle and an average temperature of 24°C. Rabbits were acclimatized for one week prior to the study.

Diet formula for animals used in the study was composed of cholesterol powder (1g/day) mixed in 1g/day of wheat bran along with routine diet of rabbits (gram whole, carrots, cucumbers, seasonal fruits) and was in strict compliance with the guidelines for the care of laboratory animals NIH Islamabad. Feeding of cholesterol powder and drugs was ensured by giving them mixed in small pellets of wheat bran after four hours fast before giving the routine diet.

Cholesterol powder one gram per day was added to the diet of the two experimental groups (group B and group C) excluding the rabbits of group A (Normal control group). All the rabbits were given tap water ad libitum for drinking.

The rabbits were randomly assigned into three groups of six animals each. The study period comprised of a total of twenty weeks after one week period for acclimatization. Animals were weighed prior to giving high cholesterol diet on day zero. Blood samples were taken on three different occasions as follows.

1. Baseline samples were collected on day zero, before starting the high cholesterol diet.
2. After 120 days of feeding on high cholesterol diet.
3. At the end of the study, on completing the treatment course for a period of 30 days.

The rabbits (n=6) in two of the experimental groups (group B and group C) were given high cholesterol diet followed by Irbesartan (40mg/kg) once daily according to the following schedule.

Group A (normal control; n=6) was the control group and received normal diet consisting of gram whole, wheat bran, green fodder, seasonal fruits and water

ad libitum for 150 days as it was normal control group and fed on normal cholesterol free diet for the whole study period. Group B (hyperlipidemic control; n=6) animals received cholesterol powder (1g/day) mixed in a diet comprising of grain whole, wheat bran, green fodder and seasonal fruits (cucumber, carrots and apples) for 120 days. Cholesterol powder was excluded from the diet for the next 30 days. Rabbits were also given tap water ad libitum for drinking.

Group C (hyperlipidemic+irbesartan; n=6) animals received the high cholesterol diet (1g/day) as per group B for 120 days and then fed on normal/routine diet without cholesterol along with Irbesartan (40mg/kg) once daily via gavage for a period of 30 days.

Fasting whole blood (4 ml) samples (n=6.0) were drawn from the tip of the ear of each animal with the help of a 5cc syringe. All the samples were transferred to separate plain clot activator tube and were let to clot at room temperature for at least 30 minutes. The samples were then centrifuged at 4500 rounds per minutes for 10 minutes. Serum was separated via an automatic micropipette for estimation of serum cholesterol, serum triglycerides and serum HDL-Cholesterol labelled accordingly in order to calculate serum LDL by using Friedwald's equation.²¹

$[\text{LDL Cholesterol mmol/L}] = [\text{Total Cholesterol}] - [\text{HDL Cholesterol}] - [\text{Triglyceride}] / 2.20$

The results of serum analysis of LDL-Cholesterol were established as means + standard error of mean. The difference between the two observations was derived using SPSS Version 20. The difference was taken as significant for a p value of 0.05.

Results

Serum LDL

The differences in means of LDL-Cholesterol values were calculated among normal control and hyperlipidemic control as well as among hyperlipidemic and treatment group.

Group A (normal control) showed unchanged levels of serum LDL when recorded on day zero, day 120 and day 150 i.e. 1.64 ± 0.5 , 1.64 ± 1.1 mmol/L and 1.64 ± 0.8 mmol/L as shown in table, p=NS.

The values of serum LDL of group B (hyperlipidemic control). Serum LDL levels on day 120 were increased significantly as compared to day zero, i.e. 3.45 ± 1.1 mmol/L versus 1.61 ± 1.1 mmol/L with p=0.0005. but

remained unchanged on day 150 in comparison to day 120. When compared with normal control group A, a significant rise was observed in group B and group C on day 150.

Group C (Irbesartan), when compared with group B (hyperlipidemic control) to assess the post treatment reduction in serum LDL levels on day 150, a statistically remarkable decline was recorded, i.e. group C (Irbesartan) showed mean values of 1.29 ± 0.2 mmol/L in comparison with group B (hyperlipidemic control) showing a mean of 3.46 ± 0.3 mmol/L on day 150, p value for group C (Irbesartan) was 0.005 as shown in table I.

Table: Comparison of serum LDL levels among group A, group B and group C on day 0, 120 and 150 in rabbits (n=6)

No. of Days	Group A	Group B	Group C
Day 0	1.64 ± 0.5	1.61 ± 1.1	1.63 ± 1.1
Day 120	1.64 ± 1.1	3.45 ± 1.1	3.46 ± 0.3
Day 150	1.64 ± 0.8	3.45 ± 0.2	1.29 ± 0.2
P value	0.005		

1n=6, Results are expressed as mean \pm SEM (Standard Error of Mean)

Discussion

In our study we found a highly significant reduction (28%) in serum LDL cholesterol with irbesartan. This favors the dual role of irbesartan, i.e. an anti hypertensive drug serving as an anti hyperlipidemic drug. Shimamura et al also demonstrated the similar results in rats as their experimental model.²² This was also revealed by Derosa et al after conducting a 12 months clinical study on 188 patients with metabolic syndrome that irbesartan has the ability to significantly reduce serum LDL cholesterol levels compared with the baseline. This role of an antihypertensive drug renders it special in respect of serum LDL cholesterol from other drugs that are specific for the treatment of hypertension.

Unlike irbesartan, when a long term therapy is conducted for primary or secondary prevention of atherosclerotic complications, angiotensin converting enzyme inhibitors have no effect on serum LDL-C.²³ Other classes of antihypertensive drugs including beta blockers, diuretics and calcium channel blockers would rather increase the serum LDL cholesterol levels.²⁴ However irbesartan does not cause any undesired derangement in the plasma LDL cholesterol after long term use and this was also

demonstrated by Kirk, (1999), although blood pressure lowering efficacy is same as those of other antihypertensive drugs.²⁵

Our study effects concerning serum LDL-Cholesterol strongly support the previous study performed on cholesterol fed rabbits.²⁶ In their study, they reported the lipid lowering effect of irbesartan and losartan. They concluded that irbesartan treatment is associated with significant reduction in plasma LDL levels compared to base line. An open multidrug comparison trial was carried out by Stella et al to study the variation in response of different ARB's on plasma lipid profile.²⁷ They demonstrated that angiotensin receptor blockers (ARB's) action on different indices of lipid profile are not same.^{28,29,30}

This conflict with Rong et al and Shimamura et al could be a reflection of use of different species, as we had rabbits instead of rats while Shimamura et al presented a clinical study. The other prospect may include the reason that animal model of Ronget al was genetically obese rats whereas we used high cholesterol diet induced hyperlipidemic rabbits. Duration of treatment could be another possibility of insignificant reduction of total cholesterol as we gave the treatment for 30 days whereas treatment period of Rong et al was 49 days. Shimamura et al gave significant results after 90 days treatment.

In the light of these findings it is clearly evident that irbesartan reduced the serum low density lipoprotein cholesterol (LDL-C) in high cholesterol diet fed rabbits. For this reason irbesartan administration can be helpful to decrease the risk of developing cardiovascular diseases because besides its antihypertensive property, irbesartan has an additional subsidy of lowering serum LDL-Cholesterol which is evident from this study. Further studies are warranted to explore the mechanism of lowering serum LDL by irbesartan in rabbits.

Conclusion

Irbesartan, an efficient antihypertensive drug, is highly effective in lowering serum LDL cholesterol. So when one has to deal with the risk factors, irbesartan with dual function, exhibiting both antihypertensive and antihyperlipidemic actions with least or no side effects can serve the humanity by treating initial hypertension as well as by reducing serum LDL-Cholesterol. As medication safety is a recognized indicator of quality of care, irbesartan can be

considered to prevent and treat life threatening cardiovascular problems safely for long duration serving at the same time for correcting both hypertension and hyperlipidemia due to high serum LDL levels.

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