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Original Article

Comparative study of atorvastatin, metformin and telmisartan on high fat induced obesity in Albino Wistar rats

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ABSTRACT

Objectives: To study the anti-obesity activity of telmisartan, atorvastatin and metformin alone and combination on high fat induced obesity in albino Wistar rats.
Materials and methods: Animals were divided into six different groups each comprising three animals. Group 1 (saline control), Group 2 (High fat diet), Group 3 (high fat diet + Atorvastatin), Group 4 (high fat diet + Telmisartan), Group 5 (high fat diet + Metformin), Group 6 (high fat diet + Atorvastatin + telmisartan + metformin).
Results: telmisartan, atorvastatin and metformin alone and combination shows anti-obesity activity on high fat diet induced obesity in albino Wister rats.

Keywords: Atorvastatin, Telmisartan, Metformin and High Fat Diet

INTRODUCTION

Obesity is a condition where a person has accumulated so much body fat that it might have a negative effect on their health. If a person's bodyweight is at least 20% higher than it should be, he or she is considered obese. If your Body Mass Index (BMI) is between 25 and 29.9 you are considered overweight. Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health[1]. People are generally considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight by the square of the person's height, is over 30 kg/m^2, with the range 25–30 kg/m^2 defined as overweight. Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis.

BMI is defined as the subject's weight divided by the square of their height and is calculated as follows.

$BMI = m / h^2$

where m and h are the subject's weight and height respectively.

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BMI is usually expressed in kilograms per square metre, resulting when weight is measured in kilograms and height in metres. To convert from pounds per square inch multiply by 703 (kg/m^2)/(lb/sq in).

PATHOPHYSIOLOGY

There are many possible pathophysiological mechanisms involved in the development and maintenance of obesity [1]. These investigators postulated that leptin was a satiety factor. In the ob/ob mouse, mutations in the leptin gene resulted in the obese phenotype opening the possibility of leptin therapy for human obesity. However, soon thereafter J. F. Caro's laboratory could not detect any mutations in the leptin gene in humans with obesity. On the contrary Leptin expression was increased proposing the possibility of Leptin-resistance in human obesity [2].

Since this discovery, many other hormonal mechanisms have been elucidated that participate in the regulation of appetite and food intake, storage patterns of adipose tissue, and development of insulin resistance. Since leptin's discovery, ghrelin, insulin, orexin, PYY 3-36, cholecystokinin, adiponectin, as well as many other mediators have been studied. The adipokines are mediators produced by adipose tissue; their action is thought to modify many obesity-related diseases. Leptin and ghrelin are considered to be complementary in their influence on appetite, with ghrelin produced by the stomach modulating short-term appetitive control (i.e. to eat when the stomach is empty and to stop when the stomach is stretched).

Leptin is produced by adipose tissue to signal fat storage reserves in the body, and mediates long-term appetitive controls (i.e. to eat more when fat storages are low and less when fat storages are high). Although administration of leptin may be effective in a small subset of obese individuals who are leptin deficient, most obese individuals are thought to be leptin resistant and have been found to have high levels of leptin [3].

This resistance is thought to explain in part why administration of leptin has not been shown to be effective in suppressing appetite in most obese people. While leptin and ghrelin are produced peripherally, they control appetite through their actions on the central nervous system. In particular, they and other appetite-related hormones act on the hypothalamus, a region of the brain central to the regulation of food intake and energy expenditure.

There are several circuits within the hypothalamus that contribute to its role in integrating appetite, the melanocortin pathway being the most well understood. The circuit begins with an area of the hypothalamus, the arcuate nucleus, that has outputs to the lateral hypothalamus (LH) and ventromedial hypothalamus (VMH), the brain's feeding and satiety centers, respectively [4]. The arcuate nucleus contains two distinct groups of neurons. The first group co expresses neuropeptide Y (NPY) and agouti-related peptide (AgRP) and has stimulatory inputs to the LH and inhibitory inputs to the VMH.

The second group coexpresses pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) and has stimulatory inputs to the VMH and inhibitory inputs to the LH. Consequently, NPY/AgRP neurons stimulate feeding and inhibit satiety, while POMC/CART neurons stimulate satiety and inhibit feeding.

Both groups of arcuate nucleus neurons are regulated in part by leptin. Leptin inhibits the NPY/AgRP group while stimulating the POMC/CART group. Thus a deficiency in leptin signaling, either via leptin deficiency or leptin resistance, leads to overfeeding and may account for some genetic and acquired forms of obesity.

ATORVASTATIN

Atorvastatin, marketed under the trade name Lipitor among others, is a member of the drug class known as statins, which are used primarily as a lipid-lowering agent and for prevention of events associated with cardiovascular disease. Like all statins, atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body. The primary uses of atorvastatin is for the treatment of dyslipidemia and the prevention of cardiovascular disease: Atorvastatin is used to lower high cholesterol levels in your blood.

Atorvastatin also helps reduce the risk of having a heart attack or stroke in people who have high blood pressure and coronary heart disease (CHD), which belongs to a group of medicines called HMG CoA reductase inhibitors. It works by reducing the amount of 'bad' cholesterol made by the liver and also raises the level of the 'good' cholesterol.

METFORMIN

Metformin, marketed under the trade name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes [5]. This is particularly true in people who are overweight. It is also used in the treatment of polycystic ovary syndrome. Limited evidence suggests metformin may prevent the CVS disease and cancer complications of diabetes [6,7]. It

is not associated with weight gain. It is taken by mouth.

Metformin is generally well tolerated [8]. Common side effects include diarrhea, nausea, and abdominal pain. It has a low risk of developing low blood sugar. High blood lactic acid levels is a concern if prescribed inappropriately and in overdose [9]. It should not be used in those with liver disease or kidney problems. While there is no clear harm if used during pregnancy insulin is generally preferred for gestational diabetes. Metformin is in the biguanide class. It works by decreasing glucose production by the liver and increasing glucose use by body tissues.

TELMISARTAN

Telmisartan (INN) is an angiotensin II receptor antagonist (angiotensin receptor blocker, ARB) used in the management of hypertension. Telmisartan is indicated in the treatment of essential hypertension. The usually effective dose telmisartan is 40–80 mg once daily. Some patients may already benefit at a daily dose of 20 mg. In cases where the target blood pressure is not achieved, telmisartan dose can be increased to a maximum of 80 mg once daily. Telmisartan is an angiotensin II receptor blocker that shows high affinity for the angiotensin II receptor type 1 (AT_1), with a binding affinity 3000 times greater for AT_1 than AT_2. It has the longest half-life of any ARB (24 hours) and the largest volume of distribution among ARBs (500 liters).

HIGH FAT DIET

This analysis included not only the obese phenotype and the degree of insulin resistance, but also changes in plasma lipid profiles, major hormones of metabolism and hepatic lipid deposition, as well as the gene expression pattern in the liver.

This was prepared by mixing coconut oil and vanaspati ghee in a ratio of 2:3 (v/v). It was given to the rats at the dose of 10 ml/kg body weight per day mixed with food.

MATERIALS AND METHODS

Animals: Six either sex Wistar albino rats of weighing range from 180 – 250 gm (for anti-obesity study) were obtained from the animal house of Swamy Vivekananda College of Pharmacy, Tiruchengode. This study was approved by our Institutional Animal Ethical Committee registration number, SVCP/IAEC/MPHARM/4/04/2016 and performed under Committee for the Purpose of Control and Supervision on Experiments on Animal guidelines. The animals were kept in the plastic cage in the animal house with 12-hour light and dark cycle. The animals were allowed free access to food pellets and tap water.

Keep rats under standard pathogen-free conditions with food and water ad libitum and regular 12:12 light–dark cycle. Make sure that the light–dark cycle is strictly followed, as checking rat after dark cycle started will disrupt the regular circadian rhythm of mice and will have impact on the metabolism of rat. Mice should be housed in the same number per cages.

Drugs: The drugs used in this study include telmisartan atorvastatin and metformin was obtained as gifted sample from Saimeera Inno Pharmaceuticals Pvt Ltd, Ambatur. The other chemicals and reagents used for the study were obtained from Department of Pharmacology, SS Institute of Pharmacy, Sankari.

Experimental Procedure

Animals were divided into six different groups each comprising three animals. Group 1 receive Normal saline as control, Group 2 receive High fat diet (10ml / kg) this was prepared by mixing coconut oil and vanaspati ghee in a ratio of 2:3(v/v). high fat diet at a dose of 10ml/kg body weight per day mixed with food, Group 3 receive high fat diet mixed with food and Atorvastatin (10mg / kg), Group 4 receive high fat diet mixed with food and Telmisartan (5mg / kg), Group 5 receive high fat diet mixed with food and Metformin (225mg / kg), Group 6 receive high fat diet mixed with food and Atorvastatin + telmisartan + metformin (10mg + 5mg + 225mg / kg = 240mg/kg).

All the animals used for the experiment were kept under observation for daily food intake, general health and behaviour. The drugs were administered to the animals in the doses given above orally, once daily. At the end of study, the animals were anaesthetized by using proper dose of ether inhalation and Blood sample was collected through retro orbital plexus, serum was separated and used for estimation of biochemical analysis.

Immediately after administration of the drugs, the animals were placed in metabolic cages individually to allow separation of urine and faeces. The urine was collected for 5 hours from each animal after administration of the drugs. During this period no water and food was made available to animals.

RESULTS

Effect of Atorvastatin, Telmisartan and Metformin and combination of Atorvastatin, Telmisartan and Metformin in body weight. The rat was treated Normal saline as control , Group 2 receive High

fat diet (10ml / kg) this was prepared by mixing coconut oil and vanaspati ghee in a ratio of 2:3(v/v). high fat diet at a dose of 10ml/kg body weight per day mixed with food, Group 3 receive high fat diet mixed with food and Atorvastatin (10mg / kg), Group 4 receive high fat diet mixed with food and Telmisartan (5mg / kg), Group 5 receive high fat diet mixed with food and Metformin (225mg / kg), Group 6 receive high fat diet mixed with food and Atorvastatin + telmisartan + metformin (10mg + 5mg + 225mg / kg = 240mg/kg).

The body weight of the animal before and after the drug treatment was measured. There was slight change in weight of the animal after drug treatment.

Effect of drugs on serum's HDL level: The blood sample of the drug treated animal was collected for HDL level was measured. The rat treated with combination of atorvastatin + HFD had decreased HDL range when compared with the other group. The rat treated with HFD showed more HDL.

Effect of drugs on serum's LDL level: Blood sample of the drug treated animal was collected for LDL level was measured. The rat treated with combination of HFD and Atorvastatin + telmisartan + metformin (10mg + 5mg + 225mg / kg = 240mg/kg) decreased LDL range when compared with the individual treating groups. The rat treated with only HFD group showed more LDL range.

Effect of drugs on serum's VLDL level: The Blood sample of the drug treated animal was collected for VLDL level was measured. The rat treated with HFD and Atorvastatin + telmisartan + metformin (10mg + 5mg + 225mg / kg = 240mg/kg) decreased VLDL range when compared with the individual treating groups. The rat treated with only HFD group showed more VLDL range.

Effect of drugs on serum's Triglyceride level: The Blood sample of the drug treated animal was collected for triglyceride level was measured. The rat treated with HFD and Combination of three drugs decreased triglyceride range when compared with the individual treating groups. The rat treated with only HFD group showed more range.

Effect of drugs on serum's Cholesterol level: The Blood sample of the drug treated animal was collected for cholesterol level was measured. The rat treated with HFD and Atorvastatin + telmisartan + metformin (10mg + 5mg + 225mg / kg = 240mg/kg) decreased cholesterol range when compared with the individual treating groups. The rat treated with only HFD group showed more.

Effect of drugs on serum's sugar level: The Blood sample of the drug treated animal was collected for sugar level was measured. The rat treated with combination of drugs atorvastatin, telmisartan and metformin (10mg + 5mg + 225mg / kg = 240mg/kg) had decreased sugar range when compared with the individual treating groups. The rat treated with high fat diet with telmisartan (5mg / kg) showed more sugar range.

Effect of Drugs in concentration of electrolyte excretion: The urine sample of the drug treated animal was collected for 5 hours and its electrolyte level was measured using suitable instruments. The concentration of sodium and potassium were measured flame photometry and chloride ion concentration was measured titrimetrically. The group treated with telmisartan (5mg / kg) had decreased concentration of sodium excretion. The group treated with metformin (225mg / kg) has more concentration of sodium excretion. The group treated with high fat diet had decreased concentration of chloride excretion. The group treated with metformin (225mg / kg) has more concentration of chloride excretion. This synergism result can encourage new formulations even in formulating polyherbal formulations. [10]

Table 1: Effect of Atorvastatin, Telmisartan and Metformin and Combination of Atorvastatin, Telmisartan and Metformin on High Fat Induced Obesity.

S.NO	GROUP	HDL (mg/dl)	VDL (mg/dl)	VLDL (mg/dl)	TRI (mg/dl)	CHO (mg/dl)	SUGAR (mg/dl)
1	GROUP-1	18	48	19	61	62	88
2	GROUP-2	24	52	21	73	78	61
3	GROUP-3	17	42	14	53	58	77
4	GROUP-4	20	47	17	57	60	85
5	GROUP-5	21	45	15	55	61	51
6	GROUP-6	19	40	12	52	57	47

DISCUSSION

There are many possible patho-physiological mechanisms involved in the development and maintenance of obesity. Animal rodent models are therefore useful tools for studying obesity as they will readily gain weight when fed high-fat diets. The type of fat should be considered when choosing a high-fat diet for an animal study. Many high-fat diets used in laboratory animal research contain more saturated fat such as lard, beef tallow, or coconut oil and these diets are quite capable of inducing obesity in susceptible strains.

Most rodents tend to become obese on high-fat diets, there can be variable responses in weight gain, glucose tolerance, insulin resistance, triglycerides and other parameters depending on the strain. Obesity is a severe metabolic disorder, characterized with increases in energy intake and a decrease in energy output concerning body weight and glucose metabolism. Obesity is associated with many important complications such as diabetes and coronary heart disease sleep apnea and pulmonary dysfunction, stroke, diseases of the gallbladder, liver and the musculoskeletal system, reproductive dysfunction, venous insufficiency, deep vein thrombosis, poor wound healing, and more.

High-fat diets Energy density In humans, a significant positive relationship has been found between the amount of dietary energy from fat and the proportion of the population who are overweight (in epidemiological studies), and in clinical studies between the level of dietary fat and body-weight gain as well as between the reduction in the dietary fat and weight loss. These associations have also been shown in animal studies. Hyperlipidemia is a metabolic disorder, specially characterized by evaluation of lipids in the blood stream and these lipids include fats, fatty acids, cholesterol, cholesterol esters, phospholipids and triglycerides.

Due to dramatic changes of living and food styles in the last century, the intake of a large, variety of high lipid snack and an excessive intake of high lipid food could result, subsequently, in hyper-lipidemia. The rat was treated Normal saline as control , Group 2 receive High fat diet (10ml / kg) this was prepared by mixing coconut oil and vanaspati ghee in a ratio of 2:3(v/v). high fat diet at a dose of 10ml/kg body weight per day mixed with food, Group 3 receive high fat diet mixed with food and Atorvastatin at the dose of 10mg / kg , Group 4 receive high fat diet mixed with food and Telmisartan at the dose of 5mg / kg , Group 5 receive high fat diet mixed with food and Metformin dose 225mg / kg , Group 6 receive high fat diet mixed with food and Atorvastatin + telmisartan + metformin combination dose of 10mg + 5mg + 225mg / kg totally 240mg/kg. The body weight of the animal before and after the drug treatment was measured. There was slight change in weight of the animal after drug treatment.

HDL removes excess cholesterol from arteries and moves it to liver for further processing or to be eliminated from the body. The highest serum HDL is the better. Therefore, the HDL is called good cholesterol. The blood sample of the treated animal was collected for HDL level was measured. The blood sample of the drug treated animal was collected for HDL level was measured. The rat treated with combination of HFD and Atorvastatin (10mg / kg) drugs had decreased HDL range when compared with the other group. The rat treated with HFD showed more HDL.

LDL cholesterol forms fatty deposits. In arterial walls, which become plaques that grow, rupture and stimulate the formation of artery blocking blood clots. If LDL cholesterol causes atherosclerosis, logic dictates that there should be a strong correlation between blood levels of LDL cholesterol and atherosclerosis. The Blood sample of the drug treated animal was collected for LDL level, HFD and Atorvastatin + telmisartan + metformin (10mg + 5mg + 225mg / kg = 240mg/kg) decreased LDL range when compared with the individual treating groups. The rat treated with only HFD group showed more LDL range.

VLDL production is directly related to the body fat. Consumption of much fat leads to the production of VLDL levels increases, resulting in the formation of large amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is insufficient, causing blockages for the normal flow of blood. The Blood sample of the drug treated animal was collected for VLDL level was measured. The rat treated with high fat diet with Atorvastatin + Telmisartan + Metformin (10mg + 5mg + 225mg / kg = 240mg/kg) decreased VLDL range when compared with the individual treating groups. The rat treated with only HFD group showed more VLDL range.

Triglycerides are mainly stored in the adipose tissue. The plasma lipoproteins are major sources of fatty acid to synthesis triglycerols. The excess of fat diet increase the TG level due to lipoprotein lipase, triacylglycerol hydrolysis, so that the accumulation in the liver becomes more evident. Which one of the causes of hardening of arteries. The Blood sample of the drug treated animal was collected for triglyceride level was measured. The rat treated with combination drugs had decreased triglyceride range when compared with the

individual treating groups. The rat treated with HFD showed more range.

Cholesterol is synthesized in all animal tissue. It acts also as a precursor for the synthesis of steroid hormones. Increased amount of cholesterol leads to cardiovascular disease particularly coronary heart disease. Reduction of 1% cholesterol produces a 2% to 3% reduction in coronary heart disease risk. The Blood sample of the drug treated animal was collected for cholesterol level was measured. The rat treated with HFD and combination of three drugs had decreased cholesterol range when compared with the individual treating groups. The rat treated with HFD showed more cholesterol range.

The drug treated animal was collected for lipid ratio level was measured. The rat treated with combination of drugs atorvastatin, telmisartan and metformin had decreased lipids ratio range when compared with the individual treating groups. The rat treated with high fat diet showed more ratio range. The Blood sample was collected for sugar level was measured. The rat treated with combination of drugs atorvastatin, telmisartan and metformin had decreased sugar range when compared with the individual treating groups. The rat treated with high fat diet with telmisartan showed more sugar range. The urine sample of the drug treated animal was collected for 5 hours and its electrolyte level was measured using suitable instruments. The concentration of sodium and potassium were measured flame photometry and chloride ion concentration was measured titrimetrically. The group treated with telmisartan had decreased concentration of sodium excretion. The group treated with metformin has more concentration of sodium excretion. The group treated with high fat diet had decreased concentration of chloride excretion. The group treated with metformin has more concentration of chloride excretion.

CONCLUSION

The study demonstrated the beneficial effect of Atorvastatin, Telmisartan and Metformin and their combination on high fat diet alone and combination induced obesity in albino Wistar rats. On the basis of the result obtained in the study, it may be concluded that the treatment with combination of Atorvastatin, Metformin and Telmisartan (10mg + 225mg + 5mg/ kg) significantly prevented the high fat diet induced obesity compared to Atorvastatin, Metformin and Telmisartan drugs alone.

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