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Ameliorative Effect of Wortmannin and Rapamycin Treatment on Obesity Markers in High Fat Diet Feed Rats.

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ABSTRACT

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Obesity is a metabolic disorder, characterized by a chronic excess of energy intake over expenditure, leading to the accumulation of that excess as fat. Obesity is associated with adipocyte hypertrophy and adipocyte hyperplasia. PI3K and mTOR, both key signaling molecules critically regulating the adipogenesis and adipocyte differentiation. Thus, the present study investigates the efficacy of wortmannin, a PI3K inhibitor and rapamycin, a mTOR inhibitor on the anthropometrical parameters (body weight, Body Mass Index, Lee index, feed intake), and fat depots in High Fat Diet (HFD)-Induced obesity. The obesity in rats was produced by feeding HFD for a period of 8 weeks. The study explored the effect of wortmannin (100µg/kg/day i.p) and rapamycin (0.75mg/kg/day i.p) on abnormal anthropometrical parameters, fat pads weight and liver weight/ body weight ratio (%) resulted from HFD in rats. The present study findings indicated that treatment with wortmannin and rapamycin significantly ($p<0.05$) reversed the abnormal effect of HFD on anthropometrical parameters, different fat pads weight and liver weight/ body weight ratio (%) as compared to Normal diet. Therefore Wortmannin & Rapamycin may serve as the potential candidates for the treatment of obesity.

Keywords: Obesity, Anthropometrical parameters, Fat pads, Wortmannin, Rapamycin.

Abrevatition: PI3K- Phosphoinositide-3-kinase, mTOR- Mammalian target of Rapamycin, BMI- Body mass index, LI- Lee index HFD- High fat diet.

INTRODUCTION

Obesity is rapidly turning into an "epidemic" afflicting much of the industrialized world, resulting in a prohibitive health and economic burden on society[1-3]. Obesity is a multifactorial, chronic disorder that has reached a pandemic proportion worldwide[4-5]. Obesity is caused by energy intake (by ingestion) exceeding energy expenditure (by basal metabolic rate, diet-induced thermogenesis and exercise), with surplus energy stored in the form of fat[6-7] and characterized by an abnormal or excessive fat accumulation which may lead to hypertrophy and hyperplasia of adipose tissue[8-9]. Nearly one third of the world's adult population (1.3 billion people) was overweight or obese in 2005 and if recent trends continue, by 2030 nearly two third of the world's adult population (3.3 billion people) could be either overweight or obese. Patients with abdominal obesity have more extensive visceral adipose tissue (VAT), anomalies of blood-glucose homoeostasis, elevated plasma triglycerides (TG) and low levels of high-density lipoprotein (HDL) cholesterol that further contributes to the later appearance of cardiovascular syndromes[10-11]. Moreover, obese and overweight patients are at higher risk from coronary artery disease, hypertension, hyperlipidemia, diabetes mellitus, cancers, gall bladder disorders, cerebrovascular accidents, osteoarthritis, restrictive pulmonary disease and sleep apnoea[12-13].

Phosphoinositide 3 kinases (PI3Ks) are lipid kinases, those exhibit their biological effect by phosphorylation of phosphoinositides[14-15]. It is earlier reported that PI3K critically regulated several cellular processes such as cell proliferation, cell differentiation, cell migration, survival and cell growth[14,16-17]. Moreover loss of PI3K signaling in the muscle results in reduced muscle weight and myocyte size, thus representing a key role of PI3K in the regulation of muscle growth[18]. Activation of PI3K/Akt pathway also plays a crucial role in the molecular mechanism underlying energy homeostasis and adiposity[19]. It is tempting to speculate that mTOR and p70s6k signaling, which are downstream targets of PI3K pathways, may be involved in adipogenesis and adipocyte differentiation[20-21]. mTOR, a 283 kDA PI3K related

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kinase (PIKK)[22] acts as a nutrient sensor[23]. In peripheral tissues, mTOR activation by amino acids, insulin and glucose promotes anabolism, including lipid accumulation in adipose tissue, skeletal muscle hypertrophy, pancreatic cell growth and protein synthesis activation. Increased or otherwise aberrant mTOR activity has been linked to development of cancer, diabetes and obesity[24- 25]. Wortmannin and rapamycin, which inhibit the activity of both PI3K and mTOR respectively, result in pronounced inhibitory effects on adipogenesis[26-27]. Thus it suggests that PI3K and mTOR, signaling molecules plays a significant role in adipocyte differentiation and adipogenesis. Therefore, the current study investigates the efficacy of wortmannin, a PI3K inhibitor and rapamycin, a mTOR inhibitor on the anthropometrical parameters (body weight, Body Mass Index, Lee index, feed intake), and fat depots in High Fat Diet (HFD)-Induced obesity.

MATERIAL AND METHODS

Animals

Male wistar rats of 7-8 weeks of age were procured from the animal facility of the Institute. The animals were housed in standard polypropylene cages (two rats/cage) and maintained under controlled room temperature (25±2˚C) with 12:12 h light and dark cycle. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India were followed and prior permission was sought from the institutional animal ethics committee for conducting the study.

Drugs

Rapamycin was purchased from Biocon LTD. (Himachal Pradesh, India). The wortmannin was purchased from Sigma Aldrich (India). All other chemicals used in the study were of analytical quality.

Induction of obesity

Obesity was developed by HFD for 8 weeks administration. The HFD contains normal chow (365gm), cholesterol (10gm), casein (250gm), lard (310gm), dl-methionine (3gm), vitamins and mineral mix (60gm), yeast powder (1gm) and sodium chloride (1gm)[28]. This quantity is for one kg of diet.

Experimental Design

Six groups of rats were employed in the present study and each group comprised of 6-7 animals. **Group I:** {Normal Control}; Normal rats were maintained on standard chow diet and water ad libitum. **Group II:** {High Fat Diet Control}; Normal rats were maintained on HFD for eight weeks to produce obesity. **Group III:** {Wortmannin 100µg/kg/day i.p, perse}[29]; Wortmannin was administered to rats on standard chow at the end of 6th week and continued upto the end of the 8th week. **Group IV:** {Rapamycin 0.75mg/kg/day i. p, perse}[30]; Rapamycin was administered to rats on standard chow at the end of 6th week continued upto the end of the 8th week. **Group V:** {Wortmannin 100µg/kg/day i.p, treated}; Wortmannin was administered to rats on HFD at the end of 6th week and continued upto the end of the 8th week. **Group VI:** {Rapamycin 0.75mg/kg/day i.p, treated}; Rapamycin was administered to rats on HFD at the end of 6th week and continued upto the end of the 8th week.

Assessment of obesity

Assessment of Anthropometric Parameters

Food intake and body weight were measured regularly. To estimate food consumption, food intake was assessed by weighing the food in each cage, including the food that was spilled on the floor of the cage. The energy values in Kcal for each diet were calculated from the macronutrient composition using values of 4 kcal/g for carbohydrate and protein while 9 kcal/g for fat. BMI[31] i.e. weight (g)/ height $(cm)^2$, Lee index[32] i.e. $(Body\ Wt)^{1/3}$/ano-nasal length (cm) x 1000, were calculated before and after the treatment as an index of obesity.

Fat Depots and Liver Weight/Body Weight Ratio (%)

To evaluate the effect of HFD and drug treatment, adipose tissue (epididymal, retroperitoneal and mesenteric fat depots) were isolated, freed from surrounding tissues, weighed individually and after that total weight was calculated[33]. In addition the liver was isolated, freed from surrounding tissues, weighed and calculated the Liver Weight/Body Weight Ratio (%).

Statistical Analysis

All values were expressed as mean ± S.D. The data obtained from various groups were statistically analyzed using one way ANOVA followed by Tukey's multiple comparison test. The p value <0.05 was considered to be statistically significant.

RESULTS

Effect of HFD on Anthropometric Parameters of Obesity

A significant increase ($p < 0.05$) in body weight, BMI, LI, feed consumption in Kcal were observed in rats after 4 week of HFD and progressively these changes increased up to 8 week when compared with age matched normal rats fed on standard diet. (**Table 1**)

Effect of HFD on Different Fat Depots and Liver Weight/Body Weight Ratio (%)

The HFD administered for 8 weeks significantly increased ($p<0.05$) body fat depots i.e. epididymal, retroperitoneal,

mesenteric fat depots, total fat depot. Moreover HFD significantly increased liver weight/body weight ratio (%) as compared to normal rats fed standard diet (**Table 2**).

Effect of wortmannin and rapamycin on HFD-Induced Changes in Anthropometric Parameters

Treatment with wortmannin and rapamycin produced a significant decrease ($p<0.05$) in body weight, BMI, LI and feed consumption in Kcal as compared to HFD group. The effect of rapamycin was not significantly ($p>0.05$) different from that of wortmannin treatment group. Moreover Wortmannin treatment produced a significantly greater decrease ($p<0.05$) in feed intake (Kcal) as compared to rapamycin treated group **(Table 3**).

Effect of wortmannin and rapamycin on HFD-Induced Changes in Different Fat Depots and Liver Weight/Body Weight Ratio (%).

The weight of different body fat depots i.e. epididymal, retroperitoneal, mesenteric fat depots and total fat were significantly increased ($p<0.05$) in HFD group as compared with age matched normal rats (**Table 2**). Treatment with wortmannin and rapamycin produced significant decrease ($p<0.05$) in body fat depots i.e. epididymal, retroperitoneal, mesenteric fat depots and total fat. Moreover, treatment with rapamycin produced significant decrease in epididymal fat depots but did not significantly decrease retroperitoneal, mesenteric fat depots and total fat as compared with wortmannin treatment group (**Table 4**). The rapamycin treatment produced significant ($p<0.05$) decrease in liver

Table 1: Effect of standard diet and HFD on the body weight, BMI, LI and feed intake in Kcal.

Parameters	Standard diet control	High fat diet
Initial Body weight (gm)	189.10 ± 10.88	190.60 ± 12.42
Final Body weight (gm)	275.10 ± 7.99	347.50 ± 20.9 [a]
Body mass index (gm/cm^2)	0.57 ± 0.025	0.81 ± 0.35 [a]
Lee index (gm/cm)	298.85 ± 8.83	339.68 ± 7.14 [a]
Feed intake (Kcal)	101.66 ± 1.34	115.34 ± 5 [a]

All Values are represented as mean ± S.D; a = P < 0.05 vs. standard diet Control on Day 56.

Table 2: Effect of standard diet and HFD on the various fat pads and liver weight/ body weight ratio (%).

Parameters	Standard diet control	High fat diet
Epididymal fat (gm)	2.56 ± 0.43	7.64 ± 0.57 [a]
Mesenteric fat (gm)	2.72 ± 0.35	7.42 ± 0.48 [a]
Retroperitoneal fat (gm)	2.82 ± 0.54	10.3 ± 1.2 [a]
Total fat	8.1 ± 0.42	25.36 ± 2.16 [a]
Liver weight/ body weight %	2.87 ± 0.21	3.97 ± 0.18 [a]

All Values are represented as mean ± S.D; a = P < 0.05 vs. standard diet control on day 56.

Table 3: Effect of various pharmacological interventions on the body weight, BMI, LI and feed intake in Kcal.

Parameters	Initial Body weight (gm)	Final Body weight (gm)	Body mass index (gm/cm^2)	Lee index (gm/cm)	Feed intake (Kcal)
Standard diet control	189.10± 10.88	275.10 ± 7.99	0.57 ± 0.025	298.85±8.83	101.66± 1.34
High fat diet	190.60± 12.42	347.5 ± 20.9 [a]	0.81 ± 0.35 [a]	339.68±7.14 [a]	115.34 ± 5 [a]
Wortmannin standard diet	184.83 ± 5.29	278.66± 7.91	0.60 ± 0.005	305.46 ± 1.56	98.88 ± 2.43
Rapamycin standard diet	185.66 ± 4.36	283.33± 7.25	0.60 ± 0.01	305.49± 4.55	101.73± 1.18
Wortmannin HFD	193.58 ± 8.45	300.6 ± 22.6 [b]	0.64 ± .02 [b]	310.51±7.24 [b]	66.49±1.3 [b,c]
Rapamycin HFD	184.37 ± 4.40	294.37±8.57 [b]	0.63 ± .02 [b]	308.53 ± 6.65 [b]	106.02 ± 2.41 [b]

All Values are represented as mean ± S.D; a = P < 0.05 vs. standard diet control, b = P < 0.05 vs. HFD, c = P < 0.05 vs. rapamycin treatment on 56 Day.

Table 4 Effect of various pharmacological interventions on the various fat pads and liver weight/body weight %.

Parameters	Epididymal fat	Mesenteric fat	Retroperitoneal fat	Total fat	Liver Weight/ body %
Standard diet control	2.56 ± 0.43	2.72 ± 0.35	2.82 ± 0.54	8.1 ± 0.42	2.87 ± 0.21
High fat diet	17.64 ± 0.57 [a]	7.42 ± 0.48	10.3 ± 1.2 [a]	25.36 ± 2.16 [a]	3.97 ± 0.18 [a]
Wortmannin standard diet	2.33 ± 0.20	2.03 ± 0.25	3.30 ± .08	7.66 ± 0.98	2.92 ± 0.23
Rapamycin standard diet	2.03 ± 0.2	1.63 ± 0.2	2.36 ± 0.32	5.70 ± 0.70	2.78 ± 0.12
Wortmannin HFD	3.95 ± 0.47 [b]	3.65 ± 0.36 [b]	4.55 ± 0.58 [b]	12.15 ± 1.36 [b]	3.70 ± 0.44
Rapamycin HFD	3.52 ± 0.5 [b,d]	3.15 ± 0.42 [b]	3.90 ± 0.62 [b]	10.57 ± 0.84 [b]	3.22 ± 0.28 [b]

All Values are represented as mean ± S.D; a = P < 0.05 vs. standard diet Control, b = P < 0.05 vs. HFD, d =P < 0.05 vs. wortmannin treatment on Day 56.

weight/body weight ratio (%). But in the case of wortmannin treated group this effect was not significant (**Table 4**).

DISCUSSION

Our study supported the previous report that HFD significantly promotes the development of various markers of obesity, like anthropometrical parameter and adipose tissue weight[34-36]. In present study, HFD group had significantly increased in the markers of obesity as compared to normal standard diet group. Our study demonstrated that treatment with both wortmannin and rapamycin significantly reversed HFD-induced markers of obesity. There was significant decrease in the body weight, BMI, LI and feed intake (Kcal) in rapamycin treated group. According to previous study absence of S6K1, an effector downstream of mTOR, protects against age- and diet-induced obesity in mice, this supports a potential explanation for the consistent effect of rapamycin on weight gain[37]. The decrease in the body weight by rapamycin may due to decrease in fat pad mass by reduced formation of new adipocytes from precursor cells (adipocyte differentiation) or decreased adipocyte size due to fat storage (adipocyte hypertrophy)[38], because there is direct correlation between the body weight and body fat. In our study rapamycin treatment decreased energy intake (Kcal) as compared to normal diet fed rats. Diet-induced obesity (DIO) increased expression of PPAR-γ leading to reduced energy expenditure[39] and it is reported that rapamycin inhibits the PPAR-γ activity. The rapamycin treatment decreases the size of adipocytes. This parameter mostly mirrors human obesity in HFD-fed rats[40].

The PI3K inhibitor, wortmannin, used in the present study reduced the body weight in HFD fed rats. It is earlier reported that insulin treatment increased more weight gain in Zucker diabetic fatty (ZDF) rat as compared to normal rats[41]. PI3K act as a downstream pathway of insulin, thus inihibition of the PI3K by wortmannin led to impaired insulin signaling, which may lead to decreased in the body weight. A growing body of evidence suggests that activation of PI3K/Akt phosphorylates two mTOR downstream translational regulatory proteins like 4EBP1 and P70S kinase involved in obesity[42-43]. The phosphoryaltion of both 4EBP1 and p70s kinase is attenuated by wortmannin a selective inhibitor of PI3K[44]. The decrease in the weight may be due to decrease in fat pad mass by reduced formation of new adipocytes from precursor cells (adipocyte differentiation) or decreased adipocyte size due to fat storage (adipocyte hypertrophy)[38], because there is direct correlation between the body weight and body fat. The effect of wortmannin on BMI and LI has not been studied earlier.

In the present study, treatment with the wortmannin decreased the BMI and LI. Wortmannin also significantly reduced the food intake and energy intake. It has been documented that PI3K cause the activation of eNOS and leads to the synthesis of NO and NO plays an important role in the regulation of energy balance since administration of nonspecific NO synthase inhibitor reduced weight gain and food intake in mice[45]. In the present study treatment with wortmannin decreased the visceral fat pads (epididymal, mesenteric and retroperitoneal) weight gain. PI3K inhibitor suppressed the expression of adipogenic markers including PPARγ2 and C/EBPα[27]. There was a slightly change in the liver weight/body weight ratio (%) after treatment with wortmannin as compared to normal diet fed rats.

The current study explored the efficacy of wortmannin, a PI3K inhibitor and Rapamycin, a mTOR inhibitor on the anthropometrical parameters (body weight, Body Mass Index, Lee index, feed intake), and fat depots in HFD-Induced obesity. So on the basis of these results, it is concluded that involvement of PI3K and mTOR signaling molecules in the

alteration of normal anthropometrical parameters in HFD as compared to normal standard diet. The convincing mechanism for anti-obesity effect may be due to inhibition of adipocytes differentiation and proliferation. Therefore PI3K and mTOR signaling molecules may be a potential target area for discovery of new drugs for treatment of obesity.

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