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Research Article

COMBINATION OF LEPTIN ANALOG AND SALBUTAMOL: TREATMENT APPROACH FOR OBESITY-INDUCED ASTHMA

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ABSTRACT

Objective: The objective of the study was to investigate the effect of leptin analog and salbutamol in obese asthmatic mice.

Methods: Obese asthmatic condition was induced by administration of hypercaloric diet for 8 weeks followed by ovalbumin-aluminum hydroxide. The animals were treated with leptin analog (0.4 mg/kg, i.p. for 14 days) and salbutamol (2 mg/kg, PO for 14 days). Biochemical parameters such as serum leptin, ghrelin, and tumor necrosis factor-α and physical parameters such as tidal volume and airflow rate were estimated to confirm the state of asthma and obesity, respectively.

Results: Elevated serum leptin and ghrelin were associated with leptin resistance in obese asthmatic mice. It was found that a significant increase in serum leptin level with animal treated with leptin analog and salbutamol when compared to animals treated with leptin analog alone. The result of respiratory parameters and serum parameters also improved with the combination of leptin analog and salbutamol. From our study, we found that salbutamol potentiates the effect of leptin analog in obese asthmatic condition.

Conclusion: Leptin analog and salbutamol are an alternative treatment approach to treat the obese asthmatic condition.

Keywords: Obesity, Asthma, Leptin analog, Salbutamol, Tumor necrosis factor alpha.

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INTRODUCTION

Obesity is linked to the imbalance between energy expenditures [1]. It is due to excessive food intake with inactive lifestyle [2-4]. Obesity is one of the vital factors to worldwide for the burden of chronic sickness and disabilities. According to reports [5,6], over 1000 million adults worldwide are morbidly obese while of 300 million of them are clinically obese. Multiple factors contribute to the etiology are sedentary lifestyle, lack of physical activity and consumption of high energy-rich diet. The various study revealed that overall 20% men and 30% of females are obese worldwide [7]. The epidemic of obesity is becoming a universal problem, imposing considerable freight on the individual and society rising morbidity and mortality [8].

Asthma, a state of inflammation of airways involves different cells and cellular components such as eosinophils, somatic cells, lymphocytes, epithelial cells macrophages, and neutrophils; plays a crucial role in pathogenesis. These inflammatory cells cause repeated episodes of wheezing, dyspnea, chest tightness, coughing, and reversible airflow obstruction [9]. The inflammation results in elevated bronchial hyperresponsiveness to stimuli [10]. As of 2009, 300 million individuals globally were affected by asthma leading to approximately 250,000 deaths per annum [11].

Epidemiologists, after a detailed study on the asthmatic spectrum, detected different asthmatic phenotypes (different identical characteristics of disease) and genotypes (different pathological origins of identical disease) [12,13]. One in every of phenotypes is obesity-induced asthma [14]. Obesity with asthma has been strongly associated with both genders [15]. Studies revealed that obesity could increase asthma severity and reduced the efficacy of standard asthma medications [16,17]. Various clinical studies showed that over 3 million adults whose body mass index higher than 25 were diagnosed with asthma [18].

It is reported that abnormal leptin and ghrelin levels are associated with obesity and asthma [19,20]. Both hormones regulate food intake through acting on neuropeptide–Y pathway [21]. Various studies showed that obesity is due to either leptin resistance or elevated serum leptin level [7,22,23]. Clinical studies also revealed a higher level of leptin in asthmatic patients [24]. Reports also revealed that the pro-inflammatory effects of leptin are responsible for asthma in the obese population [25,26]. Thus, our investigation focuses on for effect of leptin analog with salbutamol in obese asthmatic mice.

At present, there are no treatment options available for the obese asthmatic condition. Even those treatments used for asthma and obesity have numerous side effects and costly. Therefore, there is a need for identification of effective treatment approach for asthma with obesity. In this study, we investigated the effect of leptin analog and salbutamol through hypercaloric diet-induced obesity in ovalbumin (OVA)-induced asthma in Swiss albino mice.

METHODS

Swiss albino mice of female sex weight in between 24±6 g were obtained from the central animal house of Faculty of Pharmacy, Dharmsinh Desai University, Nadiad. The animal studies were approved by the Institutional Ethics Committee (DDU/FOP/06/17), ratified by the purpose of control and supervision of experimental animals (CPCSEA) by Ministry of Environment and Forests, Government of India, New Delhi, India. Animals were naïve to drug treatment and experimentation at the beginning of all studies. Animals were kept individually in polypropylene cages in an environmentally controlled room of the animal house and maintained at a temperature of 25±2°C with a 12 h dark and light cycle. 10 days of acclimatization were provided to the animal. The animals were provided with water and food *ad libitum*. Mice were fed with laboratory pellet chow diet or

special high caloric diet according to the protocol. The composition of experimental diet (g/kg diet) was according to Soni *et al.* [15].

Experimental design

A total of 24 mice were used and divided into ten groups (n=6)

Group I (OB-AS)

(Obese asthmatic control mice) Animals were given with hypercaloric diet maintained for 8 weeks, and then, the induction phase of asthma was started. Mice were sensitized with OVA conjugated to aluminum hydroxide and challenged with saline to induce asthma. The induction with OVA was done on day 1–23 and challenge was for every 7th day for 3 weeks.

Group II (OB-AS-L)

(Leptin analog treated obese asthmatic mice) Animals were treated the same as mentioned in Group-I. Then, animals were treated with leptin analog (0.4 mg/kg, i.p. for 14 days) [27].

Group III (OB-AS-S)

(Salbutamol treated obese asthmatic mice) Animals were treated the same as mentioned in Group-I. Then, animals were treated with salbutamol (2 mg/kg, PO for 14 days) [28].

Group IV (OB-AS-L-S)

(Leptin analog and salbutamol treated obese asthmatic mice) Animals were treated the same as mentioned in Group-I. Then, animals were treated with leptin analog (0.4 mg/kg, i. p. for 14 days) with salbutamol (2 mg/kg, PO for 14 days) on the same day. There were 6 h intervals between leptin analog and salbutamol treatment for this group.

At the end of the experimental period, the animal was anesthetized with ketamine, following overnight fasting. Blood was drawn by the retro-orbital method. Serum was separated by centrifugation at 4000 rpm (revolution per minute). Serum levels of leptin, ghrelin, and tumor necrosis factor- α (TNF-α) were measured using standard ELISA kits. The serum samples were stored at −70°C until analysis.

Measurement of respiratory parameters

Airflow rate was measured for the assessment of asthmatic condition. The measurement of respiratory parameters was performed as per Soni *et al.* [15].

Chemicals and diagnostic kits

Leptin analog (recombinant mouse leptin–cyt-31), Elisa kit for leptin (ELM-leptin-1 Mouse leptin Elisa 1*96 well), ghrelin (EIAM-GHR-1 Mouse EIA 1*96 well), and TNF-α (ELM- TNF-α -1 Mouse TNF-alpha Elisa 1*96 well) were purchased from Everon life science, New Delhi, India.

Statistical analysis

Statistical evaluation of analytical data was done by one-way analysis of variance followed by Tukey's test using statistical software GraphPad Prism 3.0. Data were expressed as mean ± standard error of the mean and significant was determined at $p<0.05$

RESULTS

Effect of leptin analog and salbutamol in the state of obese asthmatic condition was evaluated using respiratory and biochemical parameters such as serum leptin, ghrelin, and TNF-α. The results are represented by the graphical presentation.

Effect on airflow rate

Airflow rate was measured as respiratory parameters. It was observed that airflow rate was significantly increased in leptin analog treated an obese asthmatic animal (OB-AS-L), salbutamol treated an obese asthmatic animal (OB-AS-S), and leptin analog with salbutamol treated an obese asthmatic animal (OB-AS-L-S) when compared to an obese asthmatic animal (OB-AS) (Group I, *$p<0.05$). Airflow rate was also elevated in leptin analog with salbutamol treated obese asthmatic animals (OB-AS-L-S) when compared to leptin analog treated an obese asthmatic animal (OB-AS-L) (Group II, @ $p<0.05$) (Fig. 1).

Effect of serum leptin level

Fourteen days of administration of the drug treatment in obese asthmatic animals revealed a significant increase in serum leptin level in leptin analog treated obese asthmatic animal (OB-AS-L), and leptin analog with salbutamol treated an obese asthmatic animal (OB-AS-L-S) when compared to an obese asthmatic animal (OB-AS) (Group I, *$p<0.05$). No significant change observed in salbutamol treated an obese asthmatic animal (OB-AS-S). It was also found that elevated serum leptin level was observed in leptin analog with salbutamol treated obese asthmatic animals (OB-AS-L-S) when compared to leptin analog treated an obese asthmatic animal (OB-AS-L) (Group II, @ $p<0.05$) (Fig. 1).

Effect of serum ghrelin level

It was observed that serum ghrelin level was significantly decreased in leptin analog treated an obese asthmatic animal (OB-AS-L), salbutamol treated an obese asthmatic animal (OB-AS-S), and leptin analog with salbutamol treated an obese asthmatic animal (OB-AS-L-S) when compared to an obese asthmatic animal (OB-AS) (Group I, *$p<0.05$). It was also found that significant reduction in ghrelin level in leptin analog with salbutamol treated obese asthmatic animals (OB-AS-L-S) when compared to leptin analog treated an obese asthmatic animal (OB-AS-L) (Group II, $p<0.05$) (Fig. 1).

Effect of serum TNF-α level

TNF-α level was significantly decreased in leptin analog treated an obese asthmatic animal (OB-AS-L), salbutamol treated an obese asthmatic animal (OB-AS-S), and leptin analog with salbutamol treated an obese asthmatic animal (OB-AS-L-S) when compared to an obese asthmatic animal (OB-AS) (Group I, *$p<0.05$). It was also observed that significant reduction in TNF-α level in leptin analog with salbutamol treated obese asthmatic animals (OB-AS-L-S) when compared to leptin analog treated an obese asthmatic animal (OB-AS-L) (Group II, $p<0.05$) (Fig. 1).

DISCUSSION

Obesity is a nutritional disorder with inflammation and energy imbalance, occurring when calorie expenditure is less compared to high caloric food intake [29]. Obesity is mostly associated with abnormal physiological action of leptin [30,31]. Asthma symptoms such as dyspnea and wheezing appear as a result of an excess of thoracic and abdominal fat deposition [32]. Despite complex etiological factors for both conditions, leptin resistance was found to be one of the cause of asthmatic symptoms in obesity [33]. In the present study, we evaluated the effect of leptin analog with salbutamol to treat an obese asthmatic condition.

Hypercaloric diet has been used as a model of obesity induction in animals as its similarity with metabolic responses caused by obesity in humans [34]. It is reported that a hypercaloric diet induces significant body weight gain, adiposity, elevated serum triglycerides, and leptin [35]. Previously, it was reported that systemic leptin sensitivity was reduced after the 8th week of hypercaloric diet [36]. Hypercaloric diet consists of long-chain saturated fatty acids which are harmful lipids related to the accumulation of adipose content. These lipids bind to the toll-like receptor (TLR2 and TLR4) of microglia (cells that protect the hypothalamus) ultimately provokes the formation of inflammatory cytokines (TNF-α, interleukin [IL]-1β, and IL-6). These would consequently cause destroy the neurons for appetite homeostasis [37].

The ability of circulating adipokines, which augmented due to hypercaloric feeding, modify lung health is possible for the development of systemic inflammation. It was previously reported that the effect of hypocaloric diet-induced an alteration in respiratory airflow rate indicated breathing abnormality. This suggests that obesity may alter the condition of asthma or makes existing asthma more severe [38].

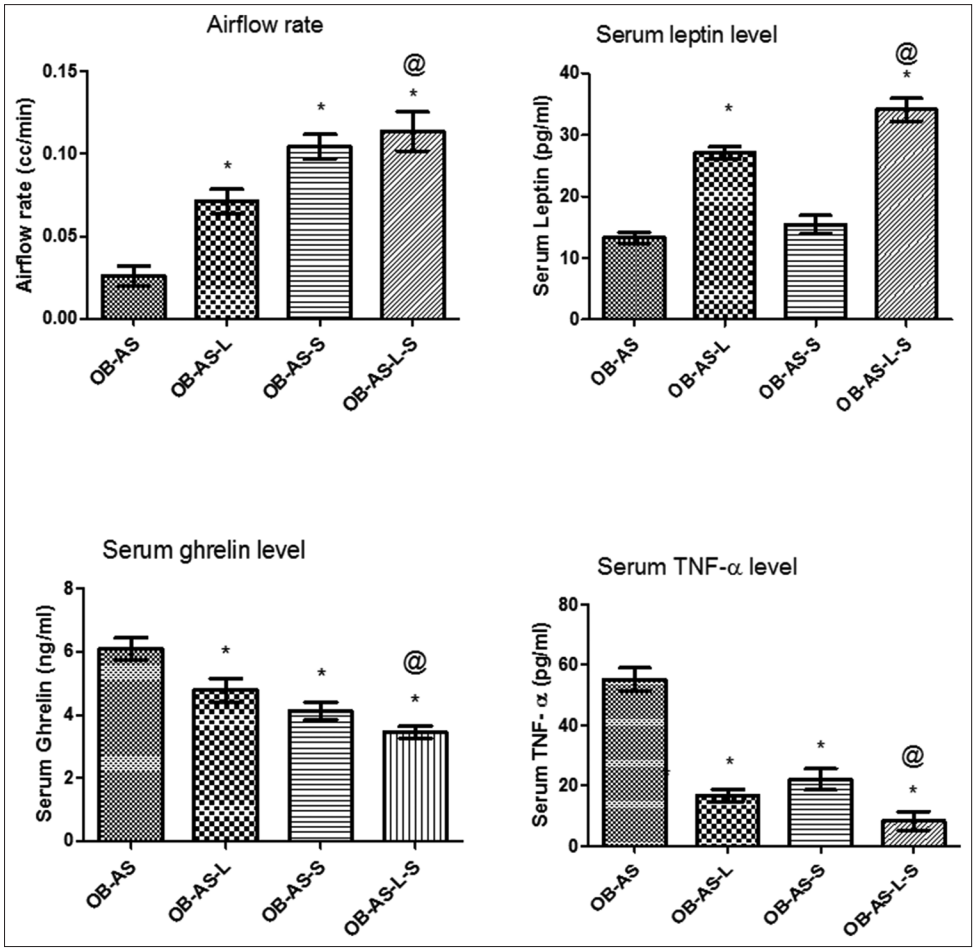

Fig. 1: Effect of leptin analog and salbutamol on serum leptin, serum ghrelin, serum tumor necrosis factor- α, and airflow rate. *p<0.05 when compared with OB-AS. @p<0.05 when compared with OB-AS-L

Leptin, an adipokine hormone, inhibits food intake and increase energy expenditure by central action on hypothalamus [39,40] while ghrelin reported to be involved in increasing food intake [41]. Leptin effect is, therefore, antagonistic to the ghrelin effect. Previous studies reported that serum leptin level and ghrelin level were elevated in obesity [42]. These both hormones action is due to their role of regulating energy homeostasis through the changes in neuropeptide Y secretion [43].

Previously, it was reported that leptin resistance was accompanied by increased in serum ghrelin and serum leptin levels [20,42,33]. Clinical and preclinical studies suggested that obesity-induced raise in leptin level and leptin resistance would be responsible for worsening asthma symptoms [33].

Obesity is an inflammatory condition characterized by increased production of inflammatory cytokines [44]. Infiltration of macrophages in adipose tissue is a major cause for the release of TNF-α [45]. Consistent with previous finding, it is found that elevated TNF-α level was responsible factor for leptin resistance in asthma with obesity [46-47]. Furthermore, respiratory parameters were also improved with leptin analog (OB-AS-L), salbutamol (OB-AS-S), and leptin analog with salbutamol (OB-AS-L-S) treated obese asthmatic animals.

Salbutamol and orlistat are most preferable treatment approach for asthma and obesity, respectively. In this study, we investigated the effect of salbutamol with leptin analog in obesity-induced asthma in animals. For that, obese asthmatic animals were treated with leptin (OB-AS-L), salbutamol (OB-AS-S), and leptin with salbutamol (OB-AS-L-S). It was observed that serum leptin level significantly elevated in obese asthmatic animals treated with leptin analog (OB-AS-L) and treated with leptin analog with salbutamol (OB-AS-L-S) when compared to obese asthmatic animals (OB-AS). It was also observed that significantly raised serum leptin level in obese asthmatic animals treated with leptin analog with salbutamol (OB-AS-L-S) when compared to obese asthmatic animals treated with leptin analog (OB-AS-L). Thus, we may suggest that salbutamol produce synergistic action with leptin analog. No significant change was seen in obese asthmatic animals treated with salbutamol (OB-AS-S) when compared to obese asthmatic animals (OB-AS).

Present investigation revealed that there was an improvement in leptin resistance by increasing leptin and decreasing ghrelin which means decreased food intake with increased fat metabolism. It was observed that serum TNF-α level significantly reduced in obese asthmatic animals treated with leptin (OB-AS-L), salbutamol (OB-AS-S), and leptin analog with salbutamol (OB-AS-L-S) when compared to obese asthmatic animals (OB-AS).

Thus, investigation of the effect of leptin analog with salbutamol revealed that leptin analog with salbutamol was more effective in improving the state of obese asthmatic condition compared to alone leptin analog. Hence, we may suggest that leptin analog with salbutamol might be an effective treatment approach for the obese asthmatic condition.

CONCLUSION

From our study, we conclude that leptin analog with salbutamol would be treatment approach for this comorbid condition and could be improving the state of leptin resistance. However, further studies are needed to determine the clinical efficacy of leptin analogs with salbutamol inpatient of asthma associated obesity.

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AUTHORS' CONTRIBUTIONS

Arun K. Soni has provided design, innovations, performed the experiment in the laboratory, preparation of manuscript and analysis of obtained data. Shrikalp S. Deshpande has provided intellectual content along with mentorship and also guarantor for genuinely work done.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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