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.

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 and 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 (CPSEA) 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)**

(Obesic 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 (ELM-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 mice**

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) 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) (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), 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). 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-S) (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-S) (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 hypercaloric 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].
Leptin, an adipokine hormone, inhibits food intake and increases energy expenditure by central action on the hypothalamus [39,40] while ghrelin is 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 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 was 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 (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 in patients 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 guarantee for genuinely work done.

CONFLICTS OF INTEREST

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

REFERENCES

1. DiPro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy. In: A Pathophysiologic Approach. 3rd ed. United Kingdom: McGraw Hill Professional; 2011.

2. Farooqi IS, O’Rahilly S. Genetics of obesity in humans. Ender Rev 2006;27:710-8.

3. Palatty PL, Saldanha E. Pharmacotherapy for weight management. J Assoc Phys India 2012;60:34-40.

4. Bollapragada MK, Shantaram M, Sunil KR. Obesity: Development, epidemiology, factors affecting, quantity, health hazards, management and natural treatment-a review. Int J Pharm Sci Res 2015;5:9-12.6.

5. Yun JW. Possible anti-obesity therapeutics from nature a review. Phytochemistry 2010;71:1625-41.

6. Anitha M, Roselin EM, Monisha DM, Karthik JC. Prevalence of obesity and overweight among medics in both male and female students. Asian J Pharm Clin Res 2016;26:289-91.

7. Wood LG, Gibson PG. Dietary factors lead to innate immune activation in asthma. Pharmacol Ther 2009;123:37-53.

8. Jeffery RW, Epstein LH, Wilson GT, Drewnowski A, Stunkard AJ, Wing RR. Long-term maintenance of weight loss: Current status. Health Psycho 2000;19:5.

9. Boulet LP. Asthma and obesity. Clin Exp Allergy 2013;43:8-21.

10. Walker BR, Colledge NR. Davidson’s principles and practice of medicine. In: With Student Consult Online Access. United Kingdom: Elsevier Health Sciences; 2013.

11. Bousquet J, Mantzouranis E, Cruz AA, Ait-Khaled N, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol 2012;129:626-38.

12. Lottvall J, Akdis CA, Bacharier LB, Bjermar L, Casale TB, Custovic A, et al. Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol 2011;127:355-60.

13. Agache I, Akdis C, Jutel M, Vichrov JC. Untangling asthma phenotypes and endotypes. Allergy 2012;67:835-46.

14. Farzan S. The asthma phenotype in the obese: Distinct or otherwise? J Allergy 2013;2013:11-14.

15. Soni AK, Deshpande SS, Suhagia BN. Gender specific correlation between obesity and asthma. Int J Pharm Res Scholar 2018;6:46-51.

16. Bateman ED, Hurrd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Res J 2008;31:143-78.

17. Soni AK, Deshpande SS. A novel hypothesis for pathophysiology of asthma in obesity. Int J Pharm Res Scholar 2017;6:612-8.

18. Baffi CW, Winnica DE, Holguin F. Asthma and obesity: Mechanisms and clinical implications. Asthma Res Pract 2015;1:1.

19. Uchida A, Zechner JF, Mani BK, Park WM, Aguirre V, Kritechevsky SB, Simonick EM, et al. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. Arch Intern Med 2005;165:777-83.

20. Arteaga-Solis E, Zee T, Emala CW, Vinson C, Wess J, Karsenty G. Inhibition of leptin regulation of parasympathetic signaling as a cause of extreme body weight-associated asthma. Cell Metab 2013;17:35-48.

21. Wasim M. Role of leptin in obesity. J Obes Weight Loss Ther 2015;5:2.

22. Myers MG Jr., Leibel RL, Seeley RJ, Schwartz MW. Obesity and leptin resistance: Distinguishing cause from effect. Trends Endocrinol Metab 2010;21:164-51.

23. Fadnavis BH, Krishnaswami S, Harris TB, Katsiaras A, Kritechevsky SB, Simonick EM, et al. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. Arch Intern Med 2005;165:777-83.

24. Arteaga-Solis E, Zee T, Emala CW, Vinson C, Wess J, Karsenty G. Inhibition of leptin regulation of parasympathetic signaling as a cause of extreme body weight-associated asthma. Cell Metab 2013;17:35-48.

25. Canöz M, Erdenen F, Uzun H, Müdderisoglu C, Aydin S. The relationship of inflammatory cytokines with asthma and obesity. Clin Invest Med 2008;31:373.

26. Martin SS, Qasim A, Reilly MP. Leptin resistance: A possible interface of inflammation and metabolism in obesity-related cardiovascular disease. J Am Coll Cardiol 2008;52:1201-10.

27. Harris RB, Zhou J, Redmond SM Jr., Smagin GN, Smith SR, Rodgers E, et al. A leptin dose-response study in obese (ob/ob) and lean (+/+7) mice. Endocrinology 1998;139:8-19.

28. Jadhav AD, Padwal SL, Jadhav RR, Jadhav SS, Pise HN, Chouke MR. Anti-inflammatory property of salbutamol on acute and chronic models of inflammation. Natl J Physiol Pharmacol 2015;3:101.

29. Van Herpen NA, Schrauwen-Hinderling VB. Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol Behav 2008;94:231-41.

30. Buettner NR, Newgard CB, Rhodes CJ, O’Doherty RM. Correction of diet-induced hyperglycemia, hyperinsulinemia, and skeletal muscle insulin resistance by moderate hyperleptinemia. Am J Physiol Endocrinol Metab 2000;279:E563-9.

31. Zhou X, De Schepper J, De Craemer D, Delhaye M, Gys G, Smits J, et al. Pituitary growth hormone release and gene expression in cafeteria-diet-induced obese rats. J Endocrinol 1998;159:165-72.

32. Ali Z, Ulrik CS. Obesity and asthma: A coincidence or a causal relationship? A systematic review. Respir Med 2013;107:1287-300.

33. Khan AR, Awad FR. Leptin resistance: A possible interface between obesity and pulmonary-related disorders. Int J Endocrinol Metabol 2016;14:e32586.

34. Rosini TC, da Silva AS, de Moraes C. Diet-induced obesity: Rodent model for the study of obesity-related disorders. Rev Assoc Méd Bras 2012;58:383-7.

35. Estadella D, Oyama LM, Dâmaso AR, Ribeiro EB, Do Nascimento CM. Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats. Nutrition 2004;20:218-4.

36. Lin S, Thomay DC, Sorellin LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6j mice. J Obes 2000;24:639.

37. Milanski M, Degnaperi G, Coope A, Morari J, Denis R, Cintra DE, et al. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamic Implications for the pathogenesis of obesity. J Neurosci 2009;29:359-70.

38. Robinson AM, Williamson DH. Comparison of glucose metabolism in the lactating mammary gland of the rat in vivo and in vitro. Effects of starvation, prolactin or insulin deficiency. Biochem J 1977;164:153-9.

39. Traylor P, Bing C. Appetite and energy balance signals from adipocytes. Philosophical transactions of the royal society. Biol Sci 2006;361:1237-49.

40. Traylor P, Bing C, Wood IS. Adipose tissue and adipokines energy regulation from the human perspective. J Nutr 2006;136:1935S-9.

41. Wasmim M. Role of leptin in obesity. J Obes Weight Loss Ther 2015;5:2.

42. Rosicka M, Krsek M, Matoulek M, Jankovská Z, Marek J, Justova V, et al. Serum ghrelin levels in obese patients: The relationship to serum leptin levels and soluble leptin receptors levels. Physiol Res 2003;52:61-6.

43. Afrah-Nazar AM, Muftedijil E, Suhary EA, Leptin and adiponectin levels of astmatic children in hilla province. Asian J Pharm Clin Res 2017;10:431-4.

44. Shoelson SE, Lee J, Yuan M. Inflammation and the IKKβ/IκB/NF-κB axis in obesity-and diet-induced insulin resistance. Int J Obes 2003;27:2849.

45. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796-808.

46. Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet DJ, et al. Multiple cytokines and acute inflammation raise mouse leptin levels: Potential role in inflammatory anorexia. J Exp Med 1997;185:171-6.

47. Mantzoros CS, Moschos S, Avramopoulos I, Kaklamani V, Loliou V, Doulgerakis DE, et al. Leptin concentrations in relation to body mass index and the tumor necrosis factor-alpha system in humans. J Clin Endocrinol Metab 1997;82:4018-27.