Increased Endothelin-1 Lung Level in Obesity Mice

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Abstract. Obesity is also strongly associated with various respiratory diseases, such as obstructive sleep apnea syndrome (OSAS), obesity hypoventilation syndrome (OHS), chronic obstructive pulmonary disease (COPD), asthma, and aspiration pneumonia. In other hand, Endothelin-1 (ET-1), a peptide chain which is the most potent vasoconstriction in human body, participates in various pathology mechanisms of respiratory disease at a certain level, but relation between them is less known. This study intends to measure the lung ET-1 levels in obese wistar rats. We used 10 male wistar rats (Ratus Norvegicus), three months in aged, divided in two groups, nonobese group and obese group which were fed with high fat and carbohydrate diet for 21 weeks. The rohrer index was evaluated twice a week to control development of obesity. Lung endothelin-1 from the two groups were assessed using Enzyme-Linked Immunosorbent Assay (ELISA). The data were then compared using independent T test. The results exhibit the significant enhancement of the endothelin-1 lung levels in the obese male rats compared with the lean male rats (p = 0.005). As a conclusion, it is clear that the levels of ET-1 lung in obese rats increase significantly. The further study is needed to find out the role of ET-1 in lung diseases-related obesity.

1. Introduction

Based on data from the World Health Organization[1] released in 2017, there has been an almost three-fold increase in the number of obese people since 1975. This fact is a worrying condition because it will also increase comorbid diseases from obesity, such as metabolic diseases[2], cardiovascular[3], and cerebrovascular[4]. Obesity is also strongly associated with various respiratory diseases, such as obstructive sleep apnea syndrome (OSAS), obesity hypoventilation syndrome (OHS), chronic obstructive pulmonary disease (COPD), asthma, and aspiration pneumonia[5].

Respiratory disorders in obese conditions can be caused by several mechanisms. The first, mechanically obesity will reduce pulmonary function, with decreased ability of chest wall compliance and increased respiratory tract resistance[6]. Second, obesity is a chronic inflammatory condition, where adipocyte cells will secrete various inflammatory mediators such as leptin and decreased anti-inflammatory mediators such as adinopectin[7] which play a role in lung inflammatory diseases such as asthma and COPD[8].

In addition, various compounds are also thought to be involved in contributing to respiratory disorders, such as TNF-α[9], interleukin-13[10], T lymphocytes[11], and a strong vasokonstor peptide, endothelin-1[12]. ET-1 is mainly produced by vascular endothelial cells and works through two
receptors namely endothelin A receptors (ETA) and endothelin B receptors (ETB) which are dispersed in almost all organs of the body, including in the lung[13]. ET-1 plays a role in several physiological processes of the body, such as regulation of vascular tone and bronchial tone in the lung[14]. However, in conditions of damage to the effects of vasodilation ET-1 as in active smokers, ET-1 also participates in various pathological mechanisms of the respiratory system by disrupting the vascular system tone system and lung immune system[15].

Weil et al (2011)[16] found an increase in ET-1 activity in arm blood vessels, but data related to ET-1 activity in the lung in obese conditions is still very limited. We suspect, there is involvement of ET-1 in the pathogenesis of obesity-related pulmonary diseases. Therefore, this study aims to look at lung ET-1 levels in obese mice.

2. Method

2.1 Ethical permission
This research has received ethical permission from the Ethics Committee of the Faculty of Medicine, Hasanuddin University with number: 234/H4.8.4.5.31/PP36-KOMITE/2018.

2.2 Handling of Try Animals
A total of 10 male Wistar rats aged 3 weeks were weaned from their mother and adapted for 1 week. The ten rats were then grouped into 2 groups, namely the non-obese group given AD2 feed as much as 15 mg/day and the obese group given CP551 15 mg/day and instant Dancow milk as much as 9 gr which was dissolved into 50 cc of water/day. At the age of 13 weeks, the amount of CP551 feed given was increased by 20 mg/day. Treatment is given for 21 weeks. Assessment of obesity is done by calculating the Rohrer index of each mouse.

2.3 Retrieval and Storage Sample
Sampling was done after lung was given 21 weeks of treatment. The rats were terminated and removed the blood. Mice are then dissected and removed all tissues including the lungs. The lungs were then crushed and then centrifuged at 3000 rpm for 15 minutes. The Sample is stored in a refrigerator with a temperature of -20 o C.

2.4 Examination of Pulmonary Endothelin-1 Levels and Statistical Analysis
Lung ET-1 examination was carried out by the enzyme-linked immunosorbent assay (ELISA) method at the Educational Hospital Laboratory by following the MyBiosource protocol. The data obtained were analyzed using SPSS. To see the significance of the changes in pulmonary ET-1 levels in obese mice compared to non-mice obesity is carried out by independent T-test.

3. Results

3.1 Characteristics of Experimental Animals
After administration of the CP551 diet and instant fortigrow dancow milk showed a greater increase in body weight and body length compared to the AD2 diet (Table 1).
Table 1. Characteristics of Try Animals

|                      | Non-Obesity | Obesity    | $P$  |
|----------------------|-------------|------------|------|
| W (gram ± SD)        | 213 ± 40.31 | 441 ± 25.56| 0.0001|
| L (cm ± SD)          | 20.1 ± 0.74 | 22.6 ± 0.54| 0.0001|
| Rohrer index (gr/cm$^2$) | 26.83 ± 1.8 | 42.07 ± 2.19| 0.0001|

$p = \text{independent sample t test}$

W = Body Weight, L = Body Length.

3.2 Serum ET-1 Levels

After giving the diet in both groups of rats, pulmonary ET-1 levels in obese group rats showed a higher value compared to the group of non-obese rats which were statistically significant (Table 2).

Table 2. Level of ET-1 Lung

|                      | Non Obesity Group | Obesity group | $P$ value |
|----------------------|-------------------|---------------|-----------|
| Lung ET-1 (pg/ml)    | 45.39 (± 7.53)    | 83 (± 20.47)  | 0.005     |

$p = \text{independent sample t test}$

4. Discussion

This study aims to look at the levels of ET-1 lung in obese mice compared to non-obese mice. This study showed an increase in pulmonary ET-1 levels almost doubled ($p < 0.05$) in obese induced mice (83 ± 20.47) compared to non-obese mice (45 ± 7.53). This is in line with a study conducted by Sylvia Del Ry et al.[17] who found that there was an increase in preproendothelin-1 expression in lung tissue of obese mice. Although it has yet to be definitely known, there are several suspected mechanisms underlying the increased levels of ET-1 lung in mice obese, such as an increase in the activity of the inflammation[18] and renin angiotensin aldosterone[19] and increased production of reactive oxygen species (ROS)[20].

Elevated levels of ET-1 in obese lung mice reinforce the researchers' expectation that ET-1 is involved in various lung diseases associated with obesity. This is possibly based on various pathological effects that can be associated with an increase in ET-1 levels shown in several studies. The prevalence of pulmonary arterial hypertension (PAH) in the obese population is higher than the non-obese population[21], which is characterized by increased pulmonary arterial pressure ≥ 25 mmHg at rest[22]. Recent studies reveal the important role of ET-1 in the pathomechanism of PAH disease [23][24], in which ET-1 plays a role in increasing pulmonary artery resistance[25]. This raises the hypothesis that ET-1 is involved in increasing the prevalence of PAH in obesity.

Obesity is known to increase the prevalence of various lung inflammatory diseases such as asthma and COPD. Production imbalances between leptin and adiponectin are thought to underlie this[8]. ET-1 is also involved in the pathogenesis of asthma and COPD through two mechanisms. The first is the bronchoconstriction effect of ET-1 and causes bronchial hypersensitivity to stimulation. Secondly, ET-1 induces overexpression of various inflammatory cells, cytokines, bronchial edema, and remodeling[8]. ET-1 was also found to increase in sputum of exacerbated COPD patients which showed a possible role of ET-1 in inflammatory changes in COPD[25]. Elevated levels of ET-1 in obese mice show a possible role of ET-1 in various lung inflammatory diseases in obesity.
5. **Research Limitation**

There are some limitations to this research.

1. We did not examine ET-1 receptors, either ETA or ETB, so that we were unable to see which receptors were the dominant activity in the lungs of obese mice.
2. We examine lung tissue without separating between the bronchi, vascular, elastin tissue, and alveoli. So that we cannot know, which network is the most dominant increase in ET-1 levels.

6. **Conclusion**

Rats suffering from obesity had higher lung ET-1 levels than lung non-obese mice. The high levels of ET-1 confirmed the alleged involvement of ET-1 in various lung diseases related to obesity, but further research is needed to confirm this.

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