Effect of Pneumothorax Duration on Oxidative Stress Level of Wistar Rat’s Lung

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ABSTRACT

Introduction. Extensive pneumothorax with a long duration of symptoms can be dangerous. Broad pneumothorax can cause hypoxemia which can cause increased production of free radicals in the lung tissue (pulmonary oxidative stress). Oxidative stress in the lungs can indicate the degree of damage or disruption of cells in the lungs due to hypoxia.

Methods. There were 4 groups each consisting of 6 mice: 24-hour pneumothoracic group (A), 48-hour pneumothoracic (B), 72-hour pneumothoracic (C) and control (D). Pneumothorax is made by air injection into the right chest pleural cavity, then on an X-ray. All samples were examined for PaO2 to ascertain their level of hypotension. After that, the rats were examined for their lungs malondialdehyde (MDA) levels to find out their stress oxidation stress levels.

Results. All groups of pneumothoracic mice experienced hypoxemia. The average MDA level was higher in the pneumothoracic group, but it was statistically significant only in the 72 hour (C) group with p = 0.031. MDA levels in each group were 1.601 ± 0.739 (A), 1.585 ± 0.714 (B), 2.256 ± 0.513 (C), 1.243 ± 0.162 (D).

Conclusion. Pneumothorax can cause pulmonary oxidative stress if the pneumothorax is large in volume and the duration of symptoms reaches 72 hours. MDA can be a marker of cell disruption or injury in cases of pneumothorax who have experienced hypoxemia.

Kata kunci: pneumothorax duration, stress oxidative, wistar rat lung.
Introduction

Pneumothorax is an emergency in the field of surgery that must be treated immediately. In developing countries, pneumothorax still occurs even though the numbers are relatively small. The incidence of spontaneous pneumothorax is 14.3 per 100,000 population per year.\textsuperscript{1} Traumatic pneumothorax occurs in 10-30\% of cases of blunt thoracic trauma and 95\% of cases of sharp trauma of thorax.\textsuperscript{2,3} In Indonesia, the incidence of pneumothorax is quite a lot and has a number high mortality. In Cipto Mangunkusumo Hospital (RSCM) from 2000-2011 there were 215 pneumothoracic patients. At the RSCM, primary spontaneous pneumothorax occurred as much as 25\%, secondary spontaneous pneumothoracic 47.1\%, traumatic pneumothoric 13.5\% and tension pneumothoric 14.4\%. The pneumothorax mortality rate is also high, as many as 33.7\% with the most causes of death are respiratory failure (45.8\%).\textsuperscript{4} Pneumothorax can be life threatening as in the tension pneumothorax condition. However, in conditions of pneumothorax with extensive volume and long duration of symptoms can also be dangerous. Large pneumothorax can cause lung pressure or collapse, causing gas exchange disruption. This results in a decrease in partial pressure of arterial oxygen (PaO\textsubscript{2}) or hypoxemia. The pathophysiological process involves a decrease in the vital lung capacity, the occurrence of intrapulmonary shunting, a decrease in the perfusion ratio of pulmonary alveoli and alveolar hypoventilation.\textsuperscript{5} Most patients with extensive pneumothorax have decreased arterial PaO\textsubscript{2} and increased differences in alveolar-arterial oxygen pressure. There is a study of 12 patients with spontaneous pneumothorax, found that arterial PaO\textsubscript{2} is below 80 mmHg (hypoxemia) in 9 patients (75\%) and below 55 mmHg in 2 patients.\textsuperscript{6} Hypoxemia can occur in pneumothorax when the area is more than 25\%. In secondary spontaneous pneumothorax hypoxemia is even easier to occur even if the area of the pneumothorax is less than 25\% due to the presence of an underlying lung disease. This long duration can affect changes in tissue conditions in the lungs. Experimental animal studies suggest that alveolar capillary membrane changes which increase its permeability in pneumothoracs with lung collapse for 72 hours, facilitating the transfer of fluid into the alveolar space (pulmonary edema).\textsuperscript{7} This mechanism is due to pulmonary hypoxia. Other animal experimental studies also say that long duration of pneumothorax is at risk of causing pulmonary edema and inflammation.

Extensive pneumothorax and long duration can be risk factors for pulmonary edema expansion (PEE). PEE can occur in large-volume pneumothoracs with a duration of symptoms that reaches more than 3 days.\textsuperscript{9} The incidence of PEE in spontaneous pneumothoracs varies,
according to existing reports including 15.6%, 16%, 29.8% and 32.5%.¹⁰⁻¹³ This figure is relatively small, but PEE mortality can reach 20%.¹⁴ This pathology is caused by rapid lung expansion and changes in lung tissue conditions due to hypoxia. Rapid lung expansion causes rapid changes in intrathoracic pressure resulting in an increase in pulmonary capillary and hydrostatic pressure. This condition is strengthened by lung conditions that have undergone regional lung tissue hypoxemia that cause cell migration and inflammatory mediators, as well as alveolar capillary permeability changes.⁹⁻¹⁵ Meanwhile, there are experimental hypoxic studies that say that hypoxia can cause increased production of free radicals or reactive oxygen species (ROS) are endogenous in lung cells (oxidative stress). The research was conducted by reducing the oxygen content inhaled. The production of ROS or excessive free radicals can also cause injury or disturbance to the alveolar epithelial cells, pulmonary vascular smooth muscle, inflammation and pulmonary edema.¹⁶⁻¹⁸ Oxidative stress can be measured by its biomarkers namely malondialdehyde (MDA).¹⁹,²⁰ Oxidative stress in the lungs can express the level of damage or disruption of cells in the lungs due to hypoxia. In pneumothorax hypoxia and hypoxemia can also occur due to lung collapse due to compressed air. The duration of the pneumothorax is also related to the duration of the hypotension. Not yet known how lung oxidative stress due to hypoxia caused by pneumothorax. Pneumothorax is likely to cause pulmonary oxidative stress due to hypoxia in the pneumothorax and it appears that the duration of the pneumothorax affects the level of oxidative stress. This study aims to determine the effect of pneumothorax duration on pulmonary oxidative stress levels, with experiments in rats.

**Methods**

This research was an in vivo experimental study with a post test only control group design. The subjects of the study were all white wistar rats that met the inclusion and exclusion criteria. The inclusion criteria were white rats (Rattus norvegicus) male wistar strain, 200-250 gram body weight, 2-3 months old, healthy and active during the adaptation period. The exclusion criteria were not proven pneumothorax after being X-rayed in pneumothorax-made rats, the existence of anatomical abnormalities and had been tested before. Retrieval of 24 research subjects was done by random allocation. When randomizing the sample groupings. All rats are numbered from 1 to 24. This number is drawn and each mouse has the same opportunity to be included in each group. The first collection was included in group 1, the second collection was included in group 2, and so on until all the mice were evenly divided.
into 4 groups (Group A-D: pneumothorax mice 24, 48, 72 hours and control).

Before pneumothorax is made, animals are anesthetized with injection of 90 mg / kgBB ketamine and intraperitoneal xylazine 10 mg / kgBB. The technique for making pneumothoraces is by injecting air into the pleural cavity. (8) (44) After shaving clean the right chest wall of the rat, an incision is made ± 0.5-1 cm in the fourth intercostal then the incision is deepened until the ribs and intercostal muscles appear. 6cc of air is injected into the pleural cavity with a syringe or abbcath which is inserted as deep as ± 0.5 cm and above the ribs in the fourth intercostal space. After that, an X-ray is done to prove pneumothorax. PaO₂ examination uses arterial blood samples taken directly from the heart with a syringe. The AGD examination results come out within 2 minutes with this i-STAT tool. The normal value of PaO₂ rat is not much different from humans. The normal value of PaO₂ rats ranges from 80.72-109.56 mmHg.

An incision is made in the midsternalis until the lung is exposed (sternotomy). Open the sternum bones of animals try carefully, not to damage lung tissue. Evacuate the right lungs of experimental animals, then the lungs are washed with PBS 1% until the washing liquid is clear. PBS 1% with pulmonary organs in centrifuge 3000 rpm, for 20 minutes, precipitates and supernatants were obtained. Supernatants are taken for examination of malondialdehyde (MDA) levels. MDA examination using ELISA technique uses the Rat MDA ELISA Kit (CloudClone) according to the work protocol of the procedure manual.

Data analysis using SPSS software for windows 25.00. Characteristics of data in the value of normality and homogeneity. The data normality test is done by the Shapiro-Wilk test because the number of samples is hom 50. The homogeneity test of the data is carried out with the Levene test. Data shows normal and homogeneous distribution if p> 0.05. After it is determined that the data distribution is normal and the data variance is the same, then the one-way ANOVA test can be performed. To find out which groups have different meanings, a post-hoc test is performed.

**Results**

After an x-ray examination, the rats then continued to PaO₂ examination. Control group was directly examined for the PaO₂ value, while the pneumothorax rat was examined for PaO₂ levels after reaching the expected time (24 hours, 48 hours and 72 hours). The results of PaO₂
are shown in table 1. It can be seen that the control group has an average value of 88.16 mmHg (normal) and the pneumothorax group has a low PaO₂ value (hypoxemia). Group A pneumothorax rats had an average PaO₂ of 47.16 mmHg, group B averaged 56.16 mmHg and group C averaged 50.5 mmHg.

| Variable | Pneumothorax group 24 hours | Pneumothorax group 48 hours | Pneumothorax group 72 hours | Control Group |
|----------|-----------------------------|-----------------------------|-----------------------------|---------------|
|          | (A)                         | (B)                         | (C)                         | (D)           |
| PaO₂ Level | 47.16 ± 1.01                | 56.16 ± 1.47                | 50.5 ± 8.16                 | 88.16 ± 2.92  |

There was an increase in MDA levels in the pneumothorax group as seen in table 2. The pneumothorax group A had an average of 1.601 nmol / g, group B 1.505 nmol / g, group C 2.256 nmol / g, while group D (control) group averaged 1.243 nmol / g.

| Variable | Pneumothorax group 24 hours | Pneumothorax group 48 hours | Pneumothorax group 72 hours | Control Group |
|----------|-----------------------------|-----------------------------|-----------------------------|---------------|
|          | (A)                         | (B)                         | (C)                         | (D)           |
| MDA Level | 1.601 ± 0.739               | 1.585 ± 0.714               | 2.256 ± 0.513               | 1.243 ± 0.162 |

In statistical analysis with one-way ANOVA test, there were significant differences in MDA levels between study groups with p <0.05. At least there are at least two groups that differ statistically according to the ANOVA test. To find out which group of rats had a significant difference, the test was continued with post hoc analysis of the Tukey test. In the post hoc analysis as shown in table 4.4, it was found that group C (72 hour pneumothorax) had a significant difference to the other groups, p value = 0.031. Group A (24-hour pneumothorax) and group B (48-hour pneumothorax evidently based on the post hoc test found no significant differences with the control group.
Table 3. Comparison of MDA Level Among the Research Groups

|                      | A      | B      | C      | D      |
|----------------------|--------|--------|--------|--------|
| Pneumothorax 24 hours group (A) |        | 1,000  | 0,238  | 0,712  |
| Pneumothorax 48 hours group (B) | 1,000  |        | 0,220  | 0,740  |
| Pneumothorax 72 hours group (C) | 0,238  | 0,220  |        | 0,031  |
| Control group (D)    | 0,712  | 0,740  | 0,031  |        |

*Post Hoc Test, p = 0,05

Discussion

In this study there was an increase in MDA in the pneumothoracic mouse group. This increase in MDA indirectly indicates the level of cell damage caused by free radicals. MDA will increase in tissue that has lesions or disease and then released into the bloodstream. MDA can be examined from lesions or tissue from the blood. In this study, MDA was taken directly from the lungs in order to obtain more accurate results. MDA from the blood can be influenced by other factors such as systemic inflammatory conditions such as severe infections or sepsis.21

The increase in MDA in the sample was only statistically significant in the 72-hour pneumothorax group. This shows that MDA levels are also increasing in line with the duration of the pneumothorax. This seems to be related to the duration of hypoxia due to pneumothorax. In the research, it was said that the level of oxidative stress also depended on the severity and duration of the hypoxia.22 Most hypoxia studies were done by inserting animal samples into oxygen-deficient spaces in the form of hypoxia chamber or hypobaric chamber. There are studies that look at the level of pulmonary oxidation stress under normobaric hypoxia conditions (inhalation of 10% O2 levels), found that MDA increases on day 5.23 There are also studies that examine hypobaric hypoxia (pressure 338 mmHg) with exposure to varying hypoxic durations (2 hours, 4 hours, 8 hours, 16 hours, 24 hours and 48 hours), it was found that MDA increased in the hypoxia group duration of 16 hours, 24 hours and 48 hours. Even further it is said that short-term hypoxia exposure (2 - 4 hours) did not show a significant effect.
In our study, MDA increased statistically at 72 hours. The difference in the results of our study with the studies above is due to differences in how to make the hypoxic condition. However, it can be concluded that hypoxia can increase oxidative stress and its duration aggravates the stress level.

An increase in MDA in the duration of 72 hours (3 days) indicates oxidative stress due to hypoxia mediated by pneumothorax. This increase means that there has been an effect of free radicals or ROS in the lung tissue. For example, free radicals or ROS in the lungs can stimulate the production of inflammatory mediators, causing inflammation of the capillaries. This inflammation can increase vascular permeability and then pulmonary edema occurs. MDA which increases on the third day implies that there has been a change in lung tissue that facilitates pulmonary edema. On this basis, MDA may be a biomarker of cell disruption or injury in hypnemic cases. Need further research on this. This study provides a discourse on the importance of assessing the status of hypoxia or hypoxemia by examining AGD in extensive pneumothoracs and the symptoms have reached almost 72 hours. Anamnesis is how long symptoms of shortness that arise suddenly are needed to estimate how long the duration of the pneumothorax. The duration of the pneumothorax can be calculated from when the patient has a complaint of sudden tightness until the patient is treated or a radiological examination as evidence of pneumothorax. In the study, the duration of spontaneous pneumothorax symptoms before being diagnosed radiologically or treated was recorded at an average of 3.8 days. In other studies an average of 5.3 days was recorded. Suspicion was needed for spontaneous pneumothorax because in spontaneous pneumothorax complaints of sudden spasms arrived often considered another disease and the diagnosis was wrong, so the duration could reach more than one day. Extensive spontaneous pneumothorax (experiencing hypoxia) and history taking, the symptoms have reached almost 72 hours, it is worth watching out for the possibility of PEE during chest tube placement. MDA examination results that show high results may be useful to increase suspicion of the emergence of PEE. This is because a high MDA states there has been a change in lung tissue due to hypoxia which will facilitate PEE.
Conclusion

Pneumothorax can cause pulmonary oxidative stress if the pneumothorax is large in volume and the duration of symptoms reaches 72 hours. The mechanism is mediated by hypoxia or hypoxemia due to pneumothorax.

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