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The Effect of Vitamin C Giving on the Degree of Leucocyte Infiltration and Edema Alveolar in Increasing the Level of Arterial Oxygen in White Rats (Rattus Norvegicus) Having Pulmonary Contusion

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Abstract

Lung contusion is the most common cases diagnosed at intrathoracic injury resulting from blunt trauma. Lung contusion usually caused excessive reactive oxygen species production, a condition that will trigger lipid peroxidase that will cause damage to the cell membrane and cell lysis. Vitamin C as an antioxidant will act as a ROS scavenger to prevent lipid peroxidase. The aim of this research is to examine the effectiveness of vitamin C to increase the level of arterial blood oxygen and explain its pathomechanism. The research used experimental method conducted in the white rats (Rattus norvegicus). Study groups were divided into three groups, i.e.control group (CG, n=9), Lung contusion (LC, n=9), and pulmonary contusion given vitamin C (VC, n=9). For the experimental group, thoracic blunt trauma model was made by dropping the cylindrical iron load with the weight of (0.25 kg) on stainless steel pipe of the right hemithorax from the height of 80 cm (E = 1.96 J). For the VC group, the vitamin C of 200 mg/kg was injected 15 minutes after the intraperitoneal trauma. For 24 hours after the treatment, the examination of the blood gas analysis and pulmonary biopsy was conducted to find out oxygenation status and histopathological examination.

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The result of the analysis indicates that the pulmonary contusion given vitamin C has better arterial PaO2 value than the pulmonary group without being given vitamin C (p = 0,019). This is caused by the decrease of leucocyte infiltration degree (p = 0.011) and alveolar oedema (p = 0,050). Vitamin C has influence as an antioxidant towards changes in leukocyte infiltration rate, alveolar edema, and blood artery oxygen level at Sprague Dawley white rat (Rattus norvegicus) suffered from lung contusion.

Keywords: lung contusion; leucocyte infiltration; alveolar oedema; PaO2.

1. Introduction

Lung contusion is the most common case diagnosed in cases of intrathoracic injury due to blunt trauma, in the adult age the incidence range are 17% to 25%, Lung contusion is associated with a progressive inflammatory response mediated by the presence of both local and systemic immunological change [2]. generally, lung injury will cause the formation of reactive oxygen species (ROS) by lung endothelial cells. In order to avoid undesirable oxidative damage caused by ROS, each organism has an antioxidant mechanism [3,4]. The effects of oxidative stress on the pathogenesis of lung contusions will increase metabolism which produces free radicals such as hydrogen peroxide (H_2O_2), superoxide anions (O2), and radical hydroxyls (OH^-). These free radicals can be eliminated by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) [5]. Clinical trials and experiments in animals have shown that certain vitamins, such as vitamin C, are powerful radical scavenger and have antioxidant activity [3]. Until now, no standard therapy has been established for patients with lung contusion [6]. This study aims to test the effectiveness of giving vitamin C to laboratory rat to increase oxygen levels of arterial blood and explain its pathomechanism.

2. Materials and Method

2.1 Collection of Samples

This research was conducted using an experimental method using a post test design with a control group. This research was conducted in 3 laboratories namely the Animal Laboratory of the Faculty of Medicine, Hasanuddin University for experimental treatment, the Clinical Pathology Laboratory for examining blood gases and the Pathology laboratory of Anatomy of the hasanudin university Hospital for histopathological examination, which was conducted in April-May 2018. Inclusion criteria: Sprague Dawley white rats, male sex, healthy white rats, weight between 250-300 grams, white rats at the age of 3-4 months. Exclusion criteria: experimental animals that died during the research process, and animals that do not have pulmonary contusions at the time of treatment. Subjects obtained from the Animal Laboratory of the Medical Faculty of Hasanuddin University were 27 rats, divided into 3 groups, each group consisted of 9 rats.

2.2 Technique Used to Induce Pulmonary Contusion

laboratory animals were made to experience pulmonary contusion using the technique described by Raghavendran and his colleagues A cylindrical load (250 grams) is dropped from a certain height (80 cm) through a verticaltube positioned on the platform. This device is supported on all four sides to minimize friction

and enable energy transfer. Then the laboratory animals will be placed under the platform, so that the trauma which being produced will cause bilateral lung contusion.

2.3 Severity of Lung Contusion

Severity of lung contusion were classified according to: Alveolar edema: 0 = no pathological anomaly (<5%), 1 = mild (<10%), 2 = moderate (15-20%), 3 = severe (>20 %); and Leukocyte infiltration: 0 = no extravascular leukocyte, 1 ≤ 10 leukocyte/field of view, 2 = 10 – 45 leukocyte/field of view, 3 ≥ 45 leukocyte / field of view

2.4 Ethical Clearence

Ethical approval for this study was obtained from Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Number; 396/H4.8.4.5.31/PP36-KOSMETIK/2018.

3. Data Analysis

Collected data were classified according to data type, then appropriate statistical method were chosen, that is SPSS 20 to evaluate data distribution using *Mann – Whitney U test*. Confidence interval used was 95 % ($p < 0,05$), and then bivariate correlation test with spearman correlation and partial t test technique were used.

4. Results

Out of the 18 laboratory animals that were made to experience lung contusions, one mouse died in the first 24 hours. Dead rat will be excluded and will be replaced by other experimental animals that fit the inclusion criteria. Out of all the lab animals made to have contusions, all of them showed the same behavior changes, their breathing patterns became faster and became less active. After 24 hours, the experimental animal will be euthanized and blood samples taken along with its lung tissue. Macroscopically we found that the lung tissue in each of the lab animals was found to have lung contusions. Out of the 3 groups of lab animals (normal, pulmonary contusions and pulmonary + vitamin C), each consisting of 9 male white rats, variable data on arterial PaO2, lung tissue leukocyte infiltration and pulmonary tissue edema score were obtained. Descriptive analysis results on the three experimental animal models of white rats can be seen in table 1.

Table 1: Descriptive analysis rsults at three experimental animal models

Variable	Statistics	Model		
		Normal(n=9)	**CnP (n=9)**	**CnP +Vit.C(n=9)**
Arterial Pa O_2	Min/Maks	73/99	45/58	50/78
	Median	95,0	54,0	62,0
	Mean(SD)	91,8(8,6)	53,2(4,1)	61,4(8,4)
Leukocyte Infiltration	Min/Maks	1/3	50/75	38/70
	Median	2,0	60,0	49,0
	Mean(SD)	21,1(0,9)	61,7(7,5)	51,3(11,7)
Edema Score (%)	Min/Maks	0,0/2,0	30,0/50,0	20,0/30,0
	Median	0,00	30,00	30,00
	Mean(SD)	0,44(0,73)	35,56(7,26)	25,56(5,27)

CnP=Contusio Pulmonum; Vit C= Vitamin C

Arterial blood gas analysis was assessed 24 hours after pulmonary contusion. Blood samples were taken from the cardiac apex (left ventricle), then examined by the Clinical Pathology Laboratory hospital of Dr. Wahidin Sudirohusodo in less than 30 minutes. To assess the effect of giving vitamin C to arterial PaO2 in models of rats experiencing pulmonary contusion, an analysis of the differences in arterial PaO2 between contusions and contusio groups that was given vitamin C. At first it was seen that the difference in the value of PaO2 between the pulmonary contusions and the normal group, to assess the effect of pulmonary contusion on arterial PaO2. The results can be seen in table 2.

Table 2: Pa O_2 differences between normal rat, Lung Contusion rat, and Lung Contusion + vitamin C at experimental animal model *Rattus norvegicus* white rat

Variable	**Model**	**PaO_2 (mmHg)**		**P values***
		Mean(SD) /Median	**Median Differences**	
Lung Contusion	Normal (=9)	91,8±8,6/95,0	-41,0	<0,001
	CnP (n=9)	53,2±4,1/54,0		
Lung Contusion + Vitamin C	Normal (n=9)	91,8±8,6/95,0	-33,0	<0,001
	CnP+VitC(n=9)	61,4±8,4/62,0		
Vitamin C at Lung Contusion	KnP (n=9)	53,2±4,1/54,0	8,0	0,019
	CnP+VitC(n=9)	61,4±8,4/62,0		

*Man Whitney U test

To assess the effect of vitamin C on inflammatory status as a measured by leukocyte infiltration in animal pulmonary contusions models of white rats, an analysis of the difference between leukocyte infiltration was carried out Contusio groups by giving it a vitamin C. Initially assessing the difference in leukocyte infiltration between the pulmonary contusions group normally to assess the effect of pulmonary contusions on leukocyte infiltration. The results can be seen in table 3.

Table 3: Leukocyte infiltration differences between lung contusion group, normal group, and contusion + vitamin C group

Variable	**Model**	**Leukocye infiltration (%)**		**P Value***
		Mean(SD) /Median	**Differences**	
Lung contusion	Normal (n=9)	2,1±0,9 /2,0	**58,0**	**<0,001**
	CnP (n=9)	61,7±7,5 /60,0		
Lung contusion + Vitamin C	Normal (n=9)	2,1 ±0,9 /2,0	**47,0**	**<0,001**
	KnP+VitC (n=9)	51,3±11,7 /49,0		
Vitamin C at lung contusion	KnP (n=9)	61,7±7,5 /60,0	**-11,0**	**0,050**
	KnP+VitC (n=9)	51,3±11,7/49,0		

*Mann Whitney U test

To assess the effect of vitamin C on alveolar edema in pulmonary contusion models in rats an analysis of differences in alveolar edema between contusion groups and contusions that was given a Vitamin C. Previously, differences in normal alveolar edema between pulmonary contusion groups were assessed to assess the effect of pulmonary contusions on alveolar edema. The results can be seen in table 4.

Table 4: Alveolar edema differences between contusion group, normal group, and contusion + vitamin C group

		Alveolar edema score (%)		
		Mean(SD)	**Differences**	
Variable	**Kelompok**			**p Value**
Lung contusion	Normal (=9)	0,44±0,73/0,0	30,0	<0,001
	KnP (n=9)	35,5±7,26/30,0		
Lung contusion + Vitamin C	Normal (n=9)	0,44±0,73/0,0	30,0	<0,001
	KnP+VitC(n=9)	25,56±5,27/30,0		
Vitamin C at Lung contusion	KnP (n=9)	35,56±7,26/30,0	0,0	0,011
	KnP+VitC(n=9)	25,56±5,27/30,0		

*Mann Whitney U test

Scores of alveolar edema in groups II and III that experienced pulmonary contusion increased significantly compared with normal group I ($p < 0.05$) indicating that the incidence of pulmonary contusions would increase the score of alveolar edema. Early supplementation of vitamin C therapy resulting a decrease in alveolar edema scores after pulmonary contusions that was statistically significant ($p = 0.011$). From the results of the bivariate correlation test in table 5, it shows that group variables, arterial PaO2, leukocyte infiltration and edema scores are related to each other significantly ($p < 0.05$).

Table 5: Bivariate correlation study results (Spearman correlation) between group at white rat experimental animal model

	Bivariate correlation	
	R	**P**
Variable correlation		
Group x PaO_2	-0,854	<0,001
Group x Leukocyte	0,840	<0,001
Group x Edema Score	0,901	<0,001
Leukocyte x Edema Score	0,865	<0,001
Leukocyte x PaO_2	-0.929	<0,001
Edema score x PaO_2	-0,875	<0,001

5. Discussion

This study shows that pulmonary contusions that was given Vitamin C have a better arterial PaO2 values than pulmonary contusions without Vitamin C. the Current treatment options for pulmonary contusion are still limited, only include oxygen administration, close observation, and management to deal with complications, so that it is very important to look for a new strategies to prevent worsening and emerging complications [7]. In this study, the laboratory animals namely Sprague Dawley strain rat (Rattus norvegicus) were made to experience pulmonary contusion with a technique that was used by Raghavendran and his colleagues [8]. In the process of making pulmonary contusions in experimental animals, the energy that was transferred is 1.96 Joules. This is in accordance with Raghavendran's research, where energy of 1.8-2.7 Joules given to rat with a body weight of about 250 grams will cause pulmonary contusions, but does not cause mortality. The effect caused by pulmonary contusion depends on the severity. Direct injury will cause damage to pulmonary vascularization resulting in alveolar bleeding, thereby interfering with alveolar perfusion. In addition, there is an inflammatory response of the tissue causing pulmonary edema which results in changes in respiratory tract resistance and leads to ventilation-perfusion imbalance (V / Q mismatch). The manifestation of this condition is the progressive worsening of gas exchange and a low P / F ratio, as shown by Türüt and his colleagues [8,9]. Vitamin C is a water soluble antioxidant that is capable of scavenging oxygen free radicals that are involved in the development or exacerbation of various diseases, including cancer, heart attack, arthritis and stroke. Cristante and his colleagues showed that vitamin C reduced the inflammatory response in rat spinal cord injury [10]. In a study conducted by Rana S about the antioxidant effects of vitamin C and vitamin E in rats experiencing pulmonary contusions, it showed that there was an increase in PaO2 levels and a decrease in PaCO2 levels that were statistically significant [3]. Based on the results of animal blood gas analysis in this study, it was found that PaO2 levels decreased significantly in the incidence of pulmonary contusions ($p < 0.001$). After administration of antioxidants in the form of vitamin C, the PaO2 level will increase significantly ($p = 0.019$). Hypoxia that occurs due to an increase in vascular resistance in lung tissue that experiences contusions causes a reduction in pulmonary blood flow through resistant alveoli. This is what underlies the reduced oxygen intake in the blood and causes a high mortality rate in trauma patients [8]. Histopathological examination of lung tissue also supports the beneficial effects caused by giving antioxidants in the form of vitamin C. Changes in pulmonary parenchyma that experience severe contusions are characterized by extensive bleeding accompanied by impaired alveoli, alveolar edema and increased leukocyte infiltration in the alveolar space. The administration of antioxidants resulted in decreased alveolar edema ($p = 0.011$) and in decreased of leukocyte infiltration but it was less significant ($p = 0.050$) in the lung tissue that experiencing contusion. This is thought to be caused by the presence of other pathways that affect the increase in leucocyte where other pathways can be proven by multivariate testing but not carried out in this study. Apart from this, the results that are less significant than leukocyte infiltration may also be influenced by data limitations and accuracy of data processing From the results of the bivariate correlation test, it was shown that group variables, arterial PaO2, leukocyte infiltration and edema scores were related to each other significantly ($p < 0.05$). This emphasizes the antioxidant potential in suppressing the inflammatory response due to blunt thoracic injury.

6. Conclusion

Giving Vitamin C to pulmonary contusions causes a decrease in leukocyte infiltration and alveolar edema, resulting increased levels of PaO2. The researcher suggested that further research be carried out with various stages of clinical trials so that the provision of Vitamin C in patients with pulmonary contusions could be implicated in clinical setting to increase PaO2 levels and reduce the incidence of ARDS.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare

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