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Hyperbaric Oxygen Effect Towards Liver Function In Rats Infected By *Plasmodium berghei*

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Abstract : Malaria is reemerging disease that causes many severe complications and even death. One of the high complications of malaria infection is liver dysfunction which may lead. HBO (Hyperbaric Oxygen) can be one of the supportive therapies for malaria because it can repair the ischemic tissue. This research employed 48 white rats (*Rattus norvegicus*) that had been infected by *Plasmodium berghei* and were divided into 6 groups. This research aims at finding out the effect of oxygen hyperbaric therapy in repairing liver function. Variable that were watched for liver function is the level of transaminase enzyme specifically in SGOT (AST) and SGPT (ALT) . The result shows that the liver function of the group which had both hyperbaric oxygen as an adjuvant therapy and Dihydroartemisinin piperaquine therapy is significant. It means that the use of hyperbaric oxygen as an adjuvant therapy and Dihydroartemisinin piperaquine therapy can decrease the level of SGOT (AST) and SGPT (ALT) significantly compared to 5 other groups of rats infected by *Plasmodium berghei*.
Keywords : HBO, malaria, SGOT, SGPT.

Introduction

Malaria is now included in emerging disease which means the disease that reappears repeatedly as health problem both in national and international level due to the increase ofmalaria is now included in emerging disease malaria cases globally[12]. Malaria disease may lead to a death, specially in the high-risk group like baby and pregnant women. Besides, malaria causes anemia directly and decreases work productivity. It is also still an endemic disease in certain area in Indonesia[13]. Liver dysfunction may happen in severe malaria infection.

Liver dysfunction may happenbecause of the decreasing of blood flowto the thrombus tissue that causes hypoxia.

Parasite in erythrocytes will head to the capillar inside the organ, sequestrate, cytoadherate, and form *rosette* that can plug the capillars and finally the liver has less oxygen[456].Several studies have shown that the malaria-causing parasite has been resistant to medicines commonly used in malaria therapy. In addition to resistance problems, treatment with the appropriate doses and medicines still causes residual symptoms in patients with malaria [45]. Therefore it is necessary to find other alternatives to overcome them. One of the alternatives is hyperbaric oxygen which can be used as an adjuvant therapy in malaria that can help improve liver function and other organs to accelerate the recovery of the sufferer and to reduce the dependence on synthetic medicines.

The use of hyperbaric oxygen (OHB) therapy is a type of treatment in which the patient inhales 100% oxygen with more than 1 ATA (2-4 ATA) pressure through the mask in certain period of time. In these conditions, the circulation of oxygen to tissues throughout the body increases 2-3 times greater than normal conditions [7]. The use of OHB has been performed on a variety of inflammatory tissues caused by ischemia, trauma, bacterial and viral infections. In some studies, OHB can decrease inflammatory mediators such as TNF α, where TNF α is one of the chemical mediators that contributes to malaria [8]. OHB may also be used for the treatment of anemia due to blood loss by increasing the oxygen bond with hemoglobin [9]. Therefore, this study was conducted to determine the effect of hyperbaric oxygen therapy in lowering levels of SGOT (AST) and SGPT (ALT) in white rats that have been infected by *Plasmodium berghei* for a certain period of time.

Experimental

This part will cover materials and methods, liver function test, hyperbaric oxygen, and treatment for the animals.

Materials and Methods

This research is an experimental research using Post Test Control Only Group Design, where the examination of liver function used is transaminase enzyme level that is SGOT (AST) and SGPT (ALT). The samples were *Rattus norvegicus* male rats of wistar strains that met the inclusion criteria of 150 grams and were healthy for 2 weeks during adaptation, with observations on smooth and slick feathers, and normal mobility.

Liver Function Test

Examination of SGOT (AST) and SGPT (ALT) employed blood samples of rats during the last day of treatment i.e. day 10. SGOT (AST) and SGPT (ALT) is an indicator to determine the liver condition, where if there is damage to the liver cells then the level of this enzyme will increase.

Determination of enzymes Activity in Serum:

Serum activities of SGOT or (AST)aspartate aminotransferase, and SGPT (ALT) alanine aminotransferase (AST) measured using ready to use (AST, ALT ELISA bioMerieux kits), by using ELISA technique[10].

Hyperbaric Oxygen

The giving of hyperbaric oxygen therapy was conducted in LAKESLA RSAL Dr Ramelan Surabaya.Two doses of hyperbaric oxygen, 1.5 ATA and 3 ATA, were given for 2 hours in a day for 10 days.

Treatment for the Animals

There are 48 male white rats (*Rattus norvegicus*)wistar strain which aged 2-3 months and were divided into 6 groups by using *simple random sampling.*

1. **First group** consists of 8 male *Rattus norvegic*rats wistar strain which were inoculated with *Plasmodium bergheis*and were treated by Dihydroartemisinin piperaquine with the doses of 3 mg/kgBW(body weight) for 3 days and hyperbaric oxygen 1.5 ATA for 2 hours during 10 days continuously (P1).
2. **Second group** consists of 8 male *Rattus norvegic*rats wistar strain which were inoculated with *Plasmodium bergheis*and were treated by Dihydroartemisinin piperaquine with the doses of 3 mg/kgBWfor 3 days and hyperbaric oxygen 3 ATA for 2 hours during 10 days continuously (P2).
3. **Thirdgroup** consists of 8 male *Rattus norvegic*rats wistar strain which were inoculated with *Plasmodium bergheis*and were treated by hyperbaric oxygen 1.5 ATA for 2 hours during 10 days continuously (P3).
4. **Fourthgroup** consists of 8 male *Rattus norvegic*rats wistar strain which were inoculated with *Plasmodium bergheis*and were treated by hyperbaric oxygen 3 ATA for 2 hours during 10 days continuously (P4).
5. **Fifthgroup** consists of 8 male *Rattus norvegic*rats wistar strain which were inoculated with *Plasmodium bergheis*and were treated by Dihydroartemisinin piperaquine with the doses of3 mg/kgBWfor 3 days (P5).
6. **Sixth group** consists of 8 male *Rattus norvegic*rats wistar strain which were inoculated with *Plasmodium bergheis*and were treated by aquadest (P6).

Ethical Clearance

This research can be conducted after obtaining the ethical approval from the team of ethical commission of Lembaga Penelitian dan Pengabdian Masyarakat Universitas Hang Tuah Surabaya (Institute of Research and Community Service of Hang Tuah University Surabaya.)

Results and Discussion

Results

Description of SGOT (AST) and SGPT (ALT) inspection results descriptively can be seen in the table below:

Table 1 Liver Function Results

GROUP	SGOT (AST)	SGPT (ALT)
P1	132	58
P1	126	75
P1	132	61
P1	140	64
AVERAGE	**132.5**	**64.5**
P2	137	57
P2	117	58
P2	126	43
P2	127	45
AVERAGE	**126.75**	**50.75**
P3	185	93
P3	174	86
P3	180	89
P3	171	95
AVERAGE	**177.5**	**90.75**
P4	187	83
P4	193	85
P4	195	83
P4	190	88
AVERAGE	**191.25**	**84.75**
P5	184	78
P5	145	74
P5	155	82
P5	157	89
AVERAGE	**160.25**	**80.75**
P6	208	130
P6	225	110
P6	223	127
P6	231	120
AVERAGE	**221.75**	**121.75**

Table Description:

P1: Group receiving dehydroartemycinand hyperbaric oxygen therapy 1.5 ATA
P2: Group receiving dehydroartemycin and hyperbaric oxygen therapy 3 ATA
P3: Group receiving hyperbaric oxygen therapy 1.5 ATA
P4: Group receiving hyperbaric oxygen therapy 3 ATA
P5: Groupreceiving dehydroartemycin
P6: Group receivingaquadest

Based on the above data, it shows that:

1. The highest SGOT level is in the P6 group and the lowest is in P2 group. SGOT levels in the P3 and P4 groups are higher than P5 (positive control) and lower than P6 (negative control).
2. The highest levels of SGPT is in the P6 group and the lowest is in P2 group. SGPT levels in the P3 and P4 groups are higher than P5 (positive control) and lower than P6 (negative control).

From the collected data above,statistical analysis is done using one way anova analysis if the requirement is fulfilled that is normal and homogenous distribution data. If the requirements are met the analysis test is followed by a post hoc LSD analysis. If the one way anova requirement is not met, the analysis uses the non-parametric test of Kruskal Wallis followed by Mann Whitney analysis.

Results of SGOT (AST) Level Analysis

SGOT inspection data use one way anova because the data requirement of normal and homogeneous distribution are fulfilled. One way anova analysis showsthatp = 0.00 <0.05, so the analysis was continued by LSD posthoc analysis to know the difference between 2 treatment groups as shown in the table below:

Table 2 LSD Posthoc Analysis Results of SGOT (AST) Level

LSD Posthoc Statistical Analysis of SGOT (AST)						
Score p	**1**	**2**	**3**	**4**	**5**	6
1		0.396	0.000	0.000	0.001	0.000
2			0.000	0.000	0.000	0.000
3				0.052	0.018	0.000
4					0.000	0.000
5						0.000
6						

Table Description:

P1: Group receiving dehydroartemycin and hyperbaric oxygen therapy 1.5 ATA
P2: Group receiving dehydroartemycin and hyperbaric oxygen therapy 3 ATA
P3: Group receiving hyperbaric oxygen therapy 1.5 ATA
P4: Group receiving hyperbaric oxygen therapy 3 ATA
P5: Group receiving dehydroartemycin
P6: Group receiving aquadest

Based on the data above, it shows that:

1. SGOT levels at P1 are higher than P2 but statistically the difference is not significant. SGOT P1 levels are significantly lower than P3 - P6.
2. SGOT levels in P2 are significantly lower than P1 and P3 - P6.
3. SGOT levels at P3 are significantly lower than P6. SGOT levels in P3 are significantly lower than P4, but SGOT P3 levels are significantly higher than P5 / positive controls.
4. SGOT levels in P4 are significantly lower than P6 / negative control, but SGOT P4 levels are significantly higher than P5 / positive control
5. SGOT levels at P5 are significantly lower than P6.

From the above results, it can be concluded that P2 (Group receiving dehydroartemycin and hyperbaric oxygen therapy 3 ATA) is the most effective in lowering SGOT levels in rats infected with *P. berghei*.

Results of SGPT (ALT) Level Analysis

SGPT examination data use one way anova because the data requirements of normal and homogeneous distribution are fulfilled. One way anova analysis showsthatp = 0.002 <0.05, so the analysis was continued by LSD posthoc analysis to know the difference between 2 treatment groups as showninthe table below:

Table 3 LSD Posthoc Analysis Results of SGPT (ALT) Level

LSD Posthoc Statistical Analysis of SGPT (ALT)						
SCORE p	**1**	**2**	**3**	**4**	**5**	6
1		0.008	0.000	0.000	0.003	0.000
2			0.000	0.000	0.000	0.000
3				0.212	0.045	0.000
4					0.400	0.000
5						0.000
6						

Table Description:

P1: Group receiving dehydroartemycin and hyperbaric oxygen therapy 1.5 ATA
P2: Group receiving dehydroartemycin and hyperbaric oxygen therapy 3 ATA
P3: Group receiving hyperbaric oxygen therapy 1.5 ATA
P4: Group receiving hyperbaric oxygen therapy 3 ATA
P5: Group receiving dehydroartemycin
P6: Group receiving aquadest

Based on the data above, it shows that:

1. SGPT levels in P1 are significantly lower than P3 - P6, and SGOT P1 levels are significantly higher than P2.
2. SGPT levels in P2 are significantly lower than P1 and P3-P6.
3. SGPT levels in P3 are significantly higher than P4 - P5, and SGOT P3 levels are significantly lower than P6.
4. SGPT levels in P4 are significantly higher than P5 but SGOT P4 levels are significantly lower than P6.
5. SGPT levels in P5 are significantly lower than P6.

From the results above, it can be concluded that P2 (Group receiving dehydroartemycin and hyperbaric oxygen therapy 3 ATA) is the most effective in lowering SGPT levels in rats infected with *P. berghei*.

Results and Discussion

In malaria there can be abnormalities in the kidneys and liver resulting from ischemia. Ischemia in malarial infections is due to decreased O2 supply due to decreased red blood cells (anemia) and the occurrence of sequestering of infected red blood cells in vascular endothelium. Anemia is one of the clinical symptoms of malaria caused by the destruction of erythrocytes containing parasites and those that do not contain parasites in the spleen, decreased survival of red blood cells and the presence of disruption of red blood cell formation[1112]. O2 supply disturbance is also caused by changes in the structure of the infected red cell membrane to form a rosette formation and sequesterization that attachment of infected red blood cells to the vascular endothelium (cytoadherence) will decrease oxygen supply[6]. Hyperbaric Oxygen therapy (HBO) can reduce the volume of bubbles in the body and increase tissue oxygen tension. Hyperbaric oxygen delivery increases the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) due to hyperoxia. Increased ROS and RNS will suppress inflammatory mediator expenditure and promote wound healing in tissue ischemia [7]. The suppression of proinflammatory cytokines (TNF-α) will prevent the occurrence of the sequestering of red blood cells in vascular endothelium [132]. Through the above mechanism, administration of HBO in malaria infection is expected to prevent the occurrence of tissue ischemia including liver organ.

One indicator to know the function of the liver is by detecting levels of SGOT (AST)and SGPT (ALT). SGPT (Serum Glutamic Pyruvate Transaminase) is an enzyme found in liver cells.

When liver cells are damaged, SGPT enzyme secreting from the liver cells into the blood circulation and will be measured through laboratory tests. SGOT (Serum Glutamic Oxaloacetic Transaminase) is a liver enzyme present in the liver parenchyma cells. SGOT will increase its chest in the blood if there is liver cell damage. However, SGOT is not specific only in the liver. SGOT can also be found in blood cells, heart cells and muscle cells, therefore an increase in SGOT does not necessarily indicate abnormalities in liver cells. SGOT (AST) and SGPT (ALT) are enzymes that can be found if there is damage (necrosis) of liver cells, as occurs in acute viral hepatitis infection, the enzymes are out of the liver cells and into the blood. The more damaged liver cells, the higher the measured SGOT (AST) and SGPT (ALT) levels in the blood[1415]. With the provision of HBO in malaria infection, hepatic tissue ischemia can be prevented through improved O2 supply and sequestering prevention[7].

The results of this study shows that group P2 (group receiving dehydroartemycin and hyperbaric oxygen therapy 3 ATA) is the most effective in lowering SGOT levels in rats infected with P. berghei. Artemisinins act more rapidly than other types of antimalarials, both in killing the parasites and in inhibiting their major metabolic processes, such as glycolysis, nucleic acid and protein synthesis[16]. These results are somewhat different from those of Tokio's 1969 study of the effect of hyperbaric oxygen administration on coronary artery embolism in experimental animals (dogs). The Tokio study showed that hyperbaric oxygen administration could lower SGOT (AST) and SGPT (ALT) levels of blood compared to control group, but the decrease was statistically less significant [14]. Similar results were demonstrated by Dewi*et al.*'s study, in which the administration of HBO combined with metformin in type 2 diabetes mellitus patients would decrease SGOT (AST) and SGPT (ALT) levels significantly between pre and post treatment [15].

Conclusion

After conducting the research about three months employing six groups of samples, we come to the conclusion that giving HBO 3 ATA combined with antimalarial (dehydroartemysinpiperaquin) can significantly lower SGOT (AST) and SGPT (ALT). It is clearly shown from the result of the research that the group which is given HBO 3 ATA combined with antimalarial (dehydroartemysinpiperaquin) is the best therapy in lowering the SGOT (AST) and SGPT (ALT) compared to five other groups. Finally, it is really suggested to other researchers to conduct such kind of research in more complex samples.

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