EVALUASI HISTOPATOLOGIS JARINGAN HEPAR KELINCI AKIBAT CEDERA REMOTE REPERFUSI ISKEMIK TUNGKAI AKUT YANG DILAKUKAN PREKONDISI ISKEMIK DAN HIPOTERMI

(Histopathological Evaluation of Rabbit Hepatic Tissue Due to Remote Ischemic Reperfusion of Acute Limbs Injury Conducted with Ischemic Precondition and Hypothermic)

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ABSTRACT

Acute limb ischemia (ALI) is an emergency that occurs due to a rapid decrease in perfusion to limbs which can result in mitochondrial dysfunction, causing disturbances in distant organs such as liver. There are various ways to reduce the effects including treatment of hypothermia and ischemic preconditions. So, this study was made to see any differences in liver tissue damage as a result of reperfusion distant ischemic injury between the untreated and treated groups of hypothermia and ischemic precondition in cases of acute limb ischemia. This study is a true-experimental, with the New Zealand White (NZW) male rabbits as the samples and it divided into three groups, untreated rabbit group (as a control), treated with ischemic preconditioning group and hypothermia group. The data were collected by taking rabbit liver biopsy samples and were analyzed in univariate and bivariate with Shapiro Wilk normality test, independent T-test and Mann Whitney. This study used 21 rabbits as sample, 3 were excluded and each group have 6 samples. Statistical analysis of the comparison of liver tissue damage scores between the control group and the ischemic precondition treatment group showed a significant difference with a p value 0.002 and the comparison of the liver tissue damage scores between the control group and the hypothermic treatment group showed significant differences with p value 0.004. It can be concluded that there are liver tissue damage as a result of ischemic reperfusion injury in experimental rabbits, and there are significant differences in liver tissue damage between the control group, the hypothermia and ischemic precondition group.

Keyword: *Acute limb ischemia, liver damage, ischemic precondition, hypothermia treatment*

ABSTRAK

Iskemik tungkai akut (Acute Limb Ischemia/ALI) merupakan suatu kegawatan yang terjadi akibat menurunnya perfusi ke tungkai secara cepat yang bisa mengakibatkan disfungsi mitokondria sehingga menyebabkan gangguan pada organ jauh seperti hepar. Terdapat berbagai cara untuk mengurangi efek, antara lain hipotermia dan prekondisi iskemik. Sehingga

penelitian ini bertujuan untuk melihat adakah perbedaan kerusakan jaringan hepar akibat cedera jauh iskemik reperfusi antara kelompok kontrol dengan kelompok yang diberikan perlakuan hipotermia dan prekondisi iskemik pada kasus iskemia tungkai akut. Penelitian ini merupakan true-eksperimental, dengan sampel kelinci New Zealand White (NZW) jantan yang dibagi ke dalam tiga kelompok yaitu kelompok tanpa perlakuan, dengan perlakuan prekondisi iskemik dan hipotermia. Pengambilan data berupa pengambilan sampel biopsi hepar kelinci. Analisis data merupakan analisis univariat dan bivariat dengan uji normalitas shapiro wilk dan uji perbandingan dengan T-test independent dan Mann Whitney. Jumlah sampel yang digunakan sebanyak 21 sampel, 3 sampel tereksklusi dan jumlah sampel pada kelompok kontrol, hipotermi dan perlakuan iskemik masing-masing sebanyak 6 sampel. Analisa statistik perbandingan skor kerusakan jaringan hepar antara kelompok kontrol dengan kelompok prekondisi iskemik menunjukkan hasil perbedaan dengan nilai p 0,002 dan perbandingan skor kerusakan jaringan hepar antara kelompok kontrol dengan kelompok hipotermi menunjukkan hasil perbedaan dengan nilai p 0,004. Sehingga dapat disimpulkan, terdapat kerusakan jaringan hepar akibat cedera iskemik reperfusi dan terdapat perbedaan bermakna kerusakan jaringan hepar antara kelompok kontrol dengan kelompok hipotermi dan prekondisi iskemik.

Kata Kunci: Iskemik tungkai akut, kerusakan hepar, prekondisi iskemik, hipotermi

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BACKGROUND

Acute limb ischemia (ALI) occurs when there is a sudden halt of blood flow to the arm or leg, mostly owing to thrombosis or emboli. When left untreated, it can threaten the viability of the limb, followed by infection, necrosis, limb loss and ultimately, death.[1] Acute limb ischemia presents as sudden lower limb ischemia that can result in amputation. The reported incidence of ALI is 1–1,5 individuals per 10,000 individuals per year.[2] Complications among ALI patients are high and despite early revascularization, 30-day mortality and amputation rates are between 10 and 15%.[3] So that ALI becomes a major health problem in the community.

Cellular damage after reperfusion of ischemic tissue is defined as ischemia–reperfusion injury (IRI).[4] Ischemia-reperfusion injury (IRI) is defined as the paradoxical exacerbation of cellular dysfunction and death, following restoration of blood flow to previously ischemic tissues. Reestablishment of blood flow is essential to salvage ischemic tissues. However, reperfusion itself paradoxically causes further damage, threatening function

and viability of the organ.[5] Vascular surgery is the main definitive treatment for such conditions like peripheral vascular disease and it is associated with subsequent injury to vital organs including the kidneys, heart, brain, intestines and lungs, with a consequent increase in both morbidity and mortality.[6]

Better knowledge of ischemic injuries is essential to reduce mitochondrial dysfunction in skeletal muscle, thereby reducing reperfusion ischemic injury. Mitochondrial dysfunction causes disturbance in distant organs such as the liver through increased oxidation stress caused by imbalance in the production and elimination of Reactive Oxygen Species (ROS).[7] Currently, there are various ways to reduce the effects of reperfusion ischemic injury, including treatment of hypothermia and ischemic preconditions. Based on the description above, this study was made to see any differences between the untreated and treated groups of hypothermia and ischemic precondition against the occurrence of reperfusion distant ischemic liver injury in cases of acute limb ischemia.

METHODS

This study is true-experimental, with New Zealand White (NZW) male rabbits as the samples and it divided into three groups, untreated rabbit group (as a control), treated with ischemic preconditioning group and hypothermic group. This study used 21 experimental animals and the inclusion criteria are the rabbits lived until the study was completed and male rabbits were obtained, kept, and recommended as experimental animals by BALITNAK Bogor Agricultural Department. The exclusion criteria are rabbits died before the end of the study and during the study 3 experimental animals were excluded. This study protocol was approved by health research ethics of the Faculty of Medicine, Indonesia University (Nomor: 882/UN2.F1/ETIK/2014).

In the control group, we only ligate the right common iliac artery for 4 hours without any treatment. For the hypothermic group, we did ligation of the right common iliac artery for 4 hours along with the hypothermic treatment using cold water pumped to a cooling pad up to 28^{o}C for 4 hours, and in the group ischemic precondition, we did the ischemic precondition for 3x5 minute before ligating the right common iliac artery for 4 hours. After that, the three groups were subjected to reperfusion for 8 hours, then a liver biopsy sample was taken with Hematoxylin Eosin (HE) staining at the 8th hour post reperfusion. The data were analyzed in univariate and bivariate using the Shapiro-Wilk normality test with independent T-test

for normal distribution and Mann-Whitney for abnormal distribution.

RESULT

In table 1, there are descriptive data of liver tissue damage that examined through biopsy examination. Ballooning hepatocyte, cytoplasma vacuolization, collapsed sinusoid, blurring intercellular border, microhemorrhagic and leukocyte infiltration are some features in damaged liver cell. The total number of damaged cells in the control group, ischemic precondition and hypothermia were 524 cells, 295 cells and 257 cells. So the control group has the highest total number of damaged cells compared to groups with ischemic preconditions and hypothermia.

Table 1. Descriptive Data of the Control Group, Ischemic Precondition Group and Hypothermia Group

	Balloning Hepatocyte	Cytoplasma Vakuolization	Collapsed Sinusoid	Blurring Intercelullar Border	Microhemorrhagic	Leukocyte Infiltration	Total
Control Group							
Rabbit 1	19	15	25	8	3	14	89
Rabbit 2	20	15	18	9	14	14	90
Rabbit 3	20	15	19	9	13	14	90
Rabbit 4	19	14	19	10	14	15	91
Rabbit 5	18	15	18	9	5	10	75
Rabbit 6	19	15	19	8	14	14	89
Ischemic Precondition							
Rabbit 1	9	5	7	1	6	6	34
Rabbit 2	17	11	16	6	16	9	65
Rabbit 3	10	7	7	1	9	6	40
Rabbit 4	7	5	6	1	7	6	32
Rabbit 5	15	10	15	6	11	8	65
Rabbit 6	15	9	16	4	9	6	59
Hypothermia Group							
Rabbit 1	9	6	8	4	9	5	41
Rabbit 2	13	7	12	0	8	6	46
Rabbit 3	16	11	16	8	15	8	84
Rabbit 4	5	3	5	1	2	4	20
Rabbit 5	7	12	8	0	2	5	34
Rabbit 6	9	6	7	0	4	6	32

Table 2. Analytic Result of Comparison Liver Tissue Damage Scores between Control Group and Ischemic Precondition Group

Variable	Subject Group		p
	Control (n)	Ischemic Precondition (n)	
Balloning Hepatocyte	6	6	0,002
CytoplasmaVakuolization	6	6	0,003
Collapsed Sinusoid	6	6	0,004
Blurring Intercelullar Border	6	6	0,003
Microhemorrhagic	6	6	0,257
Leukocyte Infiltration	6	6	0,003
Total	6	6	0,002

In table 2, the statistical analysis of comparison liver tissue damage scores between the control group and the ischemic precondition treatment group shows a significant difference with a p value 0,002.

Table 3. Analytic Result of Comparison Liver Tissue Damage Scores between Control Group and Hypothermic Group

Variable	Subject Group		p
	Control (n)	Hypothermic (n)	
Balloning Hepatocyte	6	6	<0,001
CytoplasmaVakuolization	6	6	0,003
Collapsed Sinusoid	6	6	<0,001
Blurring Intercelullar Border	6	6	0,005
Microhemorrhagic	6	6	0,106
Leukocyte Infiltration	6	6	0,002
Total	6	6	0,004

In table 3, the statistical analysis of comparison liver tissue damage scores between the control group and the hypothermic treatment group shows a significant difference with a p value 0,004.

DISCUSSION

Reperfusion injury in remote organs induced by lower limb IR has been experimentally demonstrated in several studies. The main mechanism which initiates IRI is the activation of the inflammatory response. This can lead to a complex cytokine cascade or storm that serves to perpetuate inflammatory reactions in remote organs, which can clinically manifest as multiple organ dysfunction (i.e. acute liver injury and acute lung injury). Reperfusion of acutely ischemic tissues induces the release of very powerful oxygen free radicals and cytokines.[8] With distressed mitochondria, production of injurious reactive oxygen species (ROS) is about to increased. Thus, should arterial flow be restored, these toxic material released even to systemic circulation lead to cellular damage locally and those of a significant anatomical distance. This referred to the pathogenesis of ischemia/reperfusion injury.[9]

In this study, through pathological anatomical examination, pathological changes in liver tissue were found as ballooning hepatocyte, cytoplasma vacuolization, collapsed sinusoid, blurring intercellular border, microhemorrhagic and leukocyte infiltration. Such changes described as the impact of reperfusion of a distant ischemia, in this case limb ischemia. The role of cytokines released following ischemia might be responsible to this hepatic injury through activation of Kupffer cells as shown in study on mice whereas IL6 in systemic was much higher than the portal system. IL6 is somewhat activates Kupffer cells with furthers lead to hepatic injury.[9] The results of this study are in line with other studies that suggest lower leg IR affects the liver as does reperfusion injury to the associated limb.[8]

In the descriptive data, we found that total number of damaged cells in the control group, ischemic precondition and hypothermia were 524 cells, 295 cells and 257 cells. So the control group has the highest total number of damaged cells compared to groups with ischemic preconditions and hypothermia. In statistic analysis of comparison between the groups, it was found that the comparison of liver tissue damage in control group compared with hypothermia group are significant, with a p value 0,004. The same thing happened in comparison of liver tissue damage in control group compared with ischemic preconditions group, are significant with a p value 0,002. This result suggests that the ischemic preconditions

and hypothermia could reduce distant ischemic reperfusion injury than the control group.

Ischemic preconditioning consists of brief and repetitive episodes of IRI before the induction of sustained organ ischemia and is effective in reducing the severity of tissue damage. Animal models of a number of settings of IRI have been used to investigate mechanisms of ischemic preconditioning but the basic molecular mechanisms remain unclear, probably due to the multiple signal transduction pathways involved in this phenomenon. However, it is generally recognized that brief ischemic preconditioning induces a cascade of intracellular kinases, which subsequently modify mitochondrial function.[5] The result of this study are in line with other study that ischemia preconditioning show benefit in reducing biomarkers of ischemia reperfusion injury.[6]

Hypothermia is able to decrease oxygen consumption, preventing a rapid loss of mitochondrial activity.[4] The results of this study are also in line with other study that examine the effect of hypothermia on skeletal ischemia reperfusion injury and the study found that hypothermia can reduce IRI in skeletal muscles.[10]

CONCLUSION

Based on the results of this study, it can be concluded that there are liver tissue damage as a result of ischemic reperfusion injury in experimental rabbits and there are significant differences in liver tissue damage between the control group and the hypothermia and ischemic precondition group. This result suggests that the ischemic preconditions and hypothermia could reduce distant reperfusion-ischemic injury than the control group.

ADVICE

Hope for the next researchers will be able to determine the pathway that has dominant role in the occurrence of remote ischemic reperfusion injury in acute limb ischemia, so that they can make appropriate interventions to minimize the damage that can occur. Educational institutions are expected to be able to encourage and increase the amount of research starting from molecular biology to approaching the actual clinical level problems in humans. For patient care, hope the intervention can be done in patient because it can minimize the damage that can occur due to remote ischemic reperfusion injury in acute limb ischemia.

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