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Effect of Ischemia Preconditioning and Hypothermia to Gastric Mucosal Reperfusion Injury Post Ischemia in Lower Extremities of Oryctolagus cuniculus

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Abstract

Introduction. Immediate revascularization of ischemic tissue does not always produce positive results since various reactions following formation of reactive oxygen species and activation of complement system might lead to ischemia/reperfusion injury (I/RI). It was hypothesized that ischemia preconditioning (IPC) and hypothermia (HI) have a role to reduce the impact of (I/RI).
Method. An experimental study was carried out on Oryctolagus cuniculus (New Zealand White rabbit) to find out the efficacy of IPC and HI. Subjects were divided into four groups; a control (consist of two subjects) and three treatment groups (each consist of six subjects), namely I/RI group, IPC group, and HI group. In I/RI group, right common femoral artery was ligated under anesthesia and ligation was maintained for four hours, and then released for eight hours. In IPC group, arterial ligation for two minutes and released for three minutes protocol was carried out in two cycles. In HI group, right lower extremity was wrapped with iced aluminum foil. In the last two groups mentioned, the ligation released after 4 hours and treated as in I/RI group. Subjects were sacrificed, and samples of stomach was taken through laparotomy. Histopathology exam and tissue malondialdehyde (MDA) were variables of interests. Statistical analysis was carried out using SPSS ver. 20, and significance met if $p < 0.05$.
Results. Histopathologic changes and level of tissue MDA in I/RI group was significantly higher than control group. Histopathologic changes in IPC group significantly lower than I/RI group; biochemically higher, but not significant. Histopathologic changes in HI group was lower than I/RI group, but not significant; biochemically, significantly higher than I/RI group. Histopathological IPC significantly lower than HI; biochemically IPC was lower but not significant.
Conclusion. Ischemia preconditioning plays a protective role on destructive impact of ischemia–reperfusion injury of distant organs. Hypothermia also has a role but is not as good as ischemia preconditioning.

Keywords: *ischemia/reperfusion injury, ischemia preconditioning, hypothermia, stomach, femoral*

Introduction

Many animal studies demonstrated the gastric mucosal changes in ischemia–reperfusion injury (I/RI). In most study, this I/RI induced by direct ligation the artery of target organ, one of which is a stomach. So far, study on I/RI focused on distant organs is quite minimal; as to date, the author found only a study conducted by Wang et al. On the other hand, study on I/RI is very important, since in clinical setting there's many surgical entities encountered this phenomenon, for instance, re–vascularized acute or critical limb ischemia. There were studies addressed to reduce the impact of this I/RI, namely ischemia preconditioning (IPC) and hypothermia (HI). Therefore, the need to conduct a study to reinforce prior studies is obvious.

Lower extremity was chosen as the focus of ischemia as limb referred to the most region underwent ischemia. In addition, ligation of the femoral artery is technically easy to carry out. The reason why stomach is chosen as a target distant organ was the fact that stress ulcer was a most phenomenon found as the first organ failure in critically ill patients following circulatory derangement.

The study purposed find out the evidence of by morphological derangement and oxidative stress of gastric mucosa as a systemic reperfusion injury following re–vascularized organ underwent ischemia.

Method

The investigation carried out using Oryctolagus cuniculus (New Zealand White rabbit) certified by the Veterinary Research Institute, Ministry of Agriculture of the Republic of Indonesia as the subject. These five months of 3–4 kg weighted rabbits were adapted a week prior to investigation. These subjects were divided into four groups, the control group (two rabbits) and the three treatment groups (each consist of six rabbits), i.e. I/RI group, IPC group, and HI group. The number of animal enrolled was in accordance to ethical considerations.
Intervention was carried out under sedation using 15–20 milligrams per body weight ketamine intramuscularly and diazepam 0.5 milligrams per body weight. Sedation effect will be maintained with 10 milligrams per body weight ketamine intramuscularly. In the I/RI group, ligation of the right femoral artery using silk 3.0 were maintained for four hours. Ligation is then removed, and subjects were set free in the cage for eight hours. In IPC group, a short two–minute ligation and released for three minutes protocol were applied in two cycles. Then, the treatment as in I/RI group was applied. In HI group, right lower extremity wrapped with iced foil with the temperatures maintained for 31–33ºC. Next the treatment as in I/RI group was applied. After 8 hours, subjects were sacrificed, and laparotomy was carried out to take sample of gaster; i.e. gastric

antrum and corpus. Samples were divided into two parts. First, sample was fixed in a tube filled of 10% formaldehyde buffer for morphological investigation. The second one placed in a tube filled with sterile saline solution and stored in the refrigerator for biochemical test.

Study on morphology was carried out in with hematoxylin and eosin stained under light microscope. Assessment of mucosal change carried out using modified Mohammed criteria.2 To investigate tissue MDA level, MDA assay kits was used. Test in duplo was applied.

All data were the variables of interest and has been analyzed using SPSS ver. 20. Normality test on all data was done. One–way ANOVA used to analyze tissue MDA and unpaired T test used to analyzed differences between treatment groups. Kruskal–Wallis analysis used in assessment of gastric mucosal change, and Mann–Whitney used in to analyzed differences between each treatment group. Significance met if p <0.05. The committee of ethic Faculty of Medicine, Universitas Indonesia approved the study.

Table 1. Modified Mohammed criteria in assessment of mucosal change.[2]

Grade	Criteria
0	No damage
I	Damage in mucosal surface cells
II	Grade I + damage in *gastric pit*
III	Grade II + damage in gastric glandular layer

Results

The average oxygen saturation in the ischemic limb following ligation was 67.88% while as in contralateral limb was 98.13%. Median of limb oxygen saturation after revascularization was 98% (97–99%). Subjects' characteristics were drawn in table 2.

Table 2. Subjects' Characteristic

Variable	Mean/Median
Body weight	3.225 ± 0.1982 kg
Oxygen Saturation in Ligated Extremity	67.88 ± 4.422 %
Oxygen Saturation in Contralateral Extremity	98.13 ± 1.126 %
Oxygen Saturation after Revascularization	98 (0–100) %

Morphological change of a first–degree found in both samples of control group (CG). In I/RI group there was no first–degree, out of six, there were two subjects with second–degree and four subjects with third–degree. In IPC group, out of six, three subjects with first–degree, and three subjects with second–degree. In HI group, out of six, five subjects with second–degree, and one subject with third–degree damage.

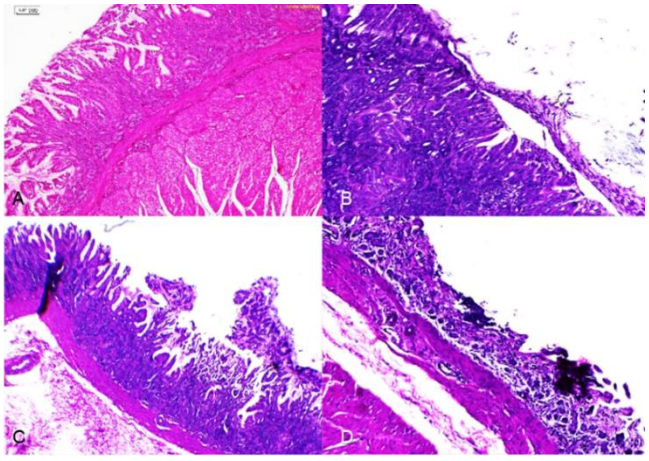

Figure 1. A. Normal gastric mucosa, B. First degree of mucosal damage. C. Second degree of mucosal damage to gastric pit. D. Third degree of mucosal damage in glandular gastric layer.

On statistical analysis, Kruskal Wallis test showed p value of 0.007. Subsequently, Mann–Whitney used to find out correlation between I/RI and control showed p value of 0.031. The difference between IPC to control showed a value of p 0.237 and HI to control of 0.021. The difference between I/RI and IPC showed p value of 0.011, and I/RI to HI of 0.093, and IPC to HI of 0.043.

Study on oxidative stress, tissue MDA level were showed increased. In both subjects of control were 0.004 ng/mL each. Mean of I/RI was 0.0115 ng/mL (+ 0.001), IPC was 0.0225 ng/mL (+ 0.014), and HI was 0.0345 ng/mL (+ 0.112). One–way ANOVA analysis showed p value of 0.004. Analysis between groups showed p value between control and I/RI of 0.001, between control and IPC and HI of 0.145 and 0.013, respectively. The p value between I/RI and IPC of 0.129, and of 0.005 between I/RI and HI. The p value between IPC and HI of 0.151.

Table 3. Scoring of histopatological change in treatment group

Treatment		p
Control	I/RI	0.031
	IPC	0.237
	HI	0.021
I/RI	IPC	0.011
	HI	0.093
IPC	HI	0.043

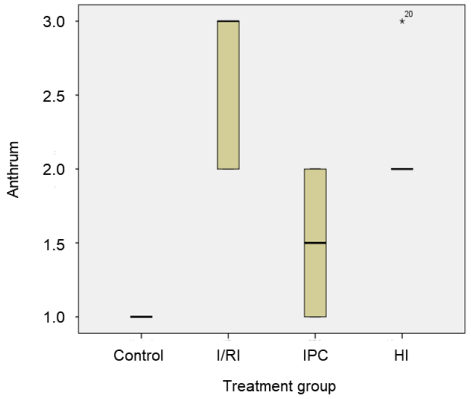

Figure 2. Median of scoring in histopathologically changes in treatment groups

Table 4. Tissue MDA levels in treatment group

Treatment		p
Control	I/RI	0.001
	IPC	0.145
	HI	0.013
I/RI	IPC	0.129
	HI	0.005
IPC	HI	0.151

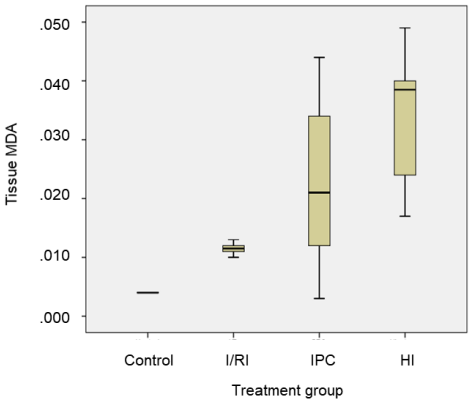

Figure 3. Tissue MDA levels in treatment groups.

Discussion

Overall, differences of the scoring representing degree of histopathology of mucosal damage and tissue MDA level were found statistically significant in treatment groups to control. Both mucosal damage of antrum and corpus in I/RI were found significantly higher than control. Tissue MDA levels were also significantly higher in the reperfusion group compared to the control. These findings suggest that ROS produced in the lower extremities approached the gastric mucosal circulation and leading to mucosal epithelial apoptosis; though apoptosis need to be proven furthermore. Mucosal integrity was found disrupted in varying degree, of which the worst found in I/RI group.

During ischemic phase, oxygen deprivation leads to inefficient cellular anaerobic metabolism with consequent energy deficit. With anaerobic glycolysis, nicotinamide adenine dinucleotide (NAD) activity in the cytoplasm persists despite a decreased pH in the gastric atmosphere. Electron transfer beyond mitochondria and the final process of oxygen dependent energy formation does not occurred. As this respiratory chain were ineffective, energy demand depends on lactic acid which is produced largely in anaerobic atmosphere from pyruvic acid bound to hydrogen ions. This increased lactic acid lead to lactic acidosis with further inactivation and mitochondrial distress. Lysis of mitochondrial membrane produce the toxin that induce cellular apoptosis.[3]

When ATP drop, cell membrane depolarization lead to change in the permeability of cell membrane. Calcium and sodium, which are normally in the extracellular compartment, are moving into the cells. Intracellular potassium moves toward extracellular compartment. High concentrations of intracellular calcium form free radicals and enzymes, such as xanthine dehydrogenase (XDH) and xanthine oxidase (XO).

Another important process in ischemia is the activity of the nuclear factor kappa beta (NFκß) that affects the transcription of nuclear DNA, which then produces an inflammatory mediator. This process activates inflammatory cytokines and receptors, including neutrophils. Neutrophils cause an increase of XO and Reactive Oxygen Species (ROS).[5]

When the perfusion restored (re–perfused), combination of oxygen, XO, and hypoxanthine forms superoxide (O2–); a free radicals which is harmless may cause enzymes contained ferro and Sulphur to be inactive and changes to a free–forming ferro leading to formation of hydroxyl ROS, which is highly reactive in inflammatory process inducing the release of cytokines in a massive number.4 In massive number, ROS lead to cellular macromolecules (such as RNA and DNA) destruction, and lead to endothelial damage, microvascular dysfunction, and apoptosis. ROS reacts with cell lipids, creating lipid peroxidase. Peroxidase is metabolized to malondialdehyde (MDA) and 4–hydroxynonenal (4–HNE).[5]

In this study, different findings between antrum and corpus were observed. The median of I/RII of the antrum group were significantly higher than the control. In the corpus group, the median of I/RI was higher than the control, though was not statistically significant. In comparison, the damage found in the antrum was significantly much severe than corpus. Gastric corpus, an area rich of parietal cells produces gastric acid were found differed to antrum where parietal cells were found minimal, dominated by mucus cells. Mucus cells produce a physical barrier between the lumen and the epithelium protects the epithelial layer from adverse factors in the gastric lumen.[6,7] Ischemia–reperfusion injury damages both parietal cells and mucus production cells, let a decrease in gastric acid production, resulted in reduced adverse effects; thus, damage to corpus region decreases in ischemia/reperfusion injury. On the other hand, damage to the mucus cells in the antrum leads to a decrease in protective function, resulted in ischemia–reperfusion process and gastric acid production which simultaneously antrum epithelial damage.

In IPC group, significant differences were found histopathologically with the I/RI group, but not significantly different from the MDA assay. IPC also has no significant difference when compared with control. Data showed that histopathologic deterioration in I/RI group was found much severe than in IPC group. However, the mean MDA in IPC was higher than the MDA in I/RI. The IPC was not significant when compared to the control group. In IPC, a “short I/RI" process occurs before pure ischemia occurs. This "short I/RI" process produces low–dose ROS, which also produces MDA as the final metabolic product, but this process does not cause tissue damage, and even has protective effect with low–dose ROS.8 Low–dose ROS releases NO, catecholamines, adenosine, bradykinin; and directly activate the intracellular kinase and induce the synthesis of protective protein. ROS also activates the NF–KB cytokine, which induces iNOS mRNA transcription 24 hours after the process and this becomes the delayed protection in organ targets.8 This protective effect is also produced by endogenous prostaglandins involving cyclooxygenase–1 and cyclooxygenase–2, nitric oxide, adenosine action on A1 receptor, and sensory nerve.[9]

In hypothermic group, there was no significant difference found histopathologically when compared to I/RI group. However, the median in the hypothermic group was lower than the median in I/RI group, which was grade 2 in hypothermia and grade 3 in I/RI. Study on MDA showed there was a significant difference between hypothermia and I/RI groups.
According to Shah et al., therapeutic hypothermia may increase the activation of antibody–initiated complement and eukaryotic cell destruction. The activation of antibody–initiated complement plays a role in reducing the benefits of therapeutic hypothermia.[10] Additionally, the therapeutic effect of hypothermia that is not optimal can also be due to the short duration of hypothermia. Research conducted by Todd and colleagues found that 1001 patients with ruptured intracranial aneurism did not benefit significantly from hypothermia treatment. The group states that the short duration of the cooling process and the warming after anesthesia ends may be the cause of the subjects not benefiting from hypothermia treatment.11 In a study conducted by Groysman involving 13 clinical studies on the use of hypothermia in acute stroke, the management began between six and 33 hours after the onset of symptoms and maintained up to a mean of 40.9 hours.[12] The study by Gunn et al showed that cooling sheep to 34ºC for 72 hours had adequate protection when performed 90 minutes after injury, and was effective but not entirely when it is started 5.5 hours after injury, and is ineffective when it is started 8.5 hours after injury.[13]

In I/RI, evacuation of mass lesions lowers intracranial pressure, and the purpose of hypothermia is to suppress reperfusion injuries and cascades produced by ischemia. The benefits of hypothermia are shown in patients treated with mild and moderate hypothermia for more than 48 hours, because the highest cerebral edema occurs 48 hours after injury.[14] Therefore, the optimum duration of hypothermia depends on the severity of the injury, and the delay before the therapeutic hypothermia. The time before re–warming is an important variable to influence the protective effect of hypothermia therapy. Hypothermia followed by rapid rehydration not only reverses the protective effect of hypothermia, but leads to traumatic pathology and its functional consequences.[15]

Considering the association between IPC and hypothermia, there appears to be a histopathologically significant difference between IPC and hypothermia. There is also no significant difference between the MDA assay of IPC and hypothermia groups. This suggests that IPC and hypothermia produce the same number of free radicals, but elicit different effects. Free radicals produced by IPC, provide a protective effect, while the free radicals from the hypothermia process, reduce the protective effect on the tissue. This study shows that IPC has an advantage in overcoming I/RI injury, although it is not significantly different.

The limitation of this study was the control that were not completely ideal. Gastric mucosal damage was found in the control, although it was a first–degree damage. Trauma and inflammation due to various manipulation may be responsible of this damage. However, conclusions were made as sampling were preceded in the same manner.

Conclusion

Product of ischemic reperfusion injury in the lower extremities approached and leading to damage in gastric mucosa. The antrum injured severely than the corpus. Ischemia preconditioning has a protective effect on the destructive effects produced by ischemic reperfusion injury of lower extremities to the gastric mucosa. Hypothermia also has a protective effect on the destructive effects produced by ischemic reperfusion injury of lower extremities to the gastric mucosa, but not as good as ischemia preconditioning.

Disclosure

This study has no conflict of interest.

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References

1. Wang T, Zhou Y–T, Chen X–N, Zhu A–X, Wu B–H. Remote ischemic postconditioning protects against gastric mucosal lesions in rats. World J Gastroenterol WJG. 2014;20(28):9519–27.
2. Mohamed A. Postulated Protective Role of Curcumin on Indomethacin–induced Acute Gastric Mucosal Damage in Adult Albino Rats (Histological and Immuohistochemical Study). Egypt J Histol. 2010;33(3):583–93.
3. Tapuria N, Kumar Y, Habib MM, Amara MA, Seifalian AM, Davidson BR. Remote Ischemic Preconditioning: A Novel Protective Method from Ischemia Reperfusion Injury—A Review. J Surg Res. 2008;150(2):304–30.
4. Breton AN. Ischemia and Reperfusion Injury: When Cells Almost Die. Vet Tech. 2010;31(7).
5. Dinu C, Diaconescu C, Avram N. Gastric Mucosa Injury Associated with Oxidative Stress. Lucr Stiinifice Med Vet TIMISOARA. 2009;XLII(2).
6. Beresford WA. Alimentary System. Histology Full Text [Internet]. [cited 2014 Oct 19]. Available from website: http://wberesford.hsc.wvu.edu/histolch24.htm
7. Anonymous. Stomach. Available from: http://gsm.utmck.edu/surgery/documents/Stomach.pdf [cited 2014 Oct 19].
8. Tapuria N, Kumar Y, Habib MM, dkk. Remote Ischemic Preconditioning: A Novel Protective Method From Cedera iskemia–reperfusi – A Review. J Surg Res2008;150:304–30.
9. Brzozowski T, Konturek PC, Konturek SJ, dkk. Ischemic Preconditioning of Remote Organs Attenuates Gastric Ischemia–Reperfusion Injury Throught Involvement of Prostaglandins and Sensory Nerves. EJP 2004;499:201–13.
10. Shah TA, Mauriello CT, Hair PS, et al. Clinical hypothermia temperatures increase complement activation and cell destruction via the classical pathway. J Translat Med. 2014;12:181.
11. Todd MM. Hindman BJ. Clarke WR. Torner JC. Mild intraoperative hypothermia during surgery for intracranial aneurysm. N Engl J Med. 2005;352:135–145.
12. Groysman LI. Emanuel BA. Kim–Tenser MA. Sung GY. Mack WJ. Therapeutic hypothermia in acute ischemic stroke. Neurosurg Focus. 2011;30:E17.
13. Gunn AJ. Cerebral hypothermia for prevention of brain injury following perinatal asphyxia. Curr Opin Pediatr. 2000;12:111–115
14. Shann F. Hypothermia for traumatic brain injury: how soon, how cold, and how long? Lancet. 2003;362:1950–1.
15. Yokobori S, Fratzen J, Bullock R. The use of hypothermia therapy in traumatic ischemic/reperfusional brain injury: Reviews of the literatures. Ther Hypothermia Temp Management. 2011;1(4): 185–92.