Ischemia/reperfusion injury and multiorgan dysfunction syndrome

Yeeta Moenadji,1 Waryanto Suharto,1 Wifanto S. Jeo,1 Retno A. Werdhani,2 Aria Kekalih,2 Aris Ramdhani,1 Benjamin Ngatio,1 Mohammad F. Ismet,1 Sinta C. Maulanisa.1
1 Department of Surgery, 2 Department of Community Medicine, 3 Training Program in Surgery, Faculty of Medicine Universitas Indonesia, dr Cipto Mangunkusumo General Hospital Jakarta.

Prologue
Ischemia is a most clinical entity found in surgical clinic and followed by multiorgan failure with high mortality rate. In many settings, this multiorgan failure is considered as the impact of sepsis.

Preface
In hypoxic cells, there’s increased production of injurious matters namely reactive oxygen species (ROS) despite attenuated internal (or, primary) antioxidant defense. Such a condition known as oxidative stress which, should it sustained, will be followed by cells injury. This cells/tissue injury may manifest temporarily lead to reversible organ dysfunction, or permanently lead to irreversible organ failure. Of interest is that such cellular injury is found in organs located at anatomical distances, concurrently and systemically.
In last decade, there’s a believe that gut is the motor of organs dysfunction (–failure). Such a believe was of the sepsis insight. Our study showed that endothelial cells is responsible for such multiorgan dysfunction (–failure).

1. Ischemia/reperfusion injury

Ischemia defined as disrupted perfusion due to lack of arterial flow lead to tissue/cells hypoxia. Inadequacy arterial flow may be found totally or partially due to either intraluminal or extraluminal etiology.1 Studies showed that hypoxemia found as oxygen saturation reaches a level which is less than 70%.2 With inadequate flow and consequent hypoxemia, oxygen uptake and utilization found to be diminished, metabolic alteration proceeded to be anaerobic lead to mitochondrial dysfunction with consequent no high energy phosphate (adenosine triphosphate, ATP) production.3,4 Following acute ischemia, cellular adaptation proceeded. In hypoxic membrane, with this structure remains, the membrane depolarized let electrical potential changed.3,5 Membrane’s ion channel in particular sodium pump and potassium channel were inefficient let substance unable to cross the membrane for an exchanges.6,7 Glucose may not able across hypoxic cells’ membrane lead to hyperglycemia – which is no correlation to diabetes mellitus – to be utilized as the main source of energy and in a hypoxic cytoplasm pyruvate may not be changed into acetyl coenzyme A of which is required for tricycles acid cycle to produce energy in aerobic atmosphere (ATP); paralleled to mitochondrial dysfunction in hypoxia.3,8,9 As result, serum lactate elevates leading to hyperlactatemia in addition to reduced pH, thus lead to metabolic acidosis.10,11 In such a condition, cellular metabolic activity is at the basal level, and hyperthermia found to be the consequence of energy insufficiency. Such a condition known as shock phase, or in metabolic perspective known as ebb phase.12 In these hypoxic cells, reactive oxygen species (ROS) which is normally found to be leaked during process of respiratory chain in mitochondrial inner matrix increased significantly.13–15 In contrast, in hypoxic cell all primary antioxidant of the first line including superoxide dismutase, catalase, and glutathione peroxidase, etc. which are produced in a normal cell were suppressed and unable to scavenges this ROS that increased in a great number.16 Imbalance of ROS production in hypoxia which is injurious with cellular antioxidant reservoir that provide protective effect known as oxidative stress.17–19

In certain period, cells may tolerate to such a change.20–24 This period is found in vary for different cells in a human body; depends on oxygen sensor gene (hypoxia inducible factor 1α, HIF1α) that control the respiratory chain activity in mitochondria.25–29 Should cellular perfusion restored in a short period, then the process is reversible. But should hypoxia continue in a longer period, there’s mismatch between ATP supply and demand and mitochondrial distress proceeded.30 Cytoplasmic changes including cells’ organelles within and all enzymes such as Na+/K+–ATPase.31 The process of membrane lipid peroxidation32 proceeds lead to ionic compartmentalization,33 i.e. a condition where there were influx of despite of efflux of extracellular ion2 as membrane’s integrity is no longer maintained. Such a condition known as cellular injury.34–36 So far, it is known occurred due structural protein breakdown (others preferred as ‘proteolysis’), which is purposed to provide energy (of non–carbohydrate carbon) – in a non–adaptive manner.37,38 As cells structure were broken, cellular changes were an irreversible one. Afterwards, condensation nuclei which is the characteristic of apoptosis might be found or cell lysis characterized necrosis as a logic consequences.39–42
Nevertheless, when the arterial flow has restored, and cellular perfusion is re-established, the products material of hypoxic cells particularly ROS which is toxic and injurious were unavoidably distributed to systemic circulation lead to cells injury; a condition known as reperfusion injury. It is postulated reperfusion injury lead to a more severe cellular injury rather than solely to ischemia as the injury found to be systemic despite local damage. There were studies on ischemia–reperfusion injury (I/RI) proceeded by directly ligating supply artery of an organ and re-perfused by release of ligation. Those studies have shown that cellular injury on I/RI is more severe. However, study focused on remote ischemia model remains less frequent.

Realizing that reperfusion injury is a serious issue following acute ischemia, there were studies focused on strategies in prevention the insult. Administration of antioxidant were reported to have efficacy to prevent reperfusion injury in animal model. Other studies showed that this reperfusion injury may be inhibited or at least to be minimized by implementation of either preconditioning or hypothermia. Preconditioning is to treat intermittent short periods of ischemia that addressed to let cells were adapted to have a true ischemia of a sufficient period. Even though there’s diversity in implementation of intermittent short period. However, another conditioning method implied following ischemia has been reported, namely postconditioning, or combination of both pre- and postconditioning. Whilst, hypothermia led to a lower energy demand, preserve superoxide dismutase that protect cells against ROS injurious effect, prevent increase of vascular resistance and attenuating inflammatory response by reducing neutrophil recruitment, thus, prevent no flow phenomenon in microcirculation.

Ischemia/reperfusion injury and multiorgan dysfunction

An experimental study carried out to find out the insults of ischemia of a significant anatomical distance to abdominal visceral organs, both solid and hollow organs, and endothelium of a great vessel of an animal model. The aim of a study was two. First, was to find the evidence of reperfusion injury due to remote ischemia. Second, to find out the evidence that ischemia may induce multiorgan failure. New Zealand white rabbits (Oryctolagus cuniculus) were used instead of rats or mice as the general physiology of rabbit is similar to that of humans, and rabbits suffer from many diseases with human equivalents. These rabbits were of 5 month old with 2.5 to 3.5 kg weight were scrutinized for eligibility, and feed well in a clean cage for a week adaptation prior to investigation in accordance to the use of animal as model.

The right limb ischemia was induced by ligation of right common femoral artery using silk 3.0 which was maintained for 4 hours period. The procedure of ligation was carried out under sedation anesthesia using ketamine intramuscular of 15–20 mg/kg body weight, in addition to 0.5 mg/kg body–weight diazepam intramuscular. Ischemia was confirmed by arterial oxygen saturation monitored in pulse oximetry. These artery ligated rabbits were set free in the cage. After 4 hours period the ligation was released, and 8 hours after ligation release these rabbits underwent laparotomy under anesthesia. We took specimens of left common femoral artery to find out endothelial injury, gastric and ileum to find out mucosal injury, and liver of three zones to find out hepatic injury (i.e. central zone of periportal, midzonal, and peripheral zones). After intervention, these rabbits were sacrificed in accordance with the regulation of veterinary research laboratory.

There were 24 rabbits enrolled, in accordance to calculation according to Federer, in consideration to 3Rs of an experimental study using animal. These subjects were divided into 4 groups, i.e. ischemia/reperfusion injury group, ischemic/preconditioning group, ischemic/hypothermia group, and control group. The procedure of ligation is as described in previous paragraph. Arterial oxygen saturation of 65–68.67% confirmed the ischemia. Arterial oxygen saturation of 98.0% confirmed that perfusion had been restored after ligation had been released.

The studies were focused on histomorphologic changes and oxidative stress. Histomorphologic changes were studied under light microscope on hematoxylin–eosin stained samples. Study on endothelial histomorphology proceeded under objective lens of 40 times on 10 high power field and addressed to find out endothelial injury in based on criteria used in the study on endothelial junction disassembly in critical burn. Grade of 0–4 had been employed, where 0 was no changes, 1 was mild disruption and 4 was the most severe involving all vessel’s wall. Study on gastric mucosa histomorphology was employing modified Mohammed criterin whereas grade 0 means no damage, grade 1 represents epithelial damage in mucosal surface, grade 2 represents grade 1 + damage of gastric pits, and grade 3 represents grade 2 + damage in gastric glandular layer. Study on ileal histomorphology employing scoring as follows: score of 1 referred to villi flattening, 2 is sub–epithelial vacuoles, 3 if congestion found, and 4 if lifting of epithelium noted. Study on hepatic tissue employing scores and classified into categories in accordance with classification of Knudsen et al., whereas grade 0 if no injury found, grade 1 should there were dilatation of hepatic sinusoidal vessels, grade 2 as there were focal necrosis on hepatic parenchyma, grade 3 if there were necrotic area in hepatic parenchyma of >10
high power fields, and grade 4 if there were injuries and necrosis on almost all area of hepatic parenchyma. To find out evidence of tissue oxidative stress, specimen were prepared for measurement of tissue malondialdehyde (MDA) level as surrogate marker, instead of serum MDA.\textsuperscript{14,79-81} Whilst, to find out evidence of cellular hypoxia tissue Hypoxia Inducible Factor (HIF)1α were measured. These parameters were statistically analyzed.

1. Study on endothelial changes in ischemia/reperfusion injury

We found endothelial injury of grade 4 in the femoral artery distal to ligation, of which was ischemic area. The ipsilateral vein distal to ligation (femoral vein) found injured of grade 3. The artery and vein of long distance of circulatory perspective (in this study were left common femoral artery and vein) were both found injured of grade 3. This finding somehow showing an insult of reperfusion injury.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Distribution of histomorphologic scores of endothelial injury in ischemia/reperfusion injury. I/R: ischemia/reperfusion injury.}
\end{figure}

Mean endothelial HIF1α increased in artery distal to ligation 0.290–0.660 (0.423 ± 0.205) (control 0.128) denoting hypoxia in ischemic area and 0.290–0.660 (0.423 ± 0.205) in artery contralateral to ligation. Whilst, in vein distal to ligation mean HIF1α was 0.169–0.315 (0.218 ± 0.083) (control 0.156) and in vein contralateral to ligation was 0.290–0.660 (0.423 ± 0.205). Meaning, signals of hypoxia were delivered to systemic circulation. Endothelial cells MDA level were increased in all samples, both of right common femoral artery [0.140–0.815 ng/mL (0.381 ng/mL ± 0.557)] and vein [0.073–0.326 ng/mL (0.147 ng/mL ± 0.094)] distal to ligation and both of left common femoral artery [0.017–0.089 ng/mL (0.042 ± 0.025)] and vein [0.030–0.094 ng/mL (0.056 ng/mL ± 0.026)]. In analysis, we found no significant difference between left and right common femoral artery, so it was veins. We also found that endothelial cells MDA level in right common artery distal to ligation was not differed significantly to those in vein.\textsuperscript{82}

Endothelial injury is thought to be responsible for a more severe insults of reperfusion injury rather than ischemia itself. Injured endothelium provokes neutrophil recruitments and further inflammatory cascade leading to a systemic response to injury. It was emphasize that the injury was not in local area of ischemia solely, but to be systemic.\textsuperscript{83} Following 4 hours ischemic period and re–perfused for 8 hours, we found endothelial lining were found disintegrated followed by edema denoting endothelial hyperpermeability. Such a condition suggesting endothelial apoptosis.\textsuperscript{84,85}

Should endothelial cells be defective, the more platelet adhered even to the basal membrane (extracellular matrix, ECM) of which contains collagen fibers and von Willebrand factor (VWF). Adhesion of platelets either directly or indirectly to collagen lead to thrombosis, which is led to plugging arterial lumen that worsen arterial flow, in addition to activation of complement factor of fibrinolytic property.\textsuperscript{86} The cascades referred to domino effect in the development of sepsis.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Production of reactive oxygen species and proposed mechanism of endothelial hyperpermeability. Increased intracellular ROS lead to cytoskeletal contraction (actin F dissociation) as well as dissociation of three endothelial junctions led to vascular leaks.}
\end{figure}
Figure 3. Activation of von Willebrand factor (vWF) in endothelial injury leading to thrombus formation. ECM: extracellular matrix, vWF: von Willebrand factor.

Nitric oxide pathway is activated lead to relaxation instead of vasoconstriction – or in other word, decreasing vascular resistance – let the flow decreased significantly.87–90 Platelet–dependent arterial thrombotic responses, then, are both a cause and a consequence of excessive oxidant stress in the vasculature, and arterial thrombotic disorders are their clinical counterpart. NO, derived both from the endothelial cell and the platelet, modulates platelet activation, adhesion, and aggregate formation, thereby serving as an important deterrent to platelet–mediated arterial thrombosis.87

2. Study on gastrointestinal mucosa in ischemia/reperfusion injury

a. Gaster

What we found in the study on gastric mucosa was disintegrated epithelial lining in addition to oxidative stress. We noted epithelial changes in gastric corpus [median of 1.5 (0–3)] were found at a lesser grade than the antrum [median of 2 (1–3)], though there were no difference significantly to control.91 Tissue MDA level were increased both in antrum and corpus with no significant difference to control.91 Tissue HIF1α was found slightly increased.

Injured parietal cells in the gastric corpus produces less gastric acid than normal, in the other hand, injured enteroendocrine cells in the antrum produces less gastrin as the logic consequences. So far, studies have shown that gastrin expression is independent to HIF1α, meaning gastrin production is not affected despite hypoxia.92 Thus, there’s remain an unanswered question in disrupted gastric homeostasis based on defense and invasion theory.93

Figure 4. Tissue Malondialdehyde (MDA) level of ischemia/reperfusion injury (I/R) of gastric mucosa

Figure 5. Histomorphologic changes of gastric epithelial injury in ischemic/reperfusion injury.
b. Ileum

Ileum is the vulnerable intestine of most, in contrast to previous concept that believe duodenum is. Unlikely duodenum which is well vascularized by at least nine branches of supply arteries, ileum which is a lymphoid tissue supplied by vasa recta is less tolerate to hypoxia. Studies have shown that M cells covering Peyer’s patches were abundantly found in ileum denoting ileum as the most part of intestine greatly deals with inflammatory response and infection. Since these two conditions require more energy, intestinal mucosa becomes more vulnerable to hypoxia. The scores of villi damage of ileum was ranged of 7–9 (7.42 ± 0.78) whereas in duodenum were of 4–7 (5.33 ± 1.53) and showed different significantly (p 0.05). Ileal tissue HIF1α increased (0.506 ng/mL, control 0.112 ng/mL) showing a difference with those in duodenal (0.259 ng/mL). Whilst, ileal MDA were found more less near to normal (0.013 nmol/mL ± 0.006, control of 0.012 nmol/mL) so were duodenal (0.014 nmol/mL).

Figure 6. The features of ileal villi histomorphologic changes in ischemic reperfusion injury. H-E stained samples under objective lens with magnification of 100 times. A. The feature is showing flattening ileal villi (arrow). B. Sub-epithelial vacuoles crowd the villi. C. Villi congestion. D. Lifting of ileal epithelial of mucosa.

Immunohistochemistry of occludin showed that level of occludin decreased slightly both in duodenum (0.175 nmol/mL+, control 0.210 nmol/mL) and ileum (0.291 nmol/mL+, control 0.324 nmol/mL). Tissue MDA level of duodenum ranged of 0.004–0.018 (0.013+0.007) and found to be increased slightly compared to control. MDA in Ileum ranged of 0.004–0.022 (0.013+0.007), also found to be increased slightly compared to control. Both were found differed not significantly (p = 0.075).

A review by Zeitouni has shown that oxygen content in the epithelial cells of intestinal mucosa is less than 2%, thus the margin of hypoxia was critical. Thus, minimal changes of arterial oxygen content may lead to decreased epithelial oxygen content. But this study has shown that at a point, we may have found histomorphologic changes particularly in villi with minimal functional changes.

Figure 7. Intestinal oxygen gradient. Despite highly vascularized and oxygenated arterial flow (80–100%), the oxygen level of is found in a gradient from sub-epithelial mucosa (4–8 %), across the epithelial and mucus layer (2–4 %), and into the severely oxygen–deficient lumen (<2 %).

3. Study on hepatic tissue in ischemia/reperfusion injury

In hepatic reperfusion injury we found sinusoid dilatation (grade I) of most, followed by focal necrosis (grade 2), and necrosis of hepatic parenchyma (grade 3) of the three hepatic zones (i.e. central, midzonal, and peripheral zone). Histopathologic changes in central zone was found of 2 (1–2), control 0 (0–1) showed a difference with p value 0.028, in midzonal was 2 (1–3), control 0 showed a difference with p value 0.012, and in peripheral zone 1 (0–2), control 0 showed a difference with p value 0.030. Hepatic tissue MDA increased (0.032 nmol/mg ± 0.007) showing significant difference to control (0.005 nmol/mg ± 0.002) with p 0.012 (sig α <0.05).

These studies on multiorgan have shown histomorphologic changes and oxidative stressed endothelial cells both in artery and vein contralateral to ischemia, epithelial cells of vissus (gastric, duodenum, ileum) and solid organ (i.e. hepatic tissue). Another finding of the study which was not reported was oxidative stress of brain parenchyma. Mean brain tissue MDA level was 0.003±0.0008, and tissue HIF1α of 0.331–0.549; both were found increased. It was clear that these findings show a concurrent multiorgan failure following eight hours reperfusion after four hours period of ischemia due to ligation of left common femoral artery. There’s no doubt that these organs’ cells injuries were found after the product of hypoxic tissues which were injurious matters were released to systemic circulation.
Ischemic/preconditioning and ischemic/hypothermia

Despite the administration of external (secondary) antioxidant or anything addressed to enhance internal (primary) antioxidant defense, ischemic/preconditioning and ischemic/hypothermia have shown its efficacy as the strategies to reduce the insult of reperfusion injury. IPC confers resistance to ischemia thus induces cells tolerance to ischemia and reduce energy demand. Whilst, hypothermia inhibits the rate of cell death so that might be a modality in management of traumatized patients aimed to prevent more tissue damage. Studies showed the merit of both ischemic/preconditioning and ischemic/hypothermia in prevention of organ damage in model of direct ischemia. Thus, we continued the study to find out the efficacy of ischemic/preconditioning and ischemic/hypothermia to prevent or to attenuate the insult of reperfusion injury in these multorgan.

In ischemic/preconditioning group, the artery was clamped using bulldog clamp for two minutes period and released for another three minutes period in two cycles prior to definitive ligation as in the first group. The procedure of ischemic/hypothermia carried out by ice application on right limb distal to ligation which was wrapped and kept for four hours period. The temperature was maintained at of 31–33 degree Celsius.

1. Study on endothelial changes in ischemic/preconditioning and ischemic/hypothermia

In preconditioned and hypothermic group, endothelial injury of right common femoral artery distal to ligation were found like those in ischemic. However, efficacy of preconditioning was shown in right common femoral vein as found in control, of which showing significance different to those in ischemic/reperfusion group.

Hypothermia also showed it efficacy, though it was less than preconditioning did. Through mechanisms described earlier, adopted cells to hypoxia as well as of low metabolic activity, thus ROS produced in a significant lesser numbers with consequent less insults.

2. Study on gastric mucosal changes in ischemic/preconditioning and ischemic/hypothermia

On the study we found gastric mucosal changes in both of ischemic/preconditioning and ischemic/hypothermia were lesser than ischemic/reperfusion injury, though we found no significant difference. In the study on oxidative stress, mean gastric mucosal MDA both of ischemic/preconditioning and ischemic/hypothermia significantly differed to ischemia/reperfusion. There was no significant difference between ischemic/preconditioning and ischemic/hypothermia.
3. Study on ileal villi changes in ischemic/preconditioning and ischemic/hypothermia

Mean ileal histomorphologic changes in ischemic/hypothermia were found lesser than ischemic reperfusion injury significantly (p = 0.002), but not in ischemic/preconditioning. Occludin of tight junction decreased in all conditions (control 0.324, ischemia reperfusion 0.291, ischemic/preconditioning 0.256, and ischemic/hypothermia 0.224) denoting preserved synthesis, but not synthesis or disassembled molecular junction. Mean villous MDA 0.013 ± 0.006, in ischemic/preconditioning 0.024 ± 0.0084, in ischemic/hypothermia 0.036 ± 0.009. Ischemic/hypothermia stressed lesser than ischemic/preconditioning.

4. Study on hepatic changes in ischemic/preconditioning and ischemic/hypothermia

Rather than ischemic reperfusion, ischemic/preconditioning and ischemic/hypothermia showed lesser injuries. The lesser one found in ischemic/preconditioning indicated tissues were well protected. An intermittent and short ischemic/reperfusion process shown to be effective to let cells were adapted adequately before a true ischemia as during this brief ischemic/reperfusion process as ROS produced in a lower dose despite MDA as metabolic end–product; of which would be less injurious. This low–dose ROS activates the release of NO, catecholamine, adenosine, bradykinin and intracellular kinase directly and induces the synthesis of protective proteins. ROS also induces NF–κβ, which induces the transcription of iNOS mRNA in 24 hours, which provides delayed protection to target organs. These protective effects are also provided by endogenous prostaglandins which consists of cyclooxygenase–1 and cyclooxygenase–2, the role of adenosine on A1 receptor, and sensory nerves. As vasodilator, NO has protective effects of anti–apoptosis, prevent platelet aggregations, and diminish inflammatory responses.

Clinical implementation

Ischemia led to cells injury of organs at anatomical distance known as reperfusion injury. Injurious matter of hypoxic cells following ischemia released to multiorgan let the cells experiencing oxidative stress though histopathologic changes were not a dominant issue. An intermittent ischemic period has shown its protective effect rather than hypothermia; thus, ischemic/preconditioning may be applied as a strategy in tissue or organ transplants. However, it is not suitable in trauma. Otherwise, ischemic/hypothermia is likely showing a merit in trauma cases.

Disclosure

Authors disclose no conflict of interest.

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