Gut Failure and Ischemia / Reperfusion Injury: Ileum or Duodenum?  
An Experimental Study on Orzyctolagus cuniculus

Preliminary Report

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Abstract

Introduction. Ischemia reperfusion injury following hypoxia/hypoxemia remains a problem in clinic of surgery, referred to a silent leading factor to high morbidity and mortality. Such a condition made an impact to visceral organ in particular gut let its function deteriorated and followed by gut dysfunction and failure shortly, and multisystem organ failure at a later date. It is postulated that ileum is a part of intestine prone to intestinal failure earlier than any other parts, for instance: duodenum. However, available data remains scanty as there no publication found. Thus, we run a preliminary study aimed to find out intestinal damage following artificial ischemia.

Method. We run an experimental study on New Zealand White (Orzyctolagus cuniculus) (n=7) by induction of ischemia. Ligation of common iliac artery is carried out for 4 hours and let the subjects live in the cage for next 8 hours. The subject is then sacrificed and a full thickness ileum and duodenum is taken through laparotomy. The samples taken referred to the object of histopathology analysis using simple staining of Hematoxylin and Eosin. Villi damage is the main focus of such a study. Such damage is assessed using scoring system based on modified from criteria of Pusponegoro.

Results. Of the seven subjects enrolled, the histopathology exams were carried out under light microscope with objective lens magnification of 40 and 100 times. The scores of intestinal villi damage found to be minimal (5.3) compared to ileum (7.4).

Conclusion. Histomorphological changes found in both of duodenum and ileum, and severe disconfiguration of intestinal villi found in the ileum. J I Bedah Indones. 2015;44:10-13.

Keywords: ischemia/reperfusion injury, ileum, duodenum.

Introduction

The impact of ischemia reperfusion injury (I/RI) on gut Ischemia reperfusion injury following hypoxia/hypoxemia remains a problem in clinic of surgery, referred to a silent leading factor to high morbidity and mortality. The basic concept of reperfusion injury is that products of anaerobe metabolism proceeded during ischemia/hypoxia including reactive oxygen species in hypoxic area just releases to systemic circulation and provokes cytokines release in affecting region.

Unfortunately, it is not just a loco–regional problems but systemic. As these injurious cellular products released to systemic circulation, the phenomenal remote cellular (organ) damage is noted and followed by multisystem organ failure.1,2

Studies showed the impact of ischemia particularly on gut referred to precipitating factor of systemic inflammatory response syndrome (SIRS) and with its continuum multisystem organ failure.3,4 However, on the former studies induced artificial ischemia was just treated directly to particular supply artery (in terms of ‘ischemia’, I) but unfortunately there’s found no published works of ischemia induced focusing on the impacts on remote organs (in terms of ‘reperfusion injury’, RI).5,6

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Referred to a proposed concept of former studies of Nieuwenhuizen and Goris as well as Leaphart and Tepas III 'gut is a motor of multisystem organ dysfunction'\textsuperscript{3,4} then we run a study to find out the impacts of I/RI on gut. The next question should be answered before we run a study is which part of gut should we focus on? It is postulated that ileum is an anatomical part of intestine prone to an intestinal failure earlier than any other parts, for instance: duodenum. However, available data regarding this susceptibility remains scanty as there no publication found questioning such a simple thinking. Hence, we run a preliminary study aimed to find out intestinal damage following induction of ischemia. This is a main study on hypoxia, particularly in I/RI in Department of Surgery, Cipto Mangunkusumo Hospital (RSCM), Faculty of Medicine Universitas Indonesia (FMUI). The objective of such a study is to find out histomorphology changes on ileum and duodenum as I/RI impact. Through these findings, the risks of gut dysfunction and gut failure following I/RI should be anticipated and prevented and managed at the early date as the best practice in the clinical setting.

Method.

An experimental study run on New Zealand White rabbit (NZW, \textit{Oryctolagus cuniculus}, \textit{n} = 7) with artificial induced ischemia. A certified male NZW of 5 month aged and 2.5–3 kg weighted is being adapted in a normal nourished in suitable temperature for a week prior to treatment according to the protocol of the laboratory of veterinary research, Institut Pertanian Bogor (IPB). In general, anorexia, hypersalivation, nasal discharge in order to control influencing factors.

Table 1. Subject characteristics

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<th>Characteristic</th>
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<td>Weight (kg)</td>
<td>2.5-3.5</td>
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<tr>
<td>Age (month)</td>
<td>5.0</td>
</tr>
<tr>
<td>Room temperature (°C)</td>
<td>25-26</td>
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<td>Frequency of meal (x/day)</td>
<td>3-4</td>
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The reason why rabbit is a chosen subject is that the caliber of vessels larger than mice, in addition to former studies has been proceeded using rabbit as a subject.

Induction of ischemia through ligation of common iliac artery using absorbable suture (polyglactin) of 3.0 is carried out for 4 hours and let the subjects live in the cage for next 8 hours. The subject is then sacrificed and a full thickness ileum and duodenum is taken through laparotomy. A 3 cm long ileum, 5 cm distance from ileoaccaal valve and 3 cm long duodenum post–pyloric of a 2 cm distance and fixed using formaldehyde of 10% solution in a tube for paraffin block. Following sample preparation and standard staining using hematoxylin eosin, the samples are object of analysis. Villi damage is the main focus of such a study. Such damage is assessed using scoring system based on modified from criteria proposed by Pusponegoro in 2004.\textsuperscript{7} The committee of ethic, Faculty of Medicine Universitas Indonesia approved this study (No. 495b/H2.F1/ETIK/2014).

Table 2. The scoring system based on modified criteria proposed by Pusponegoro

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<th>Scoring</th>
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<td>1</td>
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<tr>
<td>Blunting of vili</td>
<td>0%</td>
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<tr>
<td>Vacuolization of superficial epithelium</td>
<td>0%</td>
</tr>
<tr>
<td>Congestion</td>
<td>0%</td>
</tr>
<tr>
<td>Lifting of superficial epithelium</td>
<td>0%</td>
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Results

Those samples with blunting of villi, vacuolization of superficial epithelium, congestion, and lifting of superficial epithelium are seen in figure 1. Such scores of histomorphological changes in ileum ranged of 7-9 (mean 7.4 ± 0.79, mode 7) whilst duodenum ranged of 4-7 (mean 5.3 ± 1.53, mode 4). Statistical analysis using one sample t–test showing difference between the two groups with \( p < 0.05 \). This is showing that predominant villi histomorphological changes found in samples of ileum.
Figure 1. Histomorphological changes of duodenum and ileum on I/RI using H–E with objective magnification of 100 times. Left showing intestinal villi congestion and flattening. Right showing subepithelial vacuoles filled up the stroma of intestinal villi; lifting of epithelial mucosa is shown.

Discussion

In the samples of small intestine, both of ileum and duodenum are indicated histomorphological changes following 4 hours’ period of induced artificial ischemia by ligating the left femoral artery using absorbable suture (polyglactin) 3.0. Although in this study the factors responsible to such changes such as hypoxia and its products have not been proven yet through biochemical assay, the hypothesized remote organ ischemia and reperfusion injury is somehow proven through the evidence of intestinal villi histomorphological changes.

Based on the theory, we are now believed that ischemia just followed by reperfusion injury lead to remote organ injury. Furthermore, this simple preliminary study has proven that intestinal villi is an organ prone to such reperfusion injury at the early date; in this study, induced ischemia is maintained as much as 4 hours. Using scoring system, which is modified from the criteria proposed by Pusponegoro, we observed the histomorphological changes by all means in 8 hours of ischemic period.

Based on this scoring system, we found that the mean ileal villi of $7.4 \pm 0.79$ to maximum severe changes, disconfiguration of 12 maximum scale; is somehow severe than duodenum (mean $5.3 \pm 1.53$) and the difference is statistically significant. There’s no explanation of why the villi of ileum is more susceptible to ischemic, explicitly. Perhaps an anatomical as well as physiological approach can explain it better.

The duodenum is readily distinguished from other regions of the small intestine by the presence of submucosal Brunner’s glands, which may pack the submucosa so completely that the typical submucosal connective tissue is obscured. This Brunner’s glands provide abundant alkaline mucus to neutralize the acid contents entering from the stomach. Brunner's glands and the stomach's pyloric glands thus share a similar function. In fact, Brunner's glands look like pyloric glands that have enlarged and expanded into the submucosa of the duodenum. This alkaline atmosphere of duodenum is likely holds the villi on the atmospheric changes of acidemia found following hypoxemia. In addition, the villi are rather flatter in the duodenum than in the jejunum or ileum, and plicae are less frequent. In the other hand, the ileum has proportionally more goblet cells than more proximal sections of the small intestine. (The proportion of goblet cells generally increases as one progresses down the GI tract, with the highest proportion found in the lower tract).

The innermost layer, the mucosa, contains epithelial cells whose function is specialized for the absorption of nutrients from chyme in the lumen. The ileum also displays an increase in the amount of mucosal lymphoid tissue, which forms conspicuous clusters of lymph nodules, called Peyer's patches. These patches dot the surface of the mucosa to detect the presence of pathogens in the
chyme. At this point it is realized that, in spite of absorption particularly for lipid, the ileum is somehow a part of gut associated lymphoid tissue that is responsible to the innate immune system of gut. Any defect due to pathologic condition lead to intestinal dysfunction at the early date followed by bacterial translocation and so on. There’s also information regarding the healing process that last longer in ileum rather than upper GI parts.

We have just concluded that intestinal villi histomorphological change is found following induced artificial ischemia, which is a kind of reperfusion injury. Ileum is found to be susceptible earlier than duodenum.

Reference