CHAPTER 1 INTRODUCTION

1.1 Background of Research

Ischemia can occur in a variety of disease states or trauma as well as with a variety of surgical procedures such as vascular and endovascular surgery or organ transplant surgery. In any of these, an ischemic injury occurs when absent or greatly diminished blood flow to the tissue beds occurs for a prolonged period of time. With the initial ischemic insult, there is a conversion of muscle metabolism from aerobic to anaerobic which leads to ATP depletion and leakage of extracellular calcium into the muscle cells (Eliason and Wakefield, 2009). This condition will cause abnormalities in metabolic (Cowled and Fitridge, 2011), thrombosis (Blaisdell, 2002), and induce a proinflammatory state in the tissue beds (Collard and Gelman, 2001).

Reestablishment of blood flow, known as reperfusion, is essential to salvage ischemic tissues. However, reperfusion itself paradoxically causes further damage, threatening fuction and viability of the organs (Cowled and Fitridge, 2011). This reperfusion stage promotes proinflammatory mediators as well as reactive oxygen species (ROS) which aggravates the initial injury. This phenomenon is termed ischemia-reperfusion injury (I/R) or reperfusion injury. If severe enough, the inflammatory response after I/R may even result in the systemic inflammatory response syndrome (SIRS) or multiple organ dysfunction syndrome (MODS) (Blaisdell, 2002; Eltzschig and Collard, 2004).

According to Deitch (1992), Deitch and Goodman (1999), and also Collard and Gelman (2001) irrespective of the triggering effect, MODS follows a predictable course, generally beginning with the lungs and progressing to liver, gastrointestinal tract and kidney. Bone marrow and cardiac failure are late components of MODS, whereas neurologic manifestations can occur early or late.

The pulmonary system is the most frequently injured organ in MODS. Lung injury may rapidly progress to respiratory failure and acute respiratory distress syndrome (ARDS) (Collard and Gellman, 2001). The release of all the pro-inflammatory mediators result in inflammatory response which then promotes the sequestration of primed neutrophils in the pulmonary vasculature and followed by the formation of hyaline membranes, proteinaceous debris and thickening of alveolar wall which are the hystopathological evidences of lung injury (Barry, 2009). In this context, and frequently observed in medicine, reperfusion syndromes may become the limiting factor in clinical and /or surgical interventions and /or treatments (Van Poucke *et al*., 2006).

Previous studies have demonstrated various drugs to reduce I/R after aortic cross clamping (Hsu *et al*., 2011; Kao *et al*., 2011; Bozok *et al*., 2012). There are also some recent studies about the effects of hyperbaric oxygen therapy (HBOT), a therapy using 100% oxygen at increased atmospheric pressure in a special room, in limiting the consequences of I/R in various tissue. They stated that HBOT has beneficial effects in limiting the consequences of I/R in various tissues by attenuating proinflammatory mediators and oxidative stress induced by I/R (Kihara *et al*., 2005; Chu *et al*., 2007; Bozok *et al*, 2012; Zhou, 2012). Until now,

there is not any specific starting time for giving hyperbaric oxygen therapy to the patients with the risk of lung injury after ischemia-reperfusion. But according to Kihara *et al.* (2005) in the previous study, HBOT is effective up to 3 hours after reperfusion started and does not show any clear effects when performed more than 3 hours after reperfusion.

Based on the background above, this research entitled the effect of hyperbaric oxygen therapy starting time on lung histopathological changes after ischemia-reperfusion injury in the hind limb of rabbits (*Oryctolagus cuniculus*) as animal model has been conducted to prove the effect of hyperbaric oxygen therapy starting time to lung hystopathologycal changes after ischemia-reperfusion in the experimental animal used.

1.2 Problem Statement

How is the effectiveness of hyperbaric oxygen therapy starting time on lung histopathologycal changes after ischemia-reperfusion in the hindlimb of rabbit?

1.3 Theoretical Base

During ischemia, cellular ATP is degraded to form hypoxanthine and xanthine dehydrogenase converted to xanthine oxidase which results in the intracellular accumulation of hypoxanthine. Within the endothelium, ischemia promotes expression of certain proinflammatory gene products (*e.g.*, leukocyte adhesion molecules, cytokines) and bioactive agents (*e.g.*, endothelin, thromboxane A_2) while repressing other “protective” gene products (*e.g.*, constitutive nitric oxide

synthase, thrombomodulin) and bioactive agents (*e.g.*, prostacyclin, nitric oxide), therefore making a proinflammatory state (Maxwell and Lip, 1997; Carden and Granger, 2000; Collard and Gelman, 2001). The early ischaemic phase also activates transient eNOS which induce an initial surge of nitric oxide (NO) level (Cowled and Fitridge, 2011).

When oxygen is reintroduced during reperfusion, conversion and degradation of the excess hypoxanthine to uric acid by xanthine oxidase results in the formation and liberation of toxic reactive oxygen species (ROS) (Collard and Gelman, 2001; Cowled and Fitridge, 2011). There is also a general decline in endothelial function and loss of functional eNOS during early reperfusion, so that NO production falls, along with an increased production of ROS. ROS then cause peroxidation of the lipid structures of the cell membranes resulting in the production and systemic release of proinflammatory eicosanoids, disruption of cell permeability and ultimately cell death. ROS also activate endothelial cells, elevating the activity of the transcription factor, NF-κB, increase leukocyte activation, chemotaxis, and leukocyte-endothelial adherence (Collard and Gelman, 2001; Cowled and Fitridge, 2011). Once activated, the endothelial cell produces E-selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), endhotelial-leukocyte adhesion molecule-1 (ELAM-1), plasminogen activator inhibitor-1 (PAI-1), tissue factor and interleukin-8 (IL-8). IL-8 is a potent neutrophil chemotactic and activating factor in response to IL-1, tumour necrosis factor-alpha (TNF-α), endotoxin, histamine and hypoxia (Cowled and Fitridge, 2011).

During reperfusion, plasma thromboxane A_2 increases thus promoting vasoconstriction and platelet aggregation which coincide with a rapid rise in pulmonary artery pressure. Leukotrienes are also synthesized thus increase vascular permeability and enhance smooth muscle contraction, resulting in vasoconstriction. The lung also produces leukotrienes following reperfusion which result in increased permeability, transient pulmonary hypertension and the activation of the endothelium to produce thromboxane. The expression of numerous cytokines are also induced in this phase systematically, including TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and platelet activating factor (PAF), in association with NF-κB (Cowled and Fitridge, 2011).

Complements (iC3b and C5b-9) are activated and several proinflammatory mediators (anaphylatoxins, C3a and C5a) are also formed in this phase, altering vascular homeostasis, activating endothelial NF-κB and promotes leukocyte activation and chemotaxis. Chemotactic cytokines (IL-1, TNF-α) and ROS all induce neutrophil adherence to the endothelium by interacting CD11b/18 complex on activated neutrophils to interact with ICAM-1 on the surface of the endothelial cell to mediate firm adhesion of neutrophils then transmigrate it into the interstitial compartment (Collard *et al*., 1999; Collard and Gelman, 2001; Cowled and Fitridge, 2011). The release of all these pro-inflammatory mediators result in inflammatory response which then promotes the sequestration of primed neutrophils in the pulmonary vasculature and followed by the formation of hyaline membranes, proteinaceous debris and thickening of alveolar wall (Barry, 2009).

Previous studies have proposed that hyperbaric oxygen therapy (HBOT) can attenuate neutrophil and pro-inflammatory mediators as important factors in ischemia-reperfusion injury (I/R) (Khiabani *et al*. 2008; Zhou *et al*., 2012) and I/R-induced lung injury (Bozok *et al*., 2012). A study by Rubinstein *et al*. (2009) also presented that HBOT has antioxidative capacity, thus attenuating the oxygen-free radicals. Khiabani *et al*. (2008) shown that HBOT inhibits I/R-induced neutrophil adhesion by blocking CD18. HBOT also was demonstrated inhibiting TNF-α, thus reducing neutrophil infiltration (Chu *et al*., 2007). Meanwhile, Kihara *et al*. (2005) proposed that HBOT is effective up to 3 hours after reperfusion started.

1.4 Aim of the Research

To prove the effectiveness of hyperbaric oxygen therapy on lung histopathologycal changes after ischemia-reperfusion in the hindlimb of rabbits regardless of the starting time.

1.5 Benefit of the Research

The benefits of this research are:

1. To decrease the mortality rate of patients with ischemia-reperfusion injury which lead into multiple organs dysfunction, especially in lung.
2. To give a picture about how the hyperbaric therapy can increase the recovery level of multiple organs dysfunction in spite of the starting time, especially in lung.

3. As an input to involved parties, doctors and /or researchers, notably to get the successful way to cure or help cure ischemia-reperfusion injury.
4. Increase knowledges in the field of health as well as provide information for further research.

1.6 Hypotheses of Research

The use of hyperbaric oxygen therapy can reduce lung histopathologycal changes after ischemia-reperfusion in the hindlimb of rabbits regardless of the starting time.