Gastric Ulcers Induced by Systemic Hypoxia


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ABSTRACT

Aim: to assess the effect of systemic hypoxia on gastric mucosa and the activation of stress-responsive transcription factors induced by hypoxia.

Methods: in this experimental study, rats were allocated to control and experimental groups. The experimental group was divided into subgroups and subjected to hypoxia conditions for 1, 7, 14 or 21 days. Afterwards, histopathological evaluation and study of the protein expression of the gastric mucosa were performed.

Results: the results showed that longer exposure to hypoxic conditions leads to more severe gastric ulceration. Twenty-four hours after induction, 60% of rats had developed gastric ulcers. Seven days after induction, 80% of rats developed gastric ulcers. In the 14-day and 21-day hypoxia conditions, epithelialization (a sign of gastric ulcer healing) was observed. Evaluation of the average ulcer depth on the day of treatment showed that the greatest depth was on day 7, and the shallowest was on day 21 of treatment. Western blot analyses demonstrated that systemic hypoxia resulted in the expression of heat shock factor (HSF) and heat shock protein 70 (HSP-70), which were highest on day 7 and then regressed gradually. In control, HSF-1 and HSP-70 were not detected by Western blot analysis in the control group (normoxia).

Conclusion: in this study, systemic hypoxia caused gastric ulcers, and during the time of exposure to hypoxia, an adaptation process in the form of gastric epithelialization occurred in the rats. This development of gastric lesions was in line with the expression pattern of HSF-1 HIF-1α and HSP-70.

Key words: gastric ulcer, systemic hypoxia, heat shock factor expression.

INTRODUCTION

Gastric mucosal lesions are defects in the gastric system that extend through the muscularis mucosae. Helicobacter pylori infection, Non-steroidal anti-inflammatory drug (NSAID) use, stress ulcers and acute mucosa-damaging lesions are the most common aetiologic factors. Under normal conditions, a physiological balance exists between peptic acid secretion and gastroduodenal mucosal defence. Mucosal injury and subsequent peptic ulcers occur when the balance between the aggressive factors and the defensive mechanisms is disrupted. The imbalance between aggressive and defensive factors determines the outcomes of the gastric lesions that result from these common causes. The imbalance depends on whether there is an increase in aggressive factors or a decrease in defensive factors. The defensive mechanisms include tight intercellular junctions, mucus and bicarbonate, gastric mucosal blood flow, cellular restitution and epithelial renewal. The decrease in these mechanisms can be induced by many factors, including hypoxia. Prostaglandin enhances these protective mechanisms, and it is believed to be a major gastric mucosal defensive factor. Heat shock proteins (HSPs) have proved to be an equally effective key protective mechanism.

Cells respond to stressful conditions by activating genetic programmes whose evolutionarily conserved mechanisms have common ancestral origins, from the simplest bacteria to complex organisms, including humans. In recent years, one such genetic programme that has gained increased attention involves the families of HSPs. HSPs, also called stress proteins, are a group of proteins that are present in all cells in all life forms. They are induced when a cell undergoes various types
of environmental stress such as heat, cold and hypoxia.

Although many HSPs have multiple roles, the most important to date appears to be the capacity to act as protein chaperones or “molecular guardians” that protect vital protein structures and functions.\(^8\) Augmentation of HSP synthesis is tightly regulated by stress-inducible heat shock factors (HSFs), which are part of a transcriptional signalling cascade with both positive (e.g., HSP) and negative (e.g., proinflammatory cytokines) properties.\(^9\) HSP-70 contributes to a wide range of folding processes, including the folding and assembly of synthesized proteins, refolding of misfolded and aggregated proteins, membrane translocation of secretory proteins and control of the activity of regulatory proteins.\(^10\)

The aim of this study was to examine whether hypoxia could induce gastric ulcers in an animal model and to identify gastric mucosal changes in hypoxia conditions by histopathological and molecular evaluation. We also investigated the influence of HSP to determine the role of HSF in the gastric mucosal lesion process.

**METHODS**

**Animal Model**

This study was conducted with male Sprague-Dawley rats (8 weeks, 150–250 g). Rats were housed in metal cages with food and water ad libitum. Animals were separated into two groups: control and experimental. The experimental group were exposed to hypoxia conditions of 10% oxygen and 90% nitrogen. This group was divided into a further four groups, according to the length of exposure to the hypoxia condition: 1, 7, 14 and 21 days. At the end of their respective periods of hypoxia, the rats were anaesthetized, and blood was collected from their hearts for blood gas analysis. Next, gastric sections were removed from the animals and divided into two groups; one was frozen at –80°C for the study of protein expression, and the other was processed for histopathological evaluation.

The number of experimental animals in this study was determined with the Federer formula:

\[
(t-1)(n-1) > 15
\]

Where \( t = 5 \) is the number of treatments, and \( n \) is the number of samples per treatment group. With this formula, the number of samples obtained for each group was 5 rats.

The Research Ethical Committee, Faculty of Medicine, University of Indonesia, approved this experimental study.

**Histopathological Evaluation**

The gastric sections were embedded in paraffin, stained with haematoxylin and eosin and observed using a light microscope. A histopathology evaluation was performed, including the examination of the presence or absence of ulcers, ulcer depth, inflammatory cell infiltration and the occurrence of epithelialization of the gastric samples of each rat.

Histopathologically, ulcers are defects in the gastric mucosa; they involve the entire thickness of the mucosa and penetrate through the muscularis mucosae. Ulcers can penetrate up to the muscularis, submucosa or even the lamina propria. The ulcer depth was measured using scales installed in the microscope. The depth of an ulcer is measured from the base of the ulcer to the mucosal surface with a unit of \( \mu \text{m} \). Epithelialization is a process of closure of the ulcer with epithelial cells and is an important factor in the ulcer healing process. Epithelialization is characterized by the presence of epithelial cells accumulated on the mucosa.

**Nuclear Extraction**

The Wizard Genomic DNA Purification Kit (Promega, Madison WA, USA) was used for nuclear extraction. First, 600 µl nuclear lysis solution was put into a 15 ml centrifuge tube and placed on ice, then 10–20 mg of the gastric tissue was added, and the solution was homogenized for 10 s. The formed lysate solution was then transferred to a 1.5 ml microcentrifuge tube and incubated at 65 °C for 15–30 minutes.

**Western Blot Analysis**

Equal amounts of the proteins extracted from the gastric mucosa were subjected to 10% SDS-PAGE analysis. The separated proteins were transferred onto nitrocellulose membranes (BioRad) using Semidry Trans Blot (BioRad) for 2 hours, then soaked with Ponceau Red solution. The membranes were then soaked overnight in 10% skim milk at 2–8 °C. They were then incubated for 2 hours at 37 °C with HSF and HSP-70 antibodies (Santa Cruz Biotechnology; Santa Cruz, CA) at a concentration of 1:1000. Blots were treated with secondary antibodies, goat anti-mouse IgG (Santa Cruz Biotechnology, at a concentration 1:1000), visualized using aminoethylcarbazole tablet colorization, and documented by digital photograph.

**Statistical Analysis**

Data were analysed using SPSS 16.0 software. We used ANOVA to analyse the differences between the control group and the four treatment groups (>2 groups), and the Kruskal–Wallis test for unpaired
samples. The difference between the variables was declared significant if \( p < 0.05 \). To determine the correlations between the groups, we used Pearson’s test for normally distributed data and Spearman’s test for data that were not normally distributed.

**RESULTS**

This study used a hypoxic chamber with a 10% oxygen level and 90% nitrogen level. Ferdinal et al. used the same method and completed a blood gas analysis on samples taken from aorta.\(^{11}\) Results showed a decrease in some parameters that indicated hypoxia, such as oxygen and carbon dioxide partial pressure and oxygen saturation. (Table 1)

**Gastric Mucosal Lesion by Systemic Hypoxia**

The systemic hypoxia technique in this study successfully induced gastric mucosal lesions. The histopathological evaluation showed that ulceration in gastric sections increased with exposure to the hypoxia condition. Twenty-four hours after induction, 60% of rats developed gastric ulcers. (Table 2) Seven days after induction, 80% of rats developed gastric ulcers. Moreover, epithelialization was observed under the 14-day and 21-day hypoxia conditions. (Figure 1)

**Evaluation of the average ulcer depth on the day of treatment** showed that the greatest depth was on day 7, and the shallowest was on day 21 of treatment. (Figure 2)
Western Blot Analysis

Identification of Protein Expression of HSF-1

In the control condition without hypoxia, HSF-1 protein expression was not detected by Western blot (Figure 3). In contrast, HSF-1 protein expression was detected in rats as early as 1 day after induction and was significantly increased after 7 days of hypoxia (Figure 3). The HSF-1 protein expressions

Identification of Protein Expression of HSP-70

This study found that the protein expression of HSP-70 was first observed at day 1 and was seen most clearly in the group of experimental animals in the 7-day hypoxia group. Figure 4 shows the results of Western blot analysis using antibodies to HSP-70 (Santa Cruz); reddish-coloured bands appear at position 70 kDa. The protein expression then decreased in the 14-day and 21-day groups. There was no expression in the control group. There was strong correlation between HSF-1 protein expression and HSP-70 protein expression ($p < 0.05$, $r = 0.64$).

DISCUSSION

There are two factors that influence mucosal lesions in gastric mucosa: gastric acid secretion and defense mechanisms. The decrease in defense mechanism can be induced by systemic hypoxia. The systemic hypoxia itself can cause decreased blood flow to the gastric mucosa, leading to ischemia and then subsequent destruction of the mucosal lining. In daily practice systemic hypoxia can be found in stress ulcer patient due to sepsis shock hypovolemic or other critically patients.

The application of acetic acid onto the serosal surface of the rat stomach is one method of inducing gastric mucosal lesions. This widely used experimental model appears to be suitable for the determination of various physiological factors concerning ulcer healing and the screening of anti-ulcer drugs. Only a few models of “real” peptic ulcers are available for the study of the healing process, of which the acetic acid model introduced by Okabe and Pfeifer has received most attention.13
In this study, we used a systemic hypoxia animal model to induce gastric mucosal lesions. We created hypoxia conditions using 10% oxygen and 90% nitrogen. The animals were subjected to hypoxia conditions of 1, 7, 14 and 21 days. One of the important findings of this model was that the development of gastric mucosal lesions from peptic ulcers, and the healing process, depended on the duration of hypoxia. An advantage of animal models of hypoxia is that, in addition to seeing the impact on the stomach, we can also look at the effects of hypoxia in other organs. In addition, we often find instances of hypoxia in daily clinical practice. If we know what happens in the stomach as a result of hypoxia, we can anticipate the development of lesions in the stomach.

In this study, there was no significant difference between experimental groups in the number of rats with gastric ulcers. Further, there was no significant difference between experimental groups in the number of deep ulcers (ANOVA, $p = 0.117$). However, re-epithelialization occurred in the gastric mucosa of rats after exposed to hypoxia conditions for more than 7 days. Re-epithelialization is a sign of gastric ulcer healing. Therefore, it can be concluded that the two groups actually sustained gastric mucosal damage induced by hypoxia. Taking into account that the experimental animals with gastric mucosa that have undergone epithelialization into the group with gastric ulcer, a correlation was seen between the occurrence of ulcers and the duration of hypoxia ($r = 0.571$). However, the results of this study also proved that rats can adapt to the conditions and form epithelialization in the gastric system. Re-epithelialization, the migration of epithelial cells from the ulcer margin to restore epithelial continuity, is essential for gastric ulcer healing. Gastric ulcer healing is a complex process. The ulcer healing process is initiated by cell migration, proliferation, re-epithelialization, angiogenesis and matrix deposition. The ulcer healing process is controlled by growth factors, transcription factors and cytokines.\(^\text{14}\)

Gastric hypoxia itself leads to epithelial injury, and the outcomes after injury depend on the regeneration of this specific cell population. In general, the activation of transcription factors is one of the earliest events in the cellular response to ischaemia. Therefore, the delineation of such molecular events might provide valuable insights into the regulatory pathways that determine the changes in gene expression patterns during and after gastric ischaemia.\(^\text{15}\) The complexity of the ischaemic insult suggests coactivation of multiple transcription factor families, inducing the expression of entire gene families, some of which might lead to adaptive responses and others to maladaptive responses.\(^\text{15}\) This study focused on the functional activation, namely the HSF. Each of these transcription factors can activate a large number of genes that have been associated with cellular and molecular alterations in gastric hypoxia. HSF was one of the first transcription factors to be studied, and its activation by stresses that promote the unfolding of proteins has been well characterized. When cells are in a normal condition, HSF is in a monomeric state, but cellular stress induces trimerization of the protein.\(^\text{16}\)

In this study, we compared the HSF-1 protein expression after 1, 7, 14 and 21 days of hypoxia. The HSF-1 protein expression was detected in rats as early as 1 day after induction and was significantly increased after 7 days of hypoxia. The HSF-1 protein expressions regressed after 14 days of hypoxia.

Previous research has shown the occurrence of upregulation of HSP in Drosophila,\(^\text{17}\) Caenorhabditis elegans 18 and mammalian tissues.\(^\text{19}\) In this study, HSP-70 protein expression was detected in rats subjected to systemic hypoxia and was not seen in control rats. HSP-70 is most clearly seen in the 7-day hypoxia treatment group. This shows that hypoxia increased the expression of HSP-70. HSP-70 will be activated immediately after the stress as an adaptive response to protect cells from further damage caused by changes in protein conformation as a result of stress. Activation of HSPs is critical to adaptation to hypoxia. HSP-70 is known to be regulated by HSF. This increase in HSF transcripts is necessary for full HSP induction during hypoxia.

Statistical analysis indicated a strong significant correlation between HSF-1 and HSP-70. This is consistent with the theory that HSP-70 is a direct result of activation by HSF-1. Also, qualitatively, it can be seen that the results of Western blot analysis of the two proteins are consistent; they are highest in the 7-day treatment group.

This experimental study has provided lessons for the clinician that the occurrence of gastric ulcers would involve cellular factors that play a role not only damage but also healing. This opens up opportunities for researchers to conduct prevention and treatment of patients with gastric ulcer.

**CONCLUSION**

In this study, gastric mucosal lesions, from gastric ulcers up to epithelialization, were formed in animal models as a result of systemic hypoxia. This condition was paralleled by HSF-1 and HSP-70.
protein expression. This animal model could be used to evaluate the molecular and cellular aspects of the development of gastric ulcers and ulcer healing.

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REFERENCES