Oral Presentation (PCS-5)

# Nitric Oxide Induced Basal Cell Hyperplasia and Lamina Propria Elongation in Rat Gastroesophageal Junction

Tena Djuartina[1,2*], Bambang Pontjo Priosoeryanto[3], Ari Fahrial Syam[4] , Ahmad Aulia[5],Tri Isyani Tungga Dewi[6]

[1]Biomedical Doctoral Degree, Faculty of Medicine University Indonesia
[2]Department of Anatomy, Faculty of Medicine Catholic University Indonesia Atma Jaya
[3]Department of Veterinary Pathology, Bogor Agricultural University
[4]Department of Internal Medicine, Faculty of Medicine University Indonesia
[5]Department of Histology Faculty of Medicine University of Indonesia
[6]Veterinary teaching hospital, Bogor Agricultural University
*Corresponding author's email: tenadj@yahoo.co.id

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## INTRODUCTION

NO (Nitric Oxide) is an inorganic compound composed of nitrogen and oxygen, NO is also produced in various places on various types of mammalian cells. NO as a radical compound is important in mediating physiological and pathological events in mammals including humans [1].

GEJ (gastroesophageal junction) is a transition zone between the surface of esophagus which is covered by stratified squamous epithelium to the gastric mucosa which consists of simple columnar epithelium (z-line), where circular muscle of esophagus fuse with oblique muscle and lipid layer of the gaster. At the lower part of GEJ, there is the lower esophageal sphincter (LES) that not only allow food to move into stomach and works as an exit passage of the gas, but also inhibit reflux of any substances that potentially can cause harm to the esophagus [2].

Petersson et al, found that chronic exposure to cytotoxic levels of NO can cause inflammation, intestinal metaplasia and neoplasia. Although it is known that gastric acid, pepsin and bile acids can cause adenocarcinoma of distal esophagus and GEJ, NO exposure and nitrosative stress role in this phenomenon is yet to be fully understood and further study is needed [3].

The purpose of this was to identify and compare the histopathological changes occurring in GEJ in relation to administration of physiological concentration of nitrate dissolved in HCl and ascorbic acid. As such, the animal model used in this study can be used to study and represent the changes microscopically, because obtaining a full thickness biopsy from a human subject can be difficult to perform.

## MATERIALS AND METHODS

Forty eight male Wistar rats (*Rattus novergicus*) aged 10-12 weeks, weighing about 200-300 grams were used in this study. The animals were divided into 4 experimental groups. The control group (Group A) comprised of 12 rats were given 1 ml of water. The experimental groups (Group B,C,D) were each given 10 mM ascorbic acid dissolved in 1ml HCL pH 2.0 which is given in 0.5 ml increment with 30 minutes between each dose. Each dose is given via a 3.5 ml feeding tube, which is inserted into the lower part of esophagus sphincter. After 30 minute all experimental groups were given sodium nitrite mixed together with sodium thiocyanate in different doses (B: 1 ml sodium nitrite + 1 ml sodium thiocyanate; C: 1,5 ml sodium nitrite + 1 ml sodium thiocyanate; D: 2 ml sodium nitrite + 1 ml sodium thiocyanate) through a metal tube inserted into the upper part of esophagus sphincter. The rats were then fasted for 24 hours but drinking water were given ad libitum. Rats were than 24 hours fasted of feed but drinking water were given ad libitum. Prior to the treatment, all the rats were anesthetized using 75 mg ketamine and acepromazine 5 mg/kgbw administered via intramuscular injection. Every other day, 3 rats from each group were terminated and GEJ specimens were collected, stained with PAS and eosin (HE) and Masson's trichrome stain for histopathology analysis. The microscope used for this study is Olympus biological microscope CX-33 with Olympus DP22 microscope camera attachment.

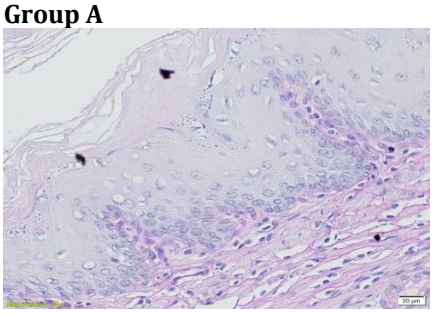
## RESULT AND DISCUSSION

Epithelial cell of esophagus is stratified squamous epithelium resistant to abrasion caused by bolus but sensitive to acid. Sub mucosa glands at the distal esophagus contribute in protection by secreting mucin and bicarbonate [4]. Stratified squamous epithelium can be divided into basal, spinosum and superficial layer. Basal layer takes about 5-15% of epithelium thickness which is about 1-3 cell layers. In sixty percent of normal

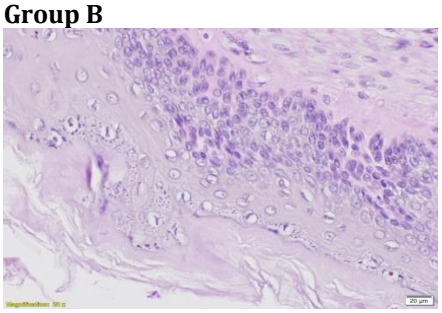
individual, it is shown 3 cm from distal esophagus that their basal layer takes more than 15% [7]. Basal layer consists of some undifferentiated basophilic layers. These cells are attached to single layer basement membrane [2].

Significant basal cells hyperplasia ($P<0.05$) were found in experimental groups when compared to control group (A-B, A-C, A-D) on fasting day 4 using one-way ANOVA, as shown by the PAS stained specimen. Basal zone hyperplasia were found in experimental groups, as shown by the PAS stained specimen. Basal zone hyperplasia most markedly occur in group D, followed by group C and B. But, mild hyperplasia was also detected in two rats from the control group (Figure 1).

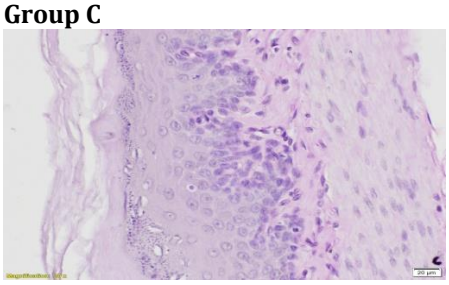
**Group A**



**Group B**



**Group C**



**Group D**

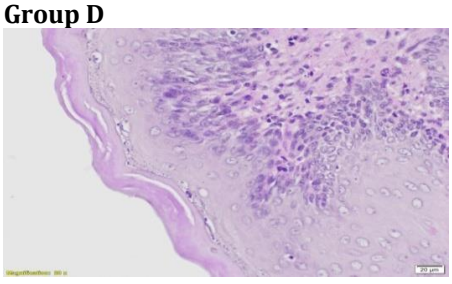


Figure 1. Increasing grades of severity in basal cell hyperplasia in rats gastroesophageal-junction. A. 30,02% B. 45,19%, C. 48.80%, D. 53,76%

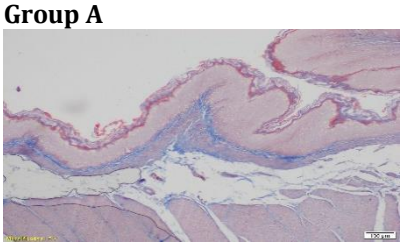
Lamina propria is non-epithelial layer which is part of esophageal mucosa. Lamina propria is located above muscularis mucosa. Lamina propria consists of loose connective tissue, vascular structure, inflammation cells and mucus secreting glands. There is an elongated structure towards epithelium that's called papilla [5]. Nitric oxide (NO) is an important radical that mediates a wide range of physiological and pathological events. It is generated at low concentrations by the constitutive enzyme nitric oxide synthase (NOS) to modulate neuromuscular and vascular functions. Higher concentrations are generated by the inducible form of the enzyme as part of the immune and inflammatory response. Sustained generation of NO by inducible NO synthesis has been implicated in the etiology of mutagenesis and neoplasia related to chronic inflammation [4,6]. Elongation of lamina propria also occurs in all of the experimental group, with group D showing the most marked increase in length ($P<0.05$) compared to control using one-way ANOVA on fasting day 6, as demonstrated in the specimen stained using Masson's trichrome stain (Figure 2).

Elongation of lamina propria that reaches the upper third of the epithelium layer is considered abnormal. Together with basal cell hyperplasia, elongation of lamina propria is an early sign that can be encountered histologically on the patient's tissue biopsy with GERD.[4] These two markers are considered sensitive but not specific because in about 60% of individuals without symptoms of reflux there is a lamina propria elongation that exceeds 1/3 of the epithelial thickness in the distal 3 cm tissue biopsy of the esophagus [2].

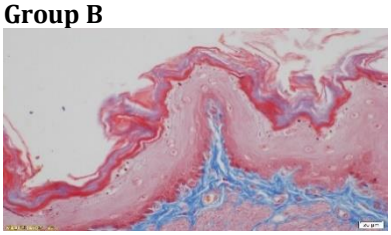
NO can diffuse through tissue membrane because NO is gaseous and lipophilic. Therefore NO produced in the lumen can directly diffuse into the surrounding epithelium and accumulate to a considerable extent that it can affects the integrity of the tissue. In addition, since NO is known to have a cytoprotective and cytotoxic effect on the tissue, which depends on the concentration level, determination of NO levels is necessary for the evaluation of its function on the tissues [1].

NO also has a lot of potential reactions that can influence various physiological and pathophysiological processes. Time, location, and production rate of NO and reactive nitrogen species (RNS), these are the factors that can help identify which NO dependent reaction is responsible for inflammatory modulation [5,7]. In-vitro studies conducted by [1,7], Barlow [8] proves that NO in the luminal disturbs the gastric barrier thus affecting the surface epithelium and disturbing intercellular space between the squamous epithelium. These changes can impair the defense function in the GEJ resulting in long-term chronic inflammation of the area causing carditis esophagitis [1,7,8].

**Group A**



**Group B**



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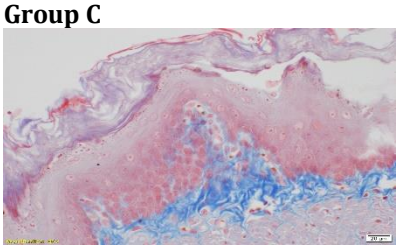

**Group D**

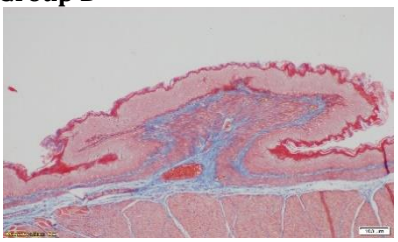

Figure 2. Group A (control) given 1 ml aqua with 52% elongation, group B (sodium nitrite 1 ml+sodium tiocyanate 1 ml) with 69% lamina propria elongation, group C (sodium nitrite 1,5 ml+sodium tiocyanate 1 ml) with 78 % lamina propria elongation, group D (sodium nitrite 2 ml+sodium tiocyanate 1ml) with 83% lamina propria elongation

Using an animal model, K Asanuma, [2] demonstrated that NO generated in the lumen diffuses into the adjacent gastric tissue to a substantial degree, leading to localized consumption of glutathione in the tissue. Nitrosative stress induced by this mechanism may be involved in the high prevalence of inflammation and metaplasia, and subsequent development of neoplastic disease at this site.

The same finding on the effect of NO as mentioned above was also found by Li & Li [9] where basal zone thickening occurs before superficial epithelial erosion, this shows that inflammation and irritation in reflux esophagitis cause cellular proliferation even before erosion.

Vieth et al, [10] found linear correlation between basal zone thickness and the degree of reflux esophagitis suffered by their patients,

Elongation of Lamina propria can be used as a marker to diagnose GERD from biopsy specimen. Lamina propria elongation that reaches upper third of epithelium layer is considered abnormal. Along with basal zone thickening, elongation of lamina propria is an early sign of GERD that can be found histologically in the biopsy specimen of GERD patients [3].

## CONCLUSION

The animal model in this study has shown that, chronic exposure to high doses of NO can cause hyperplasia of basal zone and elongation of lamina propria, which is a mark of inflammation. The animal model is used because obtaining a full thickness biopsy from GEJ in a human subject is difficult to perform.

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