Preventing NSAID-induced Gastropathy: The Role of Mucus Cells to Prevent Aspirin-Induced Acute Gastric Mucosal Damage

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ABSTRACT

Background: *Mucus is pre-epithelial gastric layer that may prevent damages due to direct contact between aspirin and gastric epithelial cells. The integrity of gastric mucosa and mucous cellular reaction may serve as primary and secondary prevention of extended aspirin-induced gastric mucosal damage. The aim of this study was to prove the function of mucus as defensive factor in rats.*

Method: *The study was conducted in twenty white rats of the Sprague-Dawley strain at Department of Pathology and Clinical Reproduction, Bogor Agricultural University, between January and December 2008. The rat in the treatment group were given 400 mg aspirin diluted in aqua bidest through intra-gatric canules; while the control group received aqua bidest only once daily for 3 days. Necropsies, macroscopic and microscopic observation were performed by counting the number of Alcian blue-periodic acid Schiff-stained mucous cells at fundus/corpus and antrum/pylorus regions. Data analysis was performed using ANOVA and Duncan test.*

Results: *The number of mucous cells with positive lesions in the treatment group was significantly different from the control group at both regions. There was no significant difference of negative lesions between treatment and control group at both regions. At antrum/pylorus region, there was no difference of negative lesions between treatment and control groups; however, both groups demonstrated significant difference of positive lesions in treatment group.*

Conclusion*: In primary prevention for gastric mucosal lesions, there is no increasing number of mucous cells in normal mucosa. Increasing number of mucous cells is a secondary prevention against extended aspirin-induced gastric mucosal damage.*

Keywords*: NSAIDs/ASA, mucus cells, gastric mucosal lesion, rat*

ABSTRAK

Latar belakang: *Mukus merupakan lapisan pre epitel untuk mencegah efek langsung asam asetil salisilat/ aspirin terhadap epitel gaster. Keutuhan mukosa merupakan pencegahan primer dan reaksi sel mukus sebagai pencegahan sekunder terhadap perluasan lesi mukosa akibat aspirin. Tujuan penelitian ini yaitu membuktikan fungsi mukus dalam pencegahan primer maupun sekunder sebagai faktor defensif pada tikus.*

Metode: *Penelitian ini dilakukan pada hewan coba tikus putih jenis Sprague-Dawley sebanyak 20 ekor di Bagian Patologi dan Klinik Reproduksi, Fakultas Kedokteran Hewan, Institut Pertanian Bogor pada bulan Januari hingga Desember 2008. Pada tikus yang mendapat perlakuan diberikan aspirin dosis 400 mg yang dilarutkan pada akuabides melalui kanul langsung ke dalam lambung, sedangkan pada kelompok kontrol hanya diberikan akuabides saja sekali sehari selama 3 hari. Nekropsi, pengamatan makroskopik dan mikroskopik lambung dilakukan dengan*

menghitung jumlah sel mukus pada regio fundus/korpus dan regio antrum/pilorus dengan pewarnaan periodik acid-Schiff (PAS) Alcian biru. Analisis statistik dilakukan dengan uji ANOVA dan Duncan.

Hasil: *Jumlah sel mukus pada kelompok perlakuan dengan lesi positif terdapat perbedaan bermakna antara kelompok kontrol pada regio fundus/korpus dan regio antrum/pilorus. Tidak terdapat perbedaan bermakna pada kelompok dengan lesi negatif baik pada kelompok yang mendapat perlakuan atau kontrol pada kedua regio. Pada regio antrum/pilorus tidak terdapat perbedaan bermakna pada kelompok perlakuan dengan lesi negatif dengan kelompok kontrol, namun kedua kelompok tersebut berbeda bermakna dengan kelompok perlakuan dengan lesi positif.*

Kesimpulan: *Pada pencegahan primer lesi mukosa gaster tidak terdapat peningkatan jumlah sel mukus pada mukosa normal. Peningkatan jumlah sel mukus merupakan pencegahan sekunder terhadap perluasan lesi mukosa gaster akibat aspirin.*

Kata kunci: *OAINS/ASA, sel mukus, lesi mukosa gaster, tikus*

INTRODUCTION

Severe gastric mucosal damage due to non-steroid anti inflammatory drugs/aspirin (NSAIDs/ASA) is caused by the imbalance between the aggressive factors and the defensive factors. Direct contact between NSAIDs and epithelial cells of gastric mucosa is the initial process of primary mechanism underlying NSAID-associated gastric mucosal damage. The gastric mucosal defense consists of pre-epithelial, epithelial and sub-epithelial barriers.[1,2] Pre-epithelial defense including the mucus layer is the fore barrier that may determine the occurrence of acute gastric mucosal damage. Lesions in gastric mucosa are mostly due to failure of epithelial mucus to protect against injury; therefore, direct contact with epithelial layer will likely to be occurred. Subsequently, inflammatory reactions which are generally initiated and amplified by inflammatory mediators may occur and can result in further damage of epithelial layer.[3,4] The condition would be exaggerated by gastric acid that may facilitate NSAID/ASA penetration into gastric epithelial cells, where they trapped within the cells causing local injury. It will cause a wide range of damage on epithelial layer starting from erosion to ulcer, either with or without bleeding. This kind of local reactions are more likely to occur in some parts of gastric mucosa, especially where the mucus layer is thin or non-existent.[1,5-8]

NSAIDs/ASA may not cause gastric mucosal damage in all cases. Therefore, it has been proposed that mucus layer as the primary fore barrier of gastric mucosa may have important role. The aim of this study was to provide evidences that mucus layer can prevent NSAIDs/ASA-induced gastric mucosal damage in rats. We hypothesized that mucus layers, particularly the number of mucous cells could prevent the occurrence of NSAIDs/ASA-induced lesions in gastric mucosa.

METHOD

The study used twenty male white rats (*Rattus norvegicus)* of the Sprague-Dawley strain as experimental animals to serve as model for human condition since their anatomical structure of gastrointestinal system is similar with human. Furthermore, it may provide evidences of affecting factors after the necropsy had been performed. The rats were 2 months old and the average weight of each rat was ± 250 g. Those experimental animals were taken from Non Ruminansia division and Satwa Harapan (NRSH), Faculty of Animal Science, Bogor Agricultural University in Bogor. The study was conducted at Department of Pathology and Clinical Reproduction, Faculty of Veterinary Science, Bogor Agricultural University between January and December 2008. The animals were categorized into two groups, i.e. the treatment and control groups. Each group consisted of 10 rats. Each rat in the treatment group was given 400 mg of acetyl salicylic acid (ASA)/aspirin as the non-steroid anti-inflammatory drug, which was consistent with the references of our literatures. We used ASA in its pure form of a white crystalline solid.

Prior to the usage of rats in this study, the rats were fed with irradiated sterile pellet at dose of 10 Kgray. Each of rat was fed as much 25 g of pellets every day. Adaptation for the rat was performed during three weeks period by administering 250 mg/kgBW of tetracyclin antibiotic for three days and 10 mg/kgBW of antihelmintic, Albendazole 5%, every week for 3 weeks. Moreover, anti-Cryptococcus drug, i.e. fluconazole was administered at dose of 50 mg/kgBW once daily for three days. Pre-eliminary study has been performed to eliminate bias which may affect the condition of gastric mucosa.

The rats are being weighed every day. ASA was administered to three-hour fasting rats in the treatment group using intra-gastric tube at dose of 400 mg ASA diluted in 2 mL aqua, once daily during three days. Similar procedures were conducted in the control group, but the rats in the control group received solution containing aqua only. Afterwards, necropsies were performed in both groups under ether anesthesia. Necropsy was conducted along the linea alba through opening of skin and fascia. The stomach was separated from its surrounding and taken out. Subsequently, macroscopic observation of gastric mucosa was done after incision along the greater curvature. After macroscopic observation had been completed, the stomach was preserved in 10% buffered neutral formalin (10% BNF) and stored for further process. Microscopic observation was performed at two gastric regions, i.e. the fundus/corpus and antrum/pylorus. Histopathological examination was done with hematoxyllin eosin (HE) and periodic acid Schiff (PAS) Alcian Blue staining at each gastric region. The number of mucous cells was counted in 10 high power field for each layer of gastric regions.

Quantitative data including the mean value of the amount of mucus cells in each high power field was analyzed by one-way ANOVA test using SPSS version 13.0. One-way ANOVA test was used to compare control group (C), treatment group with negative lesion (TNL) and treatment group with positive lesion (TPL). Significant results were further analyzed using the Duncan test, $p < 0.05$ was considered statistically significant.

RESULTS

Statistical analysis on the number of mucous cells in the fundus/corpus region demonstrated that there was a significant difference between treatment groups with positive lesions (TPL) and the control group; however, no significant difference was found between the treatment group with negative lesions (TNL) and the control group (Table 1).

Furthermore, statistical analysis in the antrum/pylorus region indicated that there were significant differences between TPL group and TNL group, and the control group. However, no significant difference was found between TNL group and the control group (Table 2).

Histopathological findings of gastric mucosa indicated an increasing number of mucous cells in the treatment group compared to the control group (Figure 1).

Table 1. The number of mucous cells in fundus/corpus region

Group	Amount of mucous cells
Control	10.43 ± 4.28[a]
Treatment group with negative lesion	16.50 ± 0.71[ab]
Treatment group with positive lesion	18.25 ± 5.06[b]

Different alphabet shows $p < 0.05$

Table 2. The number of mucous cells in anthrum/pylorus region

Group	Amount of mucous cells
Control	11.67 ± 1.97[a]
Treatment group with negative lesion	11.00 ± 2.74[a]
Treatment group with positive lesion	16.50 ± 4.43[b]

Different alphabet shows $p < 0.05$

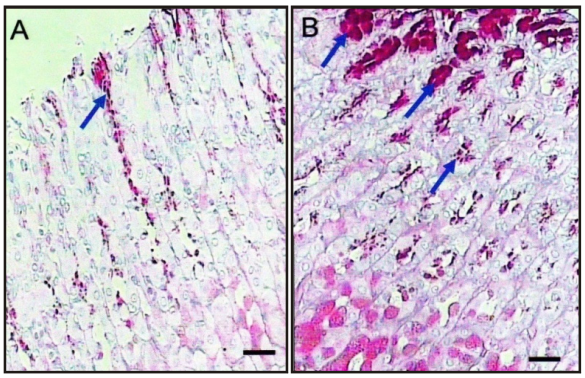

Figure 1. Histopathological findings of gastric mucosa demonstrating mucous cells in the control group (A) and treatment group (B), which shows gastric epithelial layer with higher number of mucous cells in the treatment group

DISCUSSION

Mucus as one of defensive factors has important role in protecting gastric mucosa. The protective function of mucus is determined by the activity of mucous cells activity.[3,4,5] This study attempted to provide evidences on the role of mucus in preventing gastric mucosal damage. Such study has never been conducted by other investigators. Increased activity of mucous cells was found when mucosal lesion occurred. It may become a secondary prevention strategy of our body to inhibit further expansion of the lesion. Moreover, when mucosal lesion was not found in the TNL group, the activity of mucous cells was similar to the control group. Direct contact between NSAIDs and gastric epithelial layer would bring great effect resulting in mucosal reaction that may increase the number of mucous cells. In the TPL group at fundus/corpus region, this study revealed that there was no significant difference on the number of mucous cells between TPL and control group. Such finding indicates that the important role of mucus in TPL group could not be observed due to shorter period of contact between the drugs and mucosa. Similar condition was found in TNL group.

The increased number of mucous cells induced by ASA stimulation may exert similar effect even though the mucosal lesion does not occur. Mucosal lesion may be affected by the length of contact period between ASA and gastric mucosa, the quality of mucus and bicarbonate secretion.[4,6,7,9,10]

We found that after being induced with aspirin, the number of mucous cells in the antrum/pylorus region was different from those in corpus/fundus region. There were significant differences in TPN group between the control and TNL group. In contrast, there was no significant difference between the control group and TNL group.

Such findings indicate that the number of mucous cells in both control and TNL groups was not affected by direct contact between aspirin and gastric epithelial layer. Furthermore, in TPL group, it may serve as secondary strategy to prevent expansion of lesions. Thus, mucus has important function as the fore barrier to prevent devastating effects of aspirin/ASA on gastric mucosa, particularly in the epithelial and sub-epithelial layer.[6,7,9,10] Therefore, it can be used as a strategy to prevent the occurrence of aspirin/ASA-induced mucosal lesion.

CONCLUSION

Gastric mucosal defense mechanism is highly determined by pre-epithelial component, especially mucus thickness related to mucus cells production. The production of mucus will increase when gastric mucosal lesion has occurred. However, when there is no mucosa lesion, the activity of mucous cells is similar to the control group. It indicates that mucus has an important role as fore barrier to prevent the damaging effects of aspirin on gastric epithelial through primary or secondary prevention, which could be measured as the number of mucous cells.

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