# Evaluation of Lansoprazole (An $H^+/K^+$-ATPase Inhibitor) and Azithromycin (An Antibiotic) for Control of Gastric Ulceration in Swine During Periods of Feed Deprivation

S. Melnichouk, R.M. Friendship, C.E. Dewey, and R. Bildfell


## ABSTRACT

***Helicobacter*-like organisms as well as fermentative bacteria have been implicated in gastric ulcer production in swine. Irregular feeding schedules are also considered a major risk factor. A research trial was conducted to determine whether medication with an acid secretion inhibitor (lansoprazole), either alone or in combination with an antibiotic (azithromycin), would protect pigs from gastric ulceration if the animals were subjected to a 48 h period of fasting. In a 2 × 3 factorial design, 48 pigs were fasted, while an equal number were fed ad libitum. Within these 2 study groups, pigs were randomly assigned to 1 of 3 treatments: control, 30 mg lansoprazole s.i.d. for 7 d, or lansoprazole (30 mg s.i.d. for 7 d) and azithromycin (500 mg s.i.d. for 3 d). Overall, fasted pigs were 1.9 times more likely to develop erosive or ulcerative lesions of the pars esophagea ($\chi^2 = 9.89$, $P < 0.002$). Treatment with an acid secretion inhibitor alone or in combination with an antibiotic did not protect pigs from developing gastric lesions. *Helicobacter*-like organisms were not detected in any of the stomachs. Possibly, the lansoprazole dose of 30 mg given once per day was insufficient to prevent pH levels from becoming low enough to cause damage to epithelial tissue. Alternatively other substances such as bile acids may have caused the ulcerative lesions, even though stomach acid production was suppressed.**

## RÉSUMÉ

**Des micro-organismes apparentés au genre *Helicobacter* de même que des bactéries avec un métabolisme fermentaire ont été impliqués comme cause des ulcères gastriques chez le porc. Un autre facteur de risque important est des horaires irréguliers d'alimentation. Une étude fut entreprise afin de déterminer si un traitement avec un inhibiteur de sécrétion d'acide chlorydrique (lansoprazole) seul ou combiné avec un antibiotique (azithromycine) protégerait des porcs contre l'apparition d'ulcères gastriques suite à une période de jeûne de 48 h. Dans un modèle expérimental en factoriel de 2 × 3, 6 groupes de 8 porcs ont été soumis à un jeûne alors qu'un nombre égal de porcs avaient un accès illimité à de la nourriture. À l'intérieur de ces deux groupes, les animaux furent assignés de façon aléatoire à un des trois traitements suivants : témoin, 30 mg de lansoprazole s.i.d. pour 7 j, ou lansoprazole (30 mg s.i.d. pour 7 j) et azithromycine (500 mg s.i.d. pour 3 j). De façon générale, les animaux soumis au jeûne avaient 1,9 fois plus de chance de développer des lésions érosives ou ulcératives de la partie oesophagienne de l'estomac ($\chi^2 = 9,89$, $P < 0,002$). Le traitement avec l'inhibiteur de sécrétion acide seul ou combiné à l'antibiotique n'a pas réussi à empêcher le développement de lésions gastriques chez les porcs. Des micro-organismes apparentés au genre *Helicobacter* ne furent détectés dans aucun des estomacs examinés. Il est possible que la quantité de lansoprazole administrée par jour (30 mg) était insuffisante pour modifier significativement le niveau de pH de l'estomac. Il est aussi possible que d'autres substances telles que des acides biliaires puissent avoir causés des lésions ulcératives, et ce malgré le fait que la production d'acide au niveau de l'estomac ait été empêchée.**

***(Traduit par le docteur Serge Messier)***


## INTRODUCTION

Pigs that are starved for 24 to 72 h develop erosive lesions in the pars esophagea (1–3). It is generally believed that as the stomach empties, the normal pH gradient between the acidic distal portion and the more neutral proximal region is lost. Increased fluidity and mixing results in exposure of the sensitive pars esophagea to gastric acid and pepsin. However, recent work has shown that although the pars esophagea is not protected by a mucus covering like the glandular regions of the stomach, the stratified squamous epithelium is relatively resistant to hydrochloric acid and pepsin (4,5). Several factors may work in conjunction with gastric acid to cause the rapid destruction of the epithelial tissue of the pars esophagea. Short-chain fatty acids produced by fermentative bacteria such as *Lactobacillus* and *Bacillus* are capable of causing rapid destruction of the pars esophagea at pH levels that are much higher than levels at which hydrochloric acid alone causes

Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1 (Melnichouk, Friendship, Dewey); Department of Biomedical Sciences, College of Veterinary Medicine, University of Oregon, Corvallis, Oregon 97331 USA (Bildfell).

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Address correspondence and reprint requests to Dr. R.M. Friendship, telephone: (519) 824-4120, ext. 4022, e-mail: rfriendship@ovcnet.uoguelph.ca.

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ulceration (5). A combination of these bacteria and a carbohydrate-enriched liquid diet experimentally causes erosions and gastresophageal ulcers (6).

*Helicobacter* have also been implicated in ulceration of the pars esophagea in the pig. Epidemiological studies have shown a strong relationship between the prevalence and severity of gastric ulcers and the presence of *Helicobacter heilmannii* (7–10). These bacteria almost exclusively colonize the fundic and antral regions and may stimulate G cells or parietal cells, leading to excessive acid production. Alternatively, they may interfere with acid secretion inhibitory mechanisms (11). *Helicobacter* eradication programs have been very successful in curing gastric ulcers in humans and preventing reoccurrence (12–14). Commonly, these programs utilize an effective acid secretion suppressor such as an $H^+/K^+$-ATPase inhibitor and a broad spectrum antibiotic that is effective in an acid environment. Lansoprazole, a benzimidazole compound, has been shown to effectively suppress acid secretion in human adults for 24 h at a 30 mg dose (15). Azithromycin is a new macrolide which exhibits excellent tissue distribution and extended half-life, and is far more stable than erythromycin in an acidic environment (16,17). Azithromycin has been successfully used in conjunction with an $H^+/K^+$-ATPase inhibitor to eradicate *Helicobacter* in humans (18).

The purpose of this research trial was to determine whether an acid secretion inhibitor such as lansoprazole, either alone or in combination with an antibiotic, could protect pigs from gastric ulceration if the pigs were subjected to a 48-hour period of fasting.

## MATERIALS AND METHODS

Eighty-four grower pigs were selected for the trial and placed on a coarse mash feed for 21 d prior to the start of the experiment to ensure the stomachs of the pigs would be free of ulceration. The animals were purebred Yorkshires, housed at the minimal-disease University of Guelph swine research facility at Arkell. The pigs were placed in a single room of 12 pens with 7 pigs per pen. The pigs weighed between 70 and 90 kg at the beginning of the trial. All the pigs were placed on a finely-ground pelleted feed for 1 wk prior to being assigned a treatment. In a $2 \times 3$ factorial design, half of the pens were allocated to a 48-hour fasting regimen while the pigs in the remaining 6 pens continued to be fed ad libitum for the entire experiment. Within these 2 study groups, pigs were randomly assigned to 1 of 3 treatments. The 3 subgroups, or treatments, were: group A, untreated controls; group B, pigs given 30 mg of lansoprazole (Prevacid, Abbott Laboratories, North Chicago, Illinois, USA) s.i.d., PO, for 7 d; group C, pigs given 30 mg of lansoprazole s.i.d., PO, for 7 d plus 500 mg azithromycin (Zithromax, Pfizer, New York, New York, USA), s.i.d., PO, for the first 3 d. Both drugs were fed as capsules, hidden in spoonfuls of strawberry jam. Two weeks after the completion of the treatments all pigs were euthanized and the stomachs were examined for lesions.

The stomachs were carefully removed from the carcass and opened with an incision along the greater curvature. The contents were removed and the mucosal surface was gently washed with water. All necropsy inspections were performed by a single pathologist who was kept blind to the treatment status of the pig. Gross lesions of the pars esophagea were numerically scored on a scale of 0 to 3 (19). A score of 0 was given to a stomach with a smooth glistening pars esophagea with no visible lesions; a score of 1 was given to a stomach with evidence of roughening or parakeratosis; a score of 2 was attributed to a stomach when small erosive lesions were noted; and, if these erosions were deep and/or extensive, a score of 3 was assigned.

A linear strip of epithelium (approximately 4 cm to 6 cm in length) was collected across the junction of the pars esophagea to include the junction with the glandular stomach. If an erosion or ulcer was grossly detected, the strip was oriented to include the lesion. The strip of epithelium was fixed in 10% neutral-buffered formalin and processed routinely for paraffin embedding and light microscopy. Microscopic lesions of the pars esophagea were scored on a scale of 0 to 3 (19). A score of 0 represented normal epithelial tissue, and a score of 1 was assigned to stomachs with histological evidence of parakeratosis. A score of 2 corresponded to the presence of erosions or partial-thickness epithelial loss. If epithelial loss was to, or through, the basement membrane, a score of 3 was assigned.

An in situ urease assay (20) was performed immediately after the gross examination and biopsy of the pars esophagea. The stomachs were placed in an aluminum tray and the mucosal surface was covered with a thin layer of gel-like medium consisting of 2% urea, 0.0012% phenol red, 0.3% agar, 0.01% yeast extract, 0.0091% monopotassium phosphate, and 0.00995% disodium phosphate (pH 6.7–7.0). All stomachs were visually monitored for color change for 1.5 h. The presence of urease-producing bacteria causes ammonia to be produced and a color change occurs from yellow to deep pink as the pH rises. The protocol was such that if a color change occurred, a biopsy would be taken from that specific area, otherwise a tissue biopsy (3 cm $\times$ 3 cm) was taken from the antrum and another from the cardiac region. These tissue samples were also placed in formalin and processed routinely for paraffin embedding. One set of slides was prepared by staining with hematoxylin and eosin stain and a second set was stained using Warthin-Starry silver stain method (21) which enhances visualization of *Helicobacter* spp. (22).

The effect of treatment was judged on the basis of a chi-squared ($\chi^2$) test and agreement between microscopic and macroscopic scoring was determined by calculating kappa ($\kappa$). The experimental protocol was approved by the University of Guelph Animal Care Committee and was carried out in accordance with the principles published in the Canadian Council on Animal Care *Guide to the Care and Use of Experimental Animals*.

## RESULTS

Of the 84 pigs on this trial, only 4 were categorized with normal pars esophagea based on gross pathological examination and only one based

on histological evaluation. Twenty-eight pigs demonstrated gross lesions of parakeratosis, and 24 were similarly classified by histology. Of the remaining 52 pigs examined macroscopically, 32 were classified with an ulcer score of 2 and 20 were classified with a score of 3. Histologically, 21 were given a score of 2 and 38 were categorized as a 3. Therefore, the percentages of pigs showing some evidence of erosions or ulcerations (ulcer score of 2 or 3) for gross examination and histological examination were 61.2% and 70.2%, respectively. There was a strong agreement between the gross and histologic scores ($\kappa = 0.72$), when 0 and 1 scores were categorized together and 2 and 3 scores were grouped together.

Erosive lesions and ulceration detected either on gross inspection or by microscopic examination were more likely to occur in pigs that had been fasted for 48 h compared to the pigs which had continuous access to feed. Thirty-seven of 42 (88%) fasted pigs were found to have stomach lesions categorized as 2 or 3 based on histological examination, compared to only 22 of 42 pigs (52%) fed ad libitum. This difference in prevalence of erosive or ulcerative lesions is significant ($\chi^2 = 12.81$, $P < 0.001$).

Similarly gross lesions categorized as 2 or 3 were detected in 33 (78.5%) fasted pigs and only 19 (45%) of the ad libitum feeding group ($\chi^2 = 9.89$, $P < 0.002$). Hence, fasted pigs were 1.9 times more likely to have erosions or ulcerations than ad libitum fed pigs.

The proportion of pigs with ulcer scores of 2 and 3 were similar in the 3 experimental groups, based on histological examination and on gross inspection (Table I). The lesion scores for each treatment group were also examined within the fasted and non-fasted groups, separately (Tables II and III).

Color change was not detected on any of the stomachs when the in situ urease test was administered. Tissues stained with Warthin-Starry silver stain did not reveal the presence of coiled or curved bacteria during microscopic examination. There were some rods and coccoid bacteria observed on the mucosal surface of the pars esophagea.

**TABLE I. The number of pigs in each of three treatment groups based on severity of histological lesions and macroscopic lesions of the pars esophagea**

| | Ulcer score | Control[a] | Lansoprazole[b] | Lansoprazole + azithromycin[c] |
|---|---|---|---|---|
| Histological | No lesion or parakeratosis | 7 | 13 | 5 |
| | Erosions | 6 | 5 | 10 |
| | Ulcerations | 15 | 10 | 13 |
| | Total | 28 | 28 | 28 |
| | $n = 84$ | | | |
| | $\chi^2 = 7.16$ | | | |
| | $P = 0.13$ | | | |
| Macroscopic | No lesions or parakeratosis | 10 | 12 | 10 |
| | Focal, shallow erosions | 12 | 10 | 10 |
| | Deep or diffuse ulcerations | 6 | 6 | 8 |
| | Total | 28 | 28 | 28 |
| | $n = 84$ | | | |
| | $\chi^2 = 0.90$ | | | |
| | $P = 0.92$ | | | |

[a] Control, no treatment
[b] Lansoprazole, 30 mg, PO, s.i.d., 7 d
[c] Lansoprazole, 30 mg, PO, s.i.d., 7 d and 500 mg azithromycin, PO, s.i.d., 3 d

**TABLE II. The number of pigs in each of three treatment groups divided into fasted and non-fasted categories and evaluated on the basis of histological lesions of the pars esophagea**

| | Ulcer score | Control[a] | Lansoprazole[b] | Lansoprazole + azithromycin[c] |
|---|---|---|---|---|
| Fasted for 48 h | No lesion or parakeratosis | 1 | 3 | 1 |
| | Erosions | 3 | 2 | 4 |
| | Ulcerations | 10 | 9 | 9 |
| | Total | 14 | 14 | 14 |
| | $\chi^2 = 2.34$ | | | |
| | $P = 0.67$ | | | |
| Non-fasted | No lesions or parakeratosis | 6 | 10 | 4 |
| | Erosions | 3 | 3 | 6 |
| | Ulcerations | 5 | 1 | 4 |
| | Total | 14 | 14 | 14 |
| | $\chi^2 = 6.90$ | | | |
| | $P = 0.14$ | | | |

[a] Control, no treatment
[b] Lansoprazole, 30 mg, PO, s.i.d., 7 d
[c] Lansoprazole, 30 mg, PO, s.i.d., 7 d and 500 mg azithromycin, PO, s.i.d., 3 d

**TABLE III. The number of pigs in each of three treatment groups divided into fasted and non-fasted categories and evaluated on the basis of macroscopic lesions of the pars esophagea**

| | Ulcer score | Control[a] | Lansoprazole[b] | Lansoprazole + azithromycin[c] |
|---|---|---|---|---|
| Fasted for 48 h | No lesion or parakeratosis | 3 | 3 | 3 |
| | Focal, shallow erosions | 11 | 7 | 4 |
| | Deep or diffuse ulcerations | 0 | 4 | 7 |
| | Total | 14 | 14 | 14 |
| | $\chi^2 = 10.09$ | | | |
| | $P = 0.04$ | | | |
| Non-fasted | No lesion or parakeratosis | 7 | 9 | 7 |
| | Focal, shallow erosions | 1 | 3 | 6 |
| | Deep or diffuse ulcerations | 6 | 2 | 1 |
| | Total | 14 | 14 | 14 |
| | $\chi^2 = 8.81$ | | | |
| | $P = 0.06$ | | | |

[a] Control, no treatment
[b] Lansoprazole, 30 mg, PO, s.i.d., 7 d
[c] Lansoprazole, 30 mg, PO, s.i.d., 7 d and 500 mg azithromycin, PO, s.i.d., 3 d

## DISCUSSION

The moderate agreement between gross necroscopic and histologic examination grades is similar to previous studies (19,23). Macroscopic examination often fails to identify small or superficial ulcerations that are detected by microscopic examination. In a study by Embaye et al (23), of 155 pig stomachs observed to be normal macroscopically, 50 were

found to have parakeratotic changes on the basis of histologic examination and 83 were detected with minor to severe erosions. On the other hand, because histological examination was restricted to a small section of the pars esophagea, focal erosions might not be detected by this method. Methodical histological examination of multiple serial sections of the pars esophagea would be necessary to ensure each stomach was appropriately classified. The higher percentage of ulcers found on histologic examination may also be due to the fact that grossly abnormal sections were pre-selected for histologic examination. It should be noted that no attempt at morphological quantitation was made at the gross level so that minor lesions and severe erosions were scored the same. This may have reduced the chances of detecting a difference in treatments.

A period of fasting was associated with an increase in erosive and ulcerative lesions of the pars esophagea, and this finding was observed almost 3 wk after the period of feed withdrawal. In a previous study, using endoscopic examination of pigs fed finely-ground rations, it was observed that over a similar 3-week period, ulcers did not heal but tended to become slightly more severe (24). It is possible that some of the lesions that developed during the fasting period did heal and new lesions developed after the treatment period. The induction of ulceration can occur rapidly in as little as 48 h and complete healing can occur within 14 to 21 d (25).

Treatment with 30 mg lansoprazole daily for 7 d with or without 500 mg azithromycin daily for the first 3 d of treatment did not appear to reduce the prevalence or severity of lesions of the pars esophagea at the time the stomachs were examined, 2 wk after the completion of the treatment regimen. It is possible that if the stomachs had been examined immediately after treatment, a difference might have been noted. It was beyond the scope of this study to monitor acid secretion in the stomachs during the treatment. Therefore, there is no way of knowing whether the dose of 30 mg daily of lansoprazole was sufficient to inhibit gastric acid secretion. Possibly, a higher dose would have been effective in preventing lesions, or possibly splitting the dose so that it was given 2 or 3 times during the day would have provided better results.

There are few studies which have examined the efficacy of benzimidazole compounds in controlling acid secretion in the pig. One trial proved both timoprazole and omeprazole (at a dose of 20 mg b.i.d.) were effective in preventing gastric ulceration in pigs injected with porcine somatotropin (26). Lower doses of timoprazole (5 mg b.i.d.) were also shown to be effective (26). Eaton and Krakowka (27) have reported that omeprazole given PO once daily is not effective in providing consistent achlorhydria but have shown that 5 mg, IV, q12h, is sufficient. Benzimidazole compounds act by irreversibly inhibiting $H^+/K^+$-ATPase and their effect can only be overcome by the production of new enzyme. This process takes at least 24 h to complete in humans (28) and, therefore, it is unnecessary to administer the drug more than once daily in human medicine. However, because so little work has been done in swine, human doses and regimens were used in this trial, but this may not have been appropriate.

Certainly, the lack of treatment effect may indicate that other factors besides gastric acid secretion are involved in the production of ulcers during a period of fasting. Recent studies suggest that bile acids act synergistically with hydrochloric acid during periods of fasting to produce rapid destruction of the squamous epithelia of the pars esophagea (29). High concentrations of bile acids have been shown to be associated with gastric ulcer severity (30). One would have expected that the administration of acid secretory inhibitors might have reduced the effect of bile acids by maintaining a higher pH. The findings of this trial do not provide evidence that this occurred.

The production of organic acids by a proliferation of bacteria in the pars esophagea has been suggested as a mechanism causing gastric ulceration (5,6). One might have expected that the acid inhibitory treatment would possibly encourage this scenario. Generally, the low pH of the distal stomach would inhibit the growth of *Lactobacillus* and *Bacillus*, but daily medication of lansoprazole and the sudden resumption of ad libitum feeding with finely-ground feed would be expected to be an ideal situation for the proliferation of fermentative bacteria. Likewise, one might expect the treatment with an antibiotic during the period when feed is reintroduced to reduce the effects of bacterial proliferation. However, this trial does not provide sufficient evidence to support this hypothesis. The significant difference in the proportion of pigs with severe ulceration may be due to the inaccuracy of macroscopic classification as revealed by Embaye et al (23). Histological examination revealed 10 of the 11 pigs in the control group which were classified as 2 to have deep ulcerative lesions. On a histological basis, there is no evidence of a difference between the 3 treatment subgroups either in the fasted or non-fasted groups.

*Helicobacter*-like organisms were not found in any of the 84 pigs examined suggesting that this farm may be free of these bacteria. This is a very important finding for future studies of this organism. Recent studies have suggested that *Helicobacter heilmannii* is widely distributed in the commercial swine population (7–10) and may be a source of infection for humans (31). The technique of in situ urease mapping and microscopic examination with Warthin-Starry silver stain is believed to be reliable, in that this laboratory has readily demonstrated these coiled bacteria in stomach mucosa of pigs from other herds (32). The lack of *Helicobacter* makes it impossible to judge the effectiveness of this combination of antibiotic and acid secretory inhibitor as an eradication program for swine. The fact that ulcers did occur in the absence of these organisms does suggest that if they do play a role in gastric ulceration in swine, they certainly are not the only factor involved.

This study has shown that finely-ground pelleted rations and fasting are important risk factors in the development of gastric ulcers. Acid secretory inhibitors and broad spectrum antibiotics, as they were used in this study, did not provide protection to the epithelial tissue of the pars esophagea during a period of feed withdrawal. Further research needs to be conducted to determine whether this failure in ulcer prevention is a result of ineffective acid secretion inhibition or

whether other factors are involved in causing tissue damage.

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