Effects of Combined Treatment with Aspirin and Diclofenac or Indomethacin on the Gastric Mucosa of the Rat (Reduced Damage by Proton Pump Inhibitors)

Faraj Al-Bustami¹*

Abstract

Objectives: To investigate the gastric mucosal injury caused by the combined treatment of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) and to identify the optimum methods to avoid such injury.

Methods: Sixty albino Wistar rats of 200-250gm were used in this study. The animals were divided into 8 groups. Group I served as control and received the vehicle of the used drugs (aspirin, diclofenac and indomethacin). Groups II, III, and IV received single daily dose of aspirin (4mg/kg body weight), diclofenac (2mg/kg. body weight) and indomethacin (2mg/kg body weight) respectively. Group V received aspirin and diclofenac and Group VI received aspirin and indomethacin as above. Group VII and VIII received omeprazole (2mg/kg body weight) one hour before ingestion of aspirin and diclofenac or aspirin and indomethacin as above. At the end of treatment, lesions in the stomach were evaluated macroscopically and microscopically.

Results: Macroscopic analysis showed that treatment with aspirin, diclofenac and indomethacin separately induced multiple gastric mucosal lesions which were larger and deeper following the combined treatment of aspirin and either diclofenac or indomethacin. Microscopically the lesions include increased mucus production, oedema, congestion, necrosis and hemorrhage of the fundic mucosa. After the administration of omeprazole (proton pump inhibitor) most of the mucosal lesions show evidence of partial healing.

Conclusion: Combined treatment with aspirin and diclofenac or indomethacin produced larger and deeper gastric fundic mucosal lesion than aspirin treatment alone. Omeprazole ingestion added to the above drugs significantly healed lesions compared to the control group by 75% and 70% (p=0.05).

Keywords: Aspirin, Diclofenac, Indomethacin, Gastric Ulcer Protection.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are of significant clinical value, accounting for nearly 5% of all prescribed medications ¹. Aspirin was synthesized as the first (NSAID) a century ago, and since then several types of (NSAID) have been developed.
Nonetheless (NSAID) use is often associated with gastrointestinal complications \(^2\) with approximately 15 to 30% of long-term users experiencing gastrointestinal ulcers and bleeding \(^3,4,5,6\). In the United States alone, approximately 16,500 people per year die as a result of these complications \(^7\). The anti-inflammatory action of (NSAIDs) is mediated through their inhibitory effect of cyclooxygenase (COX) activity. COX is an enzyme essential for the synthesis of prostaglandins (PGs). The inhibition of COX was believed to be the sole explanation for the gastric complications of (NSAIDs), given that PGs exert a strong protective effect on gastric mucosa \(^8,9\). Ushijima et al. \(^10\) have demonstrated that (NSAIDs) induce in vitro cell death (apoptosis and necrosis) independent of COX inhibition and have suggested that both COX inhibition and NSAID-induced cell death are required to produce gastric lesions in Vivo. There are many uncertainties about the actual state of gastric mucosal lesions due to (NSAIDs) which have been used widely in recent years \(^11\). The risk of upper gastrointestinal bleeding with aspirin is increased with old age and concomitant medication with NSAIDs \(^12\). The cardiovascular benefits of low-dose aspirin might be overcome by the risk of gastrointestinal complications, but withdrawal of aspirin therapy can precipitate a cardiovascular event \(^13\). In old patients long-term (NSAID) therapy is prescribed not only for patients with acute conditions such as trauma but also for the treatment of chronic diseases such as arthropathies, including rheumatoid arthritis and low back pain. In these patients the concomitant use of (NSAID) and aspirin is not uncommon\(^1\)

The main objective of the present study is to describe the gastric mucosal injury caused by the combined administration of aspirin and either diclofenac or indometacin and to indentify the optimum methods to avoid such injury.

### Materials and Methods

**Animals:** Sixty albino wistar rats weighing 200-250 gm of either gender, obtained from the animal house of the medical school were used for this study. Rats were housed individually in cages, maintained under standard conditions (12 hours light: 12 hour dark cycle) and were fed with standard pellet and water ad libitum.

**Drugs:** Acetyl salicylic acid (Aspirin, Bayer, Germany) diclofenac (Voltaren, Novartis, Switzerland), omeprazole (epirazole, Eipico, Egypt), indomethacin (indolag, lagap, Switzerland). These drugs and their vehicles were purchased from the corresponding companies.

Animals were randomly divided into 6 groups consisting of 10 rats each. Animals of group I were used as control, and received the vehicle. Animals of group II were given aspirin at a daily single dose of 4 mg per kg body weight. The drug was dissolved in 1% NaHCO3 and given per os. Animals of group III were given diclofenac at a single daily dose of 2.0 mg per kg body weight per os. Animals of group IV were given indomethacin in a single daily dose of 2 mg per kg body weight per os. Animals of group V were given aspirin and diclofenac as above. Animals of group VI were given aspirin and indomethacin as above. Animals of group VII were given the proton pump inhibitor omeprazole at a single daily dose of 2 mg per kg body weight followed after one hour by aspirin and diclofenac as above. Animals of group VIII were given omeprazole as above followed by aspirin and indomethacin as above. Three animals of each group were sacrificed by
cervical dislocation after 3 hours, 3 days and 3 weeks of treatment. One animal of each group was sacrificed two weeks after cessation of treatment. The stomach was taken out and cut open along the greater curvature and the grade of lesions was scored in the glandular part of the stomach with the help of a magnifying glass and a millimeter scale as described by Das and Banerjee, according to the following criteria: 
0=no pathology; 1=a small 1-2 mm ulcer; 2=medium 3-4 mm ulcer; 4=a large 5-6 mm ulcer; 8=a larger > 6mm ulcer. The sum of the total severity scores in each group of rats divided by the number of animals was expressed as the mean ulcer index.

Histopathology: After macroscopic evaluation, stomachs were preserved in 10% formaldehyde solution for histopathological studies. In each specimen, paraffin sections of 4 um in thickness were obtained, stained with one of the following methods.
1) Marks and Drysdale (PAS-hematoxylin-aaurantia) (triple stain)
2) Alcian blue, PH 2.5-Periodic acid Schiff (AB-PAS)
The above two methods are specific for mucus of the gastric glands. The severity of the histopathological changes such as changes in mucus production, congestion, oedema, haemorrhage and necrosis were expressed on an arbitrary scale as per the following changes: normal;+ little effect ++; appreciable effect; +++: severe effects; ++++: very severe effects. Ulcer injury was scored by the method described by Takeuchi et al. according to the following criteria: 1= no damage, 2= shallow damage not exceeding 25% of the mucosal depth but not exceeding 75%; 4= deep damage reaching 75% of the mucosal depth. The ulcer depth index was calculated by dividing the mean mucosal thickness by mean ulcer depth.

The ulcerated portion was measured as percentage size of the ulcer in 3 mm of gastric mucosa.

Statistical analysis: The results were expressed as mean ± S.E.M and statistical differences between several treatments and their respective control was determined by one-way analysis of variance (ANOVA), followed by Fisher's least significant difference test using the statistical package for social sciences version 14.0 software (SPSS Inc. Chicago, IL, USA) the level of significance was set at P < 0.05.

Results

Macroscopic Studies:
Three weeks after treatment, using a magnifying glass, the macroscopic observations showed that aspirin, diclofenac and indomethacine induced multiple gastric mucosal lesions, mostly 3-4mm in size, with bleeding during the observation. The mean ulcer index for aspirin was 7.4 ± 0.8, for diclofenac 9.6± 1.0 and for indomethacin 8.2 ± 0.77(P=0.001 versus control) (fig.1). As a result of a combined treatment of aspirin and diclofenac the ulcer index has increased to 16.6± 1.9 and when aspirin was given with indomethacin the ulcer index reaches 12.5±1.1 (P=0.001 versus control). Adding the proton pump inhibitor omeprazole to the combined treatment with aspirin and diclofenac and aspirin together with indomethacin significantly healed lesions compared to groups V and VI by 75% and 70% (Fig.1) (P=0.05).

Histopathological Studies:
Microscopical data were consistent with macroscopic observation. The erosive defect which appeared macroscopically appeared microscopically with congestion, oedema, necrosis and haemorrhage.
During the first few hours after aspirin treatment, the fundic gastric mucosa showed
excessive production of surface mucous which was associated with congestion and oedema (Fig 2, 3, 4). This was followed after 2-3 days by desquamation of surface mucous cells leaving the pit epithelium relatively unaffected (Fig 5). The more superficial desquamated cells showed evidence of necrosis with deeply stained pyknotic nuclei. However, some of the superficial cells appear swollen but retained a residual intracellular polarization e.g. with clearly recognizable nuclei and distinctive perinuclear and apical cytoplasmic zones (Fig 6).

After three weeks of aspirin ingestion most of the above changes have decreased or disappeared, although some patchy areas of superficial cells necrosis can still be seen (fig.7). The parietal cells underlying these superficial erosions seem to have remained unaltered by aspirin (Fig.8), the treatment with diclofenac or indomethacin produced similar but less extensive changes than with aspirin (Fig. 9). When diclofenac was given together with aspirin and after three days of treatment the fundic mucosa showed extensive mucous production associated with, oedema, congestion and necrosis. The gastric pits disappeared and these changes extended into the basal parts of the glands which showed cystic dilatations filled with secretion (Fig. 10).

After three days of combined treatment of aspirin and indomethacin, the fundic mucosa showed similar but less extensive changes to the above (aspirin and diclofenac), however after three weeks fundic mucosa showed evidence of metaplastic change in which the gastric pits were replaced by stratified squamous epithelium but no mitotic figures or pleomorphism were seen among the metaplastic cells (Fig. 11). Inbetween the patches of squamous metaplasia, the gastric mucosa appeared normal or with early metaplastic change (Fig. 11). Deep to the layers of squamous metaplasia, the gastric glands were formed mostly of parietal cells and the mucous neck cells were rarely seen (Fig. 12). Congestion of the gastric mucosa appeared in the form of dilated blood vessels between the gastri glands (Fig. 12). Two weeks after cessation of aspirin and indomethacin treatment patches of squamous metaplasia showed evidence of pit formation suggestive of the reversible nature of this change (Fig. 13). For animals subjected to PPI treatment in addition to aspirin and diclofenac or indomethacin, regenerative improvements were observed in the mucosal lesions. However some localized oedema and superficial defects were occasionally seen (Fig. 13).

Table 1. Histopathological examination of gastric lesions induced by aspirin and other NSAID

<table>
<thead>
<tr>
<th>Groups</th>
<th>Size of ulcer in 3mm of gastric mucosa %</th>
<th>Ulcer depth index</th>
<th>Surface mucous</th>
<th>Congestion</th>
<th>Oedema</th>
<th>hge</th>
<th>necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II. Aspirin</td>
<td>32.5*</td>
<td>2.78*</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>III. Diclofenac</td>
<td>39.8*</td>
<td>3.14</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>IV. indomethacin</td>
<td>28.3*</td>
<td>2.33*</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V. II + III</td>
<td>81.5†</td>
<td>1.88†</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>VI. II + IV</td>
<td>68.2†</td>
<td>1.62†</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>VII. as in V + PPI</td>
<td>24.5**</td>
<td>2.91**</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VIII. as in VI + PPI</td>
<td>20.8**</td>
<td>3.10**</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

hge = hemorrhage, statistical significance. P* < 0.01 versus controls, †P = 0.05 versus Aspirin, P** = 0.05 versus V and VI, -: normal, +: little effect, ++: appreciable effect, +++: severe effect.
<table>
<thead>
<tr>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
</tr>
<tr>
<td>II Aspirin</td>
</tr>
<tr>
<td>III diclofenac</td>
</tr>
<tr>
<td>IV indomethacin</td>
</tr>
<tr>
<td>V Aspirin + diclof</td>
</tr>
<tr>
<td>VI Aspirin + indometh</td>
</tr>
<tr>
<td>VII as In V + PPI</td>
</tr>
<tr>
<td>VIII as In VI + PPI</td>
</tr>
</tbody>
</table>

**Fig. 1:** Effects of Aspirin, diclofenac and indomethacin treatment separately or in combination on gastric mucosa (Ulcer index). Statistical significance: $p^* = 0.001$ versus control $P^† = 0.05$ versus group V and VI. $P^{**} = 0.05$ versus group II.
Fig. 3 fundic mucosa 3 hours after aspirin ingestion stained by triple stain. It shows excessive production of surface mucous and oedema (thick arrows). The pits (thin arrow) are also distended with mucous. X390

Fig. 4 fundic gastric mucosa 3 hours after aspirin ingestion stained by (AB- PAS). It shows excessive production of mucous by the pit cells (arrow). X280
Fig. 5 Fundic mucosa 3 days after aspirin ingestion stained by triple stain. It shows desquamation of cells with pyknotic nuclei (thick arrow) as well as erosion of the upper part of the pits (thin arrows). X390

Fig. 6 Fundic mucosa 3 days after aspirin ingestion stained by triple stain. It shows desquamated cells with pyknotic nuclei (thin arrow) and some desquamated cells which appear swollen and retained intracellular polarization (thick arrow) and normal parietal cells (p) x 440
Effects of Combined ...

Faraj Al-Bustami

Fig. 7 Fundic mucosa 3 weeks after aspirin ingestion stained by triple stain. Most of the pits have regenerated (arrow head) and others still shows necrosis (thin arrow).

Fig. 8 Fundic mucosa 3 weeks after diclofenac treatment stained by (AB-PAS). It shows desquamation and necrosis of the superficial parts of the gastric pits (arrows)x280
Fig. 9 Fundic mucosa 3 days after combined treatment with aspirin and diclofenac, stained by triple stain. It shows extensive mucous production, oedema and necrosis. Most of the gastric pits have disappeared and the basal parts of the glands show cystic dilatations filled with secretion (CD) x280

Fig. 10 Fundic mucosa 3 weeks after combined treatment with aspirin and indomethacin stained by triple stain. A metaplastic change of the pit cells is suggested by replacement of their cells by stratified squamous epithelium. The process involves fusion of the adjacent walls of the pits which starts at their deep parts (long arrow) and extends more superficially (arrow head) x320
Effects of Combined ...

Faraj Al-Bustami

Fig. 11 fundic mucosa two weeks after cessation of aspirin and indomethacin treatment stained by triple stain. It shows reversible metaplastic changes suggested by the reappearance of the gastric pits within the patch of metaplasia. (arrows) A large erosion can be seen lined by stratified squamous epithelium with few short pits (arrow head) x280

Fig. 12 fundic mucosa 3 weeks after combined treatment with aspirin and indomethacin stained by triple stain. A dilated blood vessel (BV) can be seen between the gastric glands-partial fusion in the deep parts of the pits is also seen (arrows) x320
Discussion

Oral administration of anti-inflammatory drugs (NSAID) induces gastroduodenal mucosal damage in experimental animals as well as in humans. As the buffered aspirin has been reported to reduce the injury caused by the non-buffered drug, aspirin in the present work was dissolved in 1% NaHCO₃.

The dose of aspirin in the range we used in this study are widely believed to be harmless to the gastric mucosa. Our study showed, however, that 4mg aspirin/kg body weight caused congestion, oedema, necrosis and haemorrhage; and these effects on the fundic mucosa were much intensified by adding either diclofenac or indomethacin to the aspirin treatment.

Diclofenac is conventionally known to provide a higher risk of upper GI hemorrhage than any other (NSAIDs) therefore, close attention should be paid to its use while a superior analgesic effect is expected. Lichtenberger and his coworkers have proposed that (NSAIDs) disrupt the hydrophobic barrier properties of the gastric mucosal surface, rendering it susceptible to attack by luminal acid. In the present study, it seems that the increased surface mucous production observed during the first few hours following aspirin and other (NSAID) treatment was unable to protect the fundic mucosa against the injurious effects of these drugs.

An important feature of the present study is
that dosing continued for longer periods than in most other studies: Whether the risk of gastric mucosal injury, necrosis and bleeding increased with time or alternatively, whether it decreased by a process of adaptation. Our study showed that the gastric mucosal injury following aspirin treatment is greatest in the first week of ingestion and adaptation occurs with prolonged ingestion. Similar results were obtained when diclofenac or indomethacin was ingested alone. However, the continued ingestion of aspirin together with diclofenac or indomethacin fundic mucosal injury was always observed and evidence of regeneration was seen only after cessation of treatment.

These results give support to previous observations that the incidence of peptic ulcers increases when (NSAIDs) which are prostaglandin inhibitors and directly injurious, are administered to patients with gastric mucosa affected by various injurious factors like aspirin ingestion or H. Pylori infection. Furthermore, delayed healing of ulcers was reported in patients who took acid-suppressant drugs while continuing to take (NSAIDs) 25. Recently it has been reported that the prevalence of gastric mucosal lesions reached 58.5% in patients without subjective symptoms, where 10.1% had peptic ulcers. This means that in patients receiving (NSAIDs) subjective symptoms are not a basis for the diagnosis of gastric mucosal lesions, especially peptic ulcers. This supports previous reports that many gastric mucosal lesions due to (NSAIDs) are asymptomatic.

Although the mechanism responsible for the metaplastic change following indomethacine ingestion is not known, the decrease in ulcer index as compared to diclofenac suggests that the metaplasia may play a protective role against ulceration. The significant decrease in the ulcer index following the ingestion of PPI (omeprazole) indicates that this drug reduces the incidence of gastric mucosal lesions in patients receiving (NSAIDs).

Recently JuSeo et al 30 reported that NSAIDs-induced acute gastric damage increased in a dose-and age-dependent manner which was accompanied by a significant increase in mucosal myeloperoxidase (MPO) levels. The latter is a biochemical indicator of the degree of mucosal neutrophil infiltration.

Experimental and limited clinical studies indicate that aging gastric mucosa has impaired mucosal defenses such as decreased mucus and bicarbonate secretion, decreased prostaglandin generation and reduced blood flow. In the present study, the excessive production of surface mucus few hours after aspirin treatment appeared as a first protective mechanism against the injurious effect of NSAIDs.

The limitations of the present study reflect the fact that it is a purely histopathological work. The underlying mechanism such as COX/Prostaglandin pathway and MPO concentration have not been evaluated. Further studies regarding the underlying detailed mechanisms and susceptibility among NSAIDs are planned in the future.

In conclusion, as it is reported that the gastric mucosal lesions are often asymptomatic in patients receiving (NSAIDs) therefore physicians should control pain with (NSAIDs) with a keen recognition that greater awareness of the risk of gastric mucosal lesion and measures for them are essential even if there are no complaints.
References


Effects of Combined ...

Faraj Al-Bustami


تأثير العلاج بالأسبرين إضافة إلى الدايكلوفيناك أو الاندوميثاسين إلى بطانة المعدة في الجرذ

فرج البسطامي

1 - كلية الطب، الجامعة الأردنية

الملخص

الهدف: معرفة الأثر والنتائج لعلاج الأسبرين وحده أو إضافة الدايكلوفيناك أو الاندوميثاسين على تلف بطانة المعدة في الجرذ وإيجاد الوسائل المناسبة لتجنب أو التخفيف من هذا التلف.

المETHODS: تم استخدام ستين جرذًا من النوع الأبيض وستربوزنترارويتين 200-250 غرام وقسمت الحيوانات إلى 8 مجموعات واستخدمت المجموعة الأولى كمجموعة تحكم ولم تعط أي علاج، حيث أثبتت دراسات سابقة أن كل الأدوية المستخدمة ليس لها أي تأثير على بطانة المعدة، أما المجموعات الثانية والثالثة والرابعة فقد أعطت جرعة يومية واحدة من الأسبرين (4 ملغم جرام لكل كيلوغرام من وزن الجسم) والدايكلوفيناك (2 ملغم لكل كيلوغرام) والاندوميثاسين (2 ملغم لكل كيلوغرام) على التوالي، وقد أعطت المجموعات الخامسة والسادسة والثامنة الأوميبرازول (2 ملغم لكل كيلوغرام) ساعة قبل إعطاء الأسبرين والدايكلوفيناك أو الأسبرين والاندوميثاسين حسب السريعة والثامنة. وتم تقسيم الفحص في غشاء المعدة عن طريق التحليل المجهرى (Microscopic) والنظري (Macroscopic).

النتائج: أظهر الفحص المجهرى أن العلاج بالأسبرين أو الدايكلوفيناك أو الاندوميثاسين يؤدي إلى حدوث تلف وترسبات في بطانة المعدة التي ظهرت بسمحة وcupping في جرح العمود الرئيسية: و من أسبرين مع الدايكلوفيناك ومع الاندوميثاسين (P=0.05) أما الفحص المجهرى فقد أظهر وجود زيادة في تفاوت الخطط السطحي واحترق وتلف وتلف في بطانة قاع المعدة، وبعد إعطاء دواء الأوميبرازول لحيوانات المجموعتين السابعة والتاسعة تم تحقيقات التسمح لقفز جذر المعدة بالمقارنة مع مجموعات التحكم (P<0.01).

الاستنتاج: إن العلاج بالدايكلوفيناك أو الاندوميثاسين إضافة إلى الأسبرين أدى إلى حدوث تلف وترسبات في بطانة قاع المعدة أكبر سمية وعما للمعالي الأسبرين وحده واضافة الأوميبرازول إلى الأدوية السابقة يؤدي إلى النتائج ملحوظة للفحص في جذر المعدة بالمقارنة مع مجموعات التحكم بنسبة تراوح بين 70-75% (P<0.01).

الكلمات الدالة: الأسبرين، الدايكلوفيناك، الاندوميثاسين، القرحة المعدية، الأوميبرازول، الوقاية.