

Ulcerogenic Effects of NSAIDs on Gastric Mucosa: Comparison between Selective and Non-Selective Cox2 Inhibitors; Indomethacin VS Rofecoxib

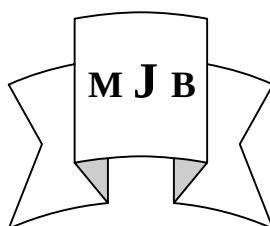
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Abstract

Background: The central pathogenic mechanism in NSAID-induced gastro-duodenal toxicity lies in their ability to inhibit the synthesis of prostaglandins by gastric mucosa through inhibition of cyclo-oxygenase enzyme (Cox). There are two isoforms of Cox enzyme: Cox-1 and Cox-2. The gastro-protective effects of prostaglandins are mediated by Cox-1 while the inflammatory effects are mediated by Cox-2. NSAIDs inhibit synthesis of prostaglandins, resulting in toxic effects on gastric mucosa and beneficial anti-inflammatory effects.

Conventional NSAID are non-selective inhibitors of cyclo-oxygenase and thus, they promote the anti-inflammatory response and at the same time inhibit gastric protective effects of prostaglandins. To overcome this problem, drugs that have little or no Cox-1 inhibitory activity have been developed and a new generation of NSAIDs has emerged.

Aim of the study: The aim of this study is to evaluate the morphological effects of Rofecoxib on the gastric mucosa by comparing them with those produced by Aspirin and Indomethacin.

Material and Methods: 40 Spargue-Dawely rats were used in this study. The animals were divided into four subgroups, each group included ten animals. Group I received no treatment and considered as control, group II received Aspirin, group III received Indomethacin, and group IV received Rofecoxib. After one month of treatment the animals were sacrificed and the gastric mucosa in each animal was examined macroscopically and microscopically.

Results: Aspirin produced the most sever gastric lesions mainly in the pylorus, which take the form of erosions and ulcerations. Rofecoxib caused the least gastric lesions mainly in the body of the stomach. Indomethacin caused an intermediate degree of gastric damage mainly in the body of the stomach.

Conclusion: Aspirin and Indomethacin produced the most sever effect on gastric mucosa, these effects take the form of gastric erosion and ulceration that involved mainly the pylorus and the body of the stomach respectively. Rofecoxib, showed the least gastric lesion as compared to Aspirin and Indomethacin.

تأثيرات مضادات الالتهاب غير الستيرويدية على مخاطية المعدة : مقارنة بين الأدوية المثبطة

لا انتقائيا وتلك المثبطة انتقائيا لعمل انزيم كوكس ٢

الخلاصة

الخلفية: أن آلية عمل مضادات الالتهاب غير الستيرويدية هي تثبيط عمل مادة البروستاغلاندين الموجود في مخاطية المعدة من خلال تثبيط انزيم السيكلوأكسي جينيز. اظهرت البحوث الحديثة ان هنالك نظيرين من هذا الانزيم: الاول يحفز تصنيع البروستاغلاندين المسؤول عن حماية مخاطية المعدة من التأثير المؤذي للحمض المعدي و الببسين، ويطلق على هذا النظير اسم COX ١ ، والثاني يحفز الاستجابة الالتهابية و مسؤول عن نشوء الاعراض والعلامات الالتهابية ويطلق عليه اسم COX٢. وعليه فان الادوية المضادة للالتهابات - غير الستيرويدية التي تثبط عمل كلا الانزيمين ستؤدي إلى التخلص من الأعراض الالتهابية ولكن في نفس الوقت

تؤدي إلى أذية العشاء المخاطي للمعدة. هذا الأمر دفع الباحثين إلى التفتيش عن مضاد التهاب غير ستيرويدي يثبط عمل الكوكس - ٢ فقط.

اهداف الدراسة: هذه الدراسة تهدف إلى دراسة دواء من هذه الأدوية هو الريفوكوكسب من جهة الاثار الجانبية على مخاطية المعدة ومقارنتها مع الأسبرين و الاندوسيد.

المواد وطريقة العمل: استخدمنا في هذه الدراسة أربعين جرذا ذكرًا حيث قسمت إلى أربعة مجاميع كل مجموعة تضم ١٠ جرذان. المجموعة الاولى استخدمت كمجموعة سيطرة اولى ولم تعط اي دواء، المجموعة الثانية اعطيت عقار الاسبرين ، المجموعة الثالثة اعطيت عقار الاندوميثاسين ، أما المجموعة الرابعة فأعطيت عقار الروفيكوكسب بجرعة ولمدة شهر. في نهاية هذه الفترة قمنا بتشريح الجرذان بعد تخديرها وقمنا باخذ المعدة من كل جرذ وفحصها عيانيا ومجهريا وتثبيت الموجودات التشريحية النسيجية.

النتائج: تسبب الاسبرين باثذ الاضرار في مخاطية المعدة. بينما تسبب الروفيكوكسب بأقل ضرر على مخاطية المعدة ، اما الأضرار التي سببها عقار الاندوسيد فكانت وسطا بين الاثنين.

الاستنتاج : إن الأدوية الجديدة من مضادات التهاب-غير الستيرويدية سببت اقل قدر من الأذى للمخاطية المعدية مقارنة بالأدوية التقليدية. ولذلك فهي أكثر أمانا للاستخدامات طويلة الأمد كما هو الحال في التهابات المفاصل المزمنة.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed medications in the world [1]. These drugs are widely favored because they are free from side effect like sedation, mental clouding, nausea, and constipation produced by traditional analgesic drugs such as opioid and related drugs [2]. Despite their beneficial role in rheumatic arthritis and inflammatory disorders, NSAID therapeutic value decreased 2 to 3 folds because of the high risk of gastro-intestinal toxicity [2].

The anti-inflammatory effect of NSADs is produced by inhibition of the synthesis of prostaglandins G/H synthase (cyclooxygenase). Two isoforms of cyclooxygenase enzyme have been recognized; Cox-1 and Cox-2. Cox-1 is predominantly expressed constitutively and functions as physiologic house keeping in most tissues including gastric mucosa, kidneys, and platelets[3,4]. Cox-2, expressed especially in macrophages and synovial cells, is induced by inflammation and mutagen stimulation [5]. The anti-inflammatory properties of NSAID is mediated through Cox-2 inhibition while the gastrointestinal

toxicity is mediated through Cox-1 inhibition [6]

Traditional NSAIDs are nonselective Cox inhibitors and differ in their relative inhibitory potency against Cox-1 and Cox-2. The important role of Cox-1 in protecting the GI tract mucosa is supported by the finding that greatest damage to GIT produced by NSAIDs therapy is mainly cause by Cox-1 inhibition [7].

The aim of this study is to compare damages to gastric mucosa caused by non-selective Cox inhibitors NSAIDs with those caused by selective Cox-2 inhibitors.

Material and Methods

1. The experimental animals: 40 healthy adult male Sprague-Dewily rats with weight ranging between 200-230gm and aged between 18-20 weeks were obtained from the National Center for Drug Control and Research/Baghdad. The animals were randomly divided into five groups, each containing 10 animals, as following:

Group I received no treatment and served as control.

Group II received Aspirin in a dose of 30mg/kg once daily for one week., considered as second control.

Group III received Indomethacine in a dose of 25mg/kg, for one month.

Group IV received Rofecoxib in a dose of 10mg/kg for one month.

The doses are the standard doses used in rats for assessment of gastric damage induced by NSAID [7]. Animals body weight were checked regularly and the dose were calculated according to it.

2. Drugs used in experiment

Aspirin tablets: acetylsalicylic acid obtained as Aspirin 100 each tablet contains acetylsalicylic acid 100mg, Batch No10n. manufactured by the State Company for Drug Industry (SDI), SAMARA-IRAQ.

Indomethacin capsules: Indols derivatives, obtained as Indomin 25 each capsule contains 25mg Indomethacine BP. Batch No.1967. Manufactured by AL-HIKMA's drug company.

Rofecoxib tablets: Selective Cox-2 inhibitor that is obtained as Dioxx 25, each tablet contains 25mg; Batch No.2-03 manufactured by Dafar Drugs Company.

Methods

1. Preparation of drugs solutions:

The drugs were diluted with normal saline to get the accurate dose for each animal according to its body weight. The drugs were administered as liquid solutions through stomach tube.

2. Operative procedure: After one month of treatment with the appropriate drug for each group, animals were sacrificed as follow:

a. The rat was anesthetized and a midline incision was made and the stomach is removed.

b. Then the stomach was opened along the greater curvature, stretched moderately by pinning on a cork board, and then the gastric mucosa was examined by naked eye and magnifying lens to:

* count the number of lesions by mm².

* determine the extent of the lesions by measuring the surface area occupied by the lesions relative to the total surface area of the gastric mucosa.

* study the distribution of ulcers and their grades in various parts of the stomach.

3. Histopathology techniques:

a. Each stomach was fixed with 10% formalin solution for 2days.

b. After fixation, three specimens were taken from each stomach; from the funds, the body, and the pylorus.

c. The sections were stained with conventional H&E stain; at least four sections are prepared from each animal.

d. The stained sections were examined under light microscope and the relevant data were registered.

e. The severity and the degree of mucosal damage are assessed according to **modified Seolny** scale, so that the following grades were obtained: Grade (0): no mucosal lesions; Grade (1): mucosal edema, congestion, and neutrophils infiltration; Grade (2): surface mucosal erosion. Grade (3): Gastric ulcerations.

4. Statistical analysis

All obtained values were expressed as mean + standard error of mean (SEM). By using Chi-square test the proportion of histopathological changes in various groups of animals were compared. P-values < 0.05 are considered significant.

Results

1. Histopathology findings:

The numbers and surface areas of stomach's lesions for each animal were calculated. The values are expressed as mean number and mean surface area; tables 1 and 2. Regarding the distribution of lesions in different parts of the stomach (funds, body, and pylorus) we found that there is significant differences in distribution of lesions between funds and body,

funds and pylorus ($P < 0.05$). The distribution of lesions according to site and severity showed significant differences between body and funds,

funds and pylorus for grade 1. The reverse was seen regarding grade 3. Table 3.

Table 1 Mean number of lesions per mm² for different groups. Aspirin is considered as second control for comparing the severity of lesions induced by other NSAID.

Group of animal	Lesion number/mm ²	% of stomach mucosa involved by lesions	P-value
I	0	0	0
II	6.40 + 0.56	100%	$P < 0.001$ as compared to normal control
III	5.50 + 0.58	85%	$P > 0.05$ as compared with aspirin
IV	0.70 + 0.23	11.25%	$P < 0.05$ as compared with aspirin

Table 2 Mean surface areas of lesions induced by different NSAID for different study groups

Group	Total area of lesion (mm ²)	% of the involved gastric mucosa	P-values
I	0	0	/
II	9.10 + 0.61	100%	$P < 0.05$ as compared with control
III	6.40 + 0.6	70.32%	$P > 0.05$
IV	0.60 + 0.15	6.59%	$P < 0.05$

2. Histopathology findings.

a. Control group. Macroscopically, the stomach revealed a normal glistening mucosa covered by a thick layer of fluffy adherent mucus.

Microscopically. The funds of the stomach revealed normal squamous non-glandular epithelium. The body revealed normal glandular epithelium with characteristic deep blue oxyntic cells at the base of the glands. The

antrum was normal showing the predominance of mucus secreting glands.

b. Aspirin and Indomethacin-treated groups. Macroscopically. The wall of the stomach appeared stretched and thinner than that in control. Numerous, tiny, pin-point petechial hemorrhages in the body and the pylorus were observed. Much irregularly distributed erosions that are

elongated, dark-red and oriented parallel to the longitudinal axis of the stomach were also observed.

Microscopically. The mucosal lesions showed variable depths; most of them have the appearance of superficial hemorrhagic erosions involving mainly the body and pylorus (figure 1), others are deeper extending throughout the mucosa to involve the sub-mucosa. There is a heavy infiltration of acute inflammatory cells (neutrophils and macrophages) involving the mucosa

and submucosa, as well as mild monocytic infiltrate. The funds of the stomach show hyperemia, but is less subjected to erosions compared with the body and pylorus. In few cases the lesion takes the form of overt gastric ulceration that involved the full thickness of the wall of the stomach (figure 2). According to the criteria adopted for assessment of the severity of the lesions the following results were obtained: tables 5 and 6.

Table 3 Aspirin-induced gastric lesions distributed according to their grades in different parts of the stomach for each case.

Case number	Funds	Body	Pylorus
1	G1	G2	G3
2	G1	G2	G3
3	G1	G2	G3
4	G1	G2	G3
5	G1	G2	G3
6	G1	G2	G3
7	G1	G2	G3
8	G1	G2	G3
9	G1	G2	G3
10	G1	G2	G3

Table 4 Indomethacin-induced gastric lesions distributed according to their grades in different parts of the stomach for each case.

Case number	Funds	Body	Pylorus
1	G1	G2	G3
2	G1	G3	G3
3	G1	G2	G2
4	G0	G3	G2
5	G1	G2	G3
6	G0	G3	G2
7	G1	G3	G3
8	G1	G3	G3
9	G0	G2	G2
10	G1	G3	G2

c. Rofecoxib-treated groups.

Macroscopically. The number of lesions is less compared to Aspirin and Indomethacine-treated group, table 1. The lesions involved mainly the body

and pylorus of the stomach and showed no specific pattern of appearance. Very small and scanty hememorrhagic spots or just hyperemic areas were observed.

Microscopically. There was a mild degree of congestion of blood vessels with a very little inflammatory infiltration. The hyperemic lesions are superficial and hardly extending beyond the superficial lining epithelium to cause frank erosions, this

is usually seen in celecoxib-treated animals. There is moderate acute inflammatory cells infiltration, as well as chronic inflammatory cells. According to Modified Sedny scale the following results were obtained, table 5.

Table 5 Rofecoxib-induced gastric lesions distributed according to their grades in different parts of the stomach for each case.

Case number	Funds	Body	Pylorus
1	G0	G1	G1
2	G0	G1	G0
3	G0	G1	G1
4	G0	G1	G1
5	G0	G1	G0
6	G0	G1	G0
7	G0	G1	G0
8	G0	G1	G0
9	G0	G1	G1
10	G0	G1	G1

Discussion

Mechanisms of NSAID-induced gastric ulceration.

NSADs produce mucosal injury via local irritating and systemic effect [8]. The later is mediated through cyclooxygenase inhibition [9]. Cyclooxygenases are membrane bound glycoproteins which catalyze arachidonic acid into prostaglandins G2 which have cytoprotective activity [10]. It has been reported that cyclooxygenases activity increases in a variety of cells after exposure to endotoxines, pro-inflammatory cytokines, growth factors, hormones, and tumor promoters [11] and is inhibited by corticosteroids. There are two isoforms of cyclooxygenases; COX-1 which is constitutively expressed and plays important role in maintenance of normal gastric mucosa and other organs functions, and COX-2, which is the inducible form, is up regulated in areas of inflammation [11].

In general, traditional NSAIDs nonspecifically reduce both cyclooxygenase isoforms, leading to both beneficial antipyretic and anti-inflammatory effects, and unwanted toxic gastrointestinal injury. NSAID that are selective COX-2 inhibitors spare the gastro-protective effects of prostaglandins which are mediated through COX-1 [12].

The result of our study showed that Aspirin produced substantial damage to the gastric mucosa in rats (tables 1, 2, 3, and 4). These effects were both quantitative and qualitative. These findings can be explained by the fact that the active Cox site is a hydrophobic channel with a series of amino acids including seiren 580, arginin 120, and tyrosine 385. Aspirin bind irreversibly to seiren 580 by acetylation, this irreversible binding makes aspirin more harmful to gastric mucosa. Most other NSAIDs reversibly bind to tyrosine 380 or arginine 120, thus causing less damage. [21]. Research works on the

effect of aspirin and NSAIDS on the gastric mucosa are in agreement with our results [13, 14].

Indomethacin produced ulceration in 70% of cases; these results are in agreement with other studies [15]. Our study has also shown that Rofecoxib causes ulceration in only 5% of case, this finding is in agreement with that obtained by Goldstein et al. [16]. These wide ranges of differences between aspirin and Indomethacin on one hand and Rofecoxib on the other hand can be attributed to the fact that aspirin and indomethacin are non-selective Cox-1 and Cox-2 inhibitors [17].

Since members of NSAIDs differ in their ability and selectivity in inhibiting Cox-2 enzyme it follows that their ulcerogenic effects on gastric mucosa are variable. Aspirin and indomethacin are considered as equally selective inhibitors for Cox-1 and Cox-2 enzymes therefore, they induced the highest percentage of ulcerations [18]. Rofecoxib is highly selective Cox-2 inhibitors [17], therefore it produced very little gastric damage because it preserves the gastro protective effect of Cox-1.

Distribution of ulcers according to grades and locations.

Funds. Our study has shown that aspirin causes lesions in the funds in 100% of case, and these lesions were of grade 1 in 100% of cases. Indomethacin produced fundic lesions in 70% of cases, and these were of grade 1 in 100% of the cases.. Rofecoxib produced no lesion in the funds. The explanation for resistance of funds to the injurious effect of NSAID is that, the funds represents the area of keratinizing squamous epithelium of the stomach in mouse. The epithelial cells of this region have tight apical junction making the mucosa impermeable to back diffusion

of hydrogen ion, and thus more resistant to the effects of NSAID [19].

Body. Aspirin produced lesions in the body of the stomach in 100% of cases however, these lesions were variable: grade 1 in 0%, grade 2 in 60%, and grade 3 in 40%. Indomethacin affected the body by the same extent as aspirin (100%), but the severity of lesions was more than those produced by aspirin; grade 1 in 0%, grade 2 in 40%, and grade 3 in 60%. These findings can be explained by the fact that the body of the stomach contains the largest number of acid and pepsin-containing glands that when exposed to damage by NSAID will lead to more production of acid and pepsin and thus more damage [20]. Rofecoxib affected the body by 100% of the cases, but lesions are almost of grade1; our results are consistent with those reported by Cappelle et al, [21].

Pylorus. Aspirin produced lesion in pylorus by 100% of the cases and represented by the following grades: grade 1 in 0%, grade 2 in 20%, grade 3 in 80%. Indomethacin produced lesion in the pylorus by 100% of the cases; grade 1 in 0%, grade 2 in 50%, and grade 3 in 50%. These differences between aspirin and indomethacin in gastric toxicity can be explained by that Aspirin is a potent inhibitor of COX-1 and COX-2. Suppression of prostaglandin synthesis via inhibition of COX-1 can cause an increase in gastric acidity and a decrease in gastric and duodenal secretion of bicarbonate. In addition, aspirin is especially known for its ability to cause local toxicity through a mechanism known as ion trapping in which the drug becomes concentrated in the mucosa [21]. In this case aspirin may have caused further damage by increasing the overall acidity in the body and pylorus.

Recently [22], it has been shown that prostanoid synthesis was greater in pyloric mucosa than it was in duodenal

mucosa, which means that Cox-1 is highly expressed in pyloric mucosa. Since Aspirin has a high ratio of Cox-1/ Cox-2 selectivity, its toxic effect on pyloric mucosa is more than that produced by indomethacin, though the later has more sever effect on the body than does Aspirin.

Rofecoxib effected pylorus by 50% of cases as follow: grade 1 in 100. This can be explained by the fact that the different percentage of induction of ulcer by different members of NSAIDs is due to different ratios of cox-1/ Cox-2 selectivity [18].

Conclusions

1. Aspirin and Indomethacin produced lesions in gastric mucosa by 100% of the cases. These lesions take the form of gastric erosion and ulceration and involved especially the body and the pylorus of the stomach...
2. Aspirin produced more sever lesions in the pylorus than Indomethacin. While Indomethacin produced more sever lesions in the body than did Aspirin.
3. Rofecoxib produced lesions that are confined to the pylorus and are exclusively of grade 1.
5. New generation of NSAIDs showed less gastric toxicity compared to traditional generation.

References

1. Carson JJ, Willet LR: Toxicity of NSAID: An overview of the epidemiological evidence. *J Drugs* 46(suppl.):243-248, 1993.
2. Gabriel SE, Jaak L, and Bombardier C: Risk of serious gastrointestinal complications related to NSAID. *N Engl. J. Med* 303: 136-138. 1980.
3. Wolf MM: NSAID and gastric mucosa. *N Engl. J. Med* 15: 37-48, 1996.
4. Shigeta JL, Takahashi S, Okabe S: Role of cyclooxygenase-2 in the

healing of gastric ulcer in rats. *J. pharmacol. Experiment* 286: 1383-1390, 1998.

5. Kargman S, Charleson, Cartweigh M, et al: Characterization of prostaglandin G/H synthase 1&2 in rat, dog, monkey, and human gastrointestinal tract. *Gastroenterology* 111: 445-454, 1996.
6. Needleman P, Isakso PC: The discovery and function of Cox-2. *Rheumatol.* 24 (suppl 49.): 6-8, 1997.
7. Gies GS: Update on clinical development with celecoxib; what can we expect? *J. Rheumatol.* 26: 31-36, 1999.
8. Engelhard G, Homma D, et al: Anti-inflammatory, analgesic, antipyretic, and related properties of meloxicam a new NSAID with favorable gastrointestinal tolerance. *Inflamm. Res. J:* 423-433, 1995.
9. Ganong WF: Textbook of Medical Physiology. *Appleton and Lang. Norwalk. Connecticut. 14th edition, PP88-89, and PP, 408-421, 1988.*
10. Sun DC, Chen JK: Experimental ulcer production in the pylorus legated. *J. Clin. Gastroentrol.* 27(suppl.): 21-27, 1998.
11. Cryer B, Gold Schmidt M, Redferm, et al: Comparison of salicylate and aspirin on mucosal injury and gastroduodenal mucosal prostaglandin. *Gastroenterology* 99: 1616-1621, 1990.
12. Vane JR, Mitchell JA, Appleton I, et al: Inducible isoform of cyclooxygenase and nitric oxide synthase in inflammation. *Proc. Natl. Acad. SCI .USA* 91: 2046 -2050. 1994.
13. Schema JM: Pathogenesis of gastrointestinal injury due to NSAIDs: Implication for prevention and therapy. *Semin. Arthritis. Rheum.* 21: 201-210, 1992.
14. Sarosiek J, Mazuta K, Slomiany A, et al: effect of acetyl salicylic acid on gastric mucin viscosity, permeability for hydrogen ion, and

susceptibility for pepsin. *Biochem. Pharmacol.* 35:4291-4295, 1986.

15. Goldstein KD, Correa MY, et al: Reduced incidence of gastrointestinal ulcer with celecoxib. *American Journal of Gastroenterology* 96 (400): 1020-1026, 2001.

16. Cryer B, Dubois A: The advantage of highly selective inhibitors of cyclooxygenase enzymes. *Prot Lipid Med.*56: 341-261, 1998.

17. Cannon GW: Cyclooxygenase-2 selective inhibition. *Drugs Today. J.* 35(7):487- 496, 1999.

18. Brain D, Golden MD, Steven B, Abramson MD, et al: Selective cox-2 inhibitors for arthritis. *J. of Rheumatology* 21:1346-1349, 2000.

19. Tsujii M, Kawaka S, and Dubois RN: cyclooxygenase-2 expression in human colon cancer cells increases

metastatic potential Porch Natl. *Acad.SCI USA* 112: 387-397, 1997.

20. Vane JR, Botting RM: New insight in to the mode of action of anti-inflammatory drugs associated with upper gastrointestinal toxicity. *Gastroenterology Clin North Am* 29: 97, 2000.

21. Suma Reed: Non-steroidal anti-inflammatory drug-induced duodenal ulceration and perforation in mature Rottweiler. *Can Vet J.* 43(12): 971-972, 2002 December.

22. Wooten JG, Blikslager AT, Rayan KA, Marks SL, Law JM, Lascelles BD: Cyclooxygenase expression and prostanoid production in pyloric and duodenal mucosae in dogs after administration of non-steroidal anti-inflammatory drugs. *American journal of veterinary research* 69 (4):457-464. April 2008.

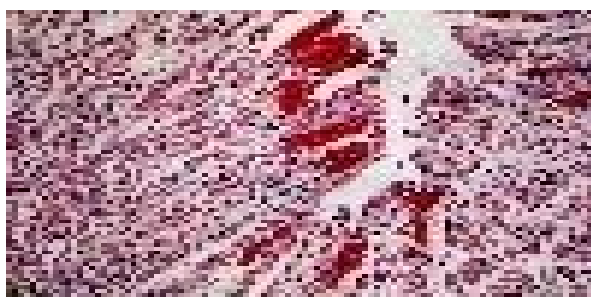


Figure 1 The body of the stomach; Hemorrhagic erosions involving the superficial part of the gastric mucosa. X40.



Figure 2 Pylorus; Deep ulceration involving the entire thickness of the gastric wall, X40.