Effects of etoricoxib on the esophagogastroduodenal mucosa

Efeitos do etoricoxibe na mucosa esofagogastroduodenal

Marília Daniela Ferreira Carvalho, MD, Ítalo Medeiros Azevedo, Aldo Cunha Medeiros, MD, PhD.

Research performed at Department of Surgery, Federal University of Rio Grande do Norte (UFRN), Brazil.
Financial support: CNPq, Brazil.
Conflict of interest: None.
Correspondence address: Aldo Cunha Medeiros, Department of Surgery, Federal University of Rio Grande do Norte, at Ave. Nilo Peçanha 620, Natal, RN, Brazil, Email: aldom@uol.com.br
Submitted: 08 October 2011. Accepted, after review: 11 December 2011.

ABSTRACT

Purpose: The objective of this study was to compare the effects of etoricoxib with diclofenac in the mucosa of the esophagus, stomach and duodenum. **Methods:** Eighteen Wistar rats were randomly divided into 3 groups of 6 each. Group 1 (control) rats received 0.9% saline solution orally. In group 2, the animals were treated daily with etoricoxib 15mg/kg/day orally. Group 3 rats, were treated with 15 mg/kg of sodium diclofenac all by gavage. On the 15th day, the animals were killed with sodium thiopental (100mg/kg) i.p. and esophagus, stomach and duodenum were removed, immersed in 10% formalin for 48 hours for fixation. 5µm sections were prepared and stained with hematoxylin and eosine. The histopathological findings mucosal edema, inflammatory infiltration, mucosal metaplasia and hemorrhage were quantified according to their intensity from 0 to +4, and converted into scores. Statistically analysis was done by ANOVA and Tukey test, considering differences significant at $p<0.05$. **Results:** In group 1 (control) scores obtained for the esophagus, stomach, duodenum were respectively: 1, 2, 1. Group 2 (etoricoxib) scores corresponded to 4, 7 and 3 respectively, and those of group 3 (diclofenac): 11, 24, 10. So, aggression to the esofagogastroduodenal mucosa was less intense with the use of etoricoxib than with diclofenac, and the diference was statistically significant ($p<0.05$). Comparing the scores, the etoricoxib was four folds more aggressive in the esophagus, 3.5 folds in the stomach and three folds in the duodenum. This contrasts with diclofenac theatment, which was respectively 11, 12 and 10 folds more aggressive in esophagus, stomach and duodenum than the observed in the control group. **Conclusion:** The data showed the advantage of etoricoxib compared to diclofenac. It was demonstrated that the esofagogastroduodenal mucosal injury caused by etoricoxib was significantly lower than that caused by the use of diclofenac.

Key words: Etoricoxib. Diclofenac. Esophagus. Stomach. Duodenum. Mucosa. Injury. Rats.

RESUMO

Objetivo: O objetivo deste estudo foi comparar os efeitos do etoricoxibe com diclofenaco na mucosa do esôfago, estômago e duodeno. **Métodos:** Dezoito ratos Wistar foram divididos aleatoriamente em 3 grupos de 6 cada. Grupo 1 (controle) - os ratos receberam solução salina 0,9% por via oral. No grupo 2, os animais foram tratados diariamente com etoricoxibe 15mg/kg/dia por via oral. Os ratos do Grupo 3 foram tratados com 15 mg/kg de diclofenaco de sódio, todos por gavagem. No 15º dia de tratamento, os animais foram mortos com tiopental sódico i.p. (100mg/kg) e foram removidos esôfago, estômago e duodeno, em seguida imerso em formalina a 10% durante 48 horas para a fixação. Seções de 5mμ foram preparadas e coradas com hematoxilina e eosina. O histopatológico examinou edema da mucosa, infiltração inflamatória, metaplasia de mucosa e presença de hemorragia, que foram quantificados de acordo com a sua intensidade de 0 a +4, e convertidos em escores. Análise estatística foi feita pelo ANOVA e teste de Tukey, com significância de $p<0,05$. **Resultados:** No grupo 1 (controle) os escores obtidos para o esôfago, duodeno, estômago, foram respectivamente: 1, 2, 1. No grupo 2 (etoricoxib) os escores corresponderam a 4, 7 e 3, respectivamente, e nos órgãos do grupo 3 (diclofenac): 11, 24, 10. A agressão à mucosa esofagogastroduodenal foi menos intensa com o uso de etoricoxibe do que com diclofenaco, e a diferença foi estatisticamente significante ($p<0,05$). **Conclusão:** Os dados confirmaram vantagem de etoricoxibe em comparação com o diclofenaco. Foi demonstrado que a lesão da mucosa esofagogastroduodenal causada por etoricoxibe foi significativamente menor do que a causada pelo tratamento com diclofenaco.

Descritores: Etoricoxibe. Diclofenaco. Esôfago. Estômago. Duodeno. Mucosa. Lesão. Ratos.

INTRODUCTION

The non-steroids antiiflammatory drugs (NSAIDs) are used for nonspecific symptomatic treatments that, besides the antiiflamatory action have analgesic, antipyretic and antithrombotic properties. In 1971, Vane and Botting formulated a hypothesis, that was later repeated and improved, on the mechanism by which these agents relieve pain and inflammation[1]. These drugs inhibit the cyclooxygenase enzyme (COX), which catalyzes the conversion of arachidonic acid into prostaglandins, mediators responsible for the production of inflammatory signs (swelling, pain and erythema). There are two COX isoforms with different tissue distributions and

physiological functions. COX-1 is constitutively expressed in many tissues and maintain homeostasis in major organ systems, especially the gastric and renal sites, while COX-2 is essentially pro-inflammatory and is expressed only in response to stimuli such as mitogens, cytokines and growth factors. NSAIDs have varying degrees of inhibition for COX-1 and COX-2[2]. If on its therapeutical dose, a given anti-inflammatory drug mainly inhibits COX-2, with little interference with COX-1 activity, this agent is called COX-2 specific inhibitor. These compounds hade been entitled coxibs. Etoricoxib, as well as other drugs from this group, saves COX-1 (cytoprotective enzyme), and potentially has an advantage over non-selective NSAIDs COX-2, therefore operate effectively as anti-inflammatory. In theory, it has not adverse effects consequential to the non-selective enzyme inhibition, such as heartburn, diarrhea, nausea, vomiting and gastritis, gastric perforation, peptic ulcers, bleeding, and nefropaty[3,4].

An effective treatment for pain involves enormous challenges for doctors and patients. The goals of clinical management are the effective relief of the signs and symptoms minimizing disability, associated with many conditions such as rheumatoid arthritis, dysmenorrhea, postoperative pain and osteoartrosis[5]. For this purpose, etoricoxib has been widely used, likely due to lower incidence of gastrointestinal complications compared to traditional NSAIDs, with particular benefits for its chronic users.

Based on these informations, the aim of this study was to compare the effects of etoricoxib with diclofenac in the mucosa of the esophagus, stomach and duodenum of rats.

METHODS

The animals were obtained from Center of Health Sciences of the Federal University of Rio Grande do Norte (UFRN), Brazil, and they were housed with free access to food and water, maintained under constant environmental conditions (23±2ºC; 12h/12h of light/dark cycle). The study was performed in healthy male *Wistar* rats (weight range: 185–280 g). The experiments were performed according to the Guide for the Care and Use of Experimental Animals and were approved by the Ethical Committee for Using Animals of UFRN.

The animals were randomly divided into 3 groups of 6 each, observed in individual cages with food (Labina Purina®) and water *ad libitum*. In group 1 (control) 0.9% saline solution was administered orally. In group 2 (n = 6), the animals were treated daily with etoricoxib 15 mg/kg/day orally, always at the same time in the morning. Group 3 (n = 6) rats were treated with 15 mg/kg of sodium diclofenac in the same conditions as group 1 and 2. On the 15th day of treatment, the animals received intraperiponial lethal dose of sodium thiopental (100mg/kg). They were submitted to depilation of the abdominal wall, antisepsis with 70% alcohol and a 6 cm median thoracolaparotomy was performed. The terminal esophagus, stomach and duodenum

were removed. The specimens were fixed in 10% formaline, cut as 5 µm tissue sections, dehydrated in ethanol and xylene and stained with hematoxylin and eosin. All specimens were examined by the same pathologist, who had no knowledge of the study groups. Score measurements were made using light micrographs (100X) of the stained sections. The histopathological findings such as mucosal edema, inflammatory infiltration, mucosal metaplasia, presence of hemorrhage were quantified according to their intensity from 0 to +4, and converted into scores. Data were statistically analyzed using the BioEstat 2.0 by ANOVA and Tukey test, considering differences significant at $p<0.05$.

RESULTS

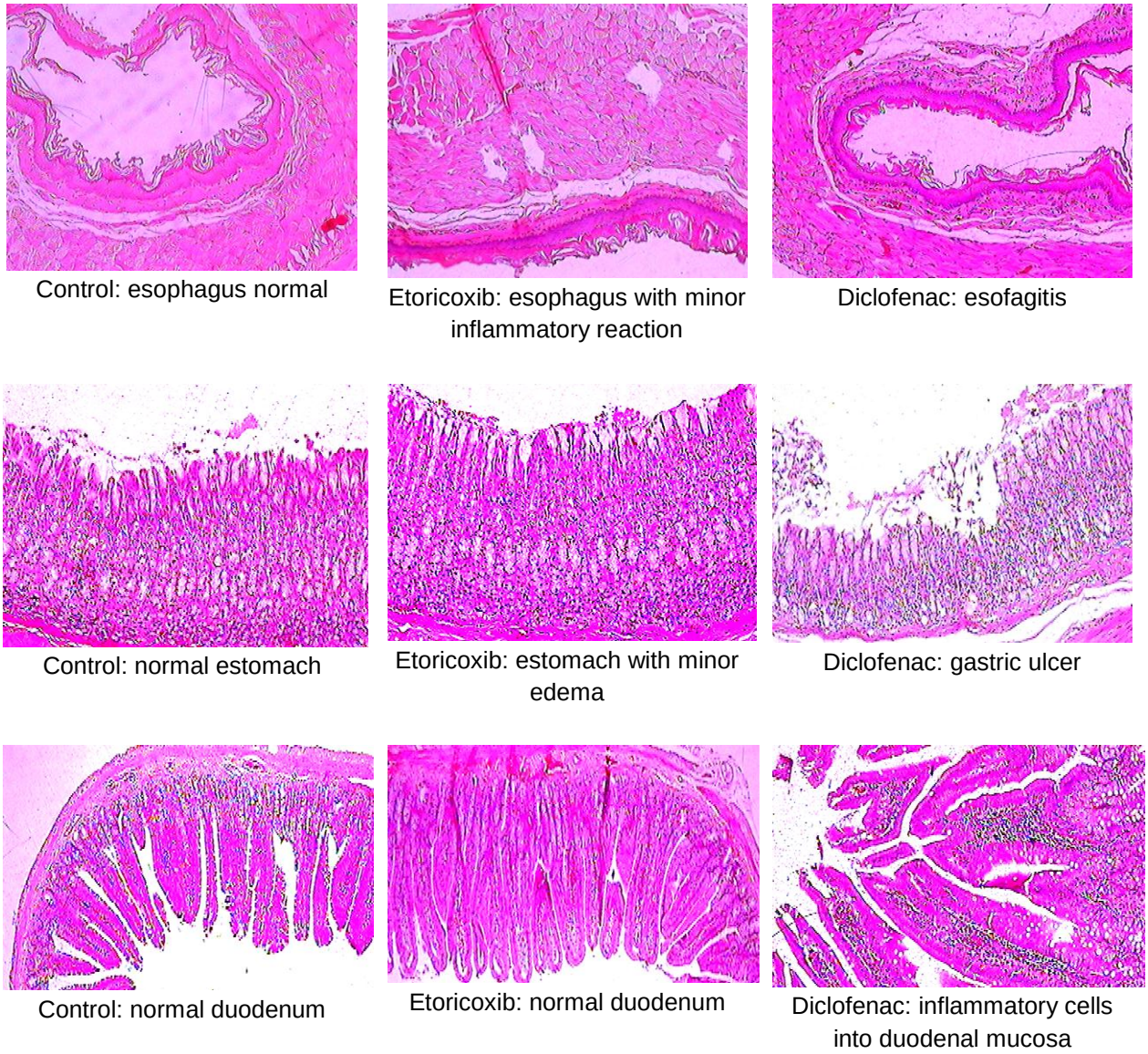
By histopathological analysis of the blades, the following scores values were found: on group 1 (control) scores obtained for the esophagus, stomach, duodenum were: 1, 2, 1; on group 2 (etoricoxib) the scores were 4, 7, 3 respectively; and the animals of group 3 (diclofenac) had scores 11, 24, 10 for esophagus, stomach and duodenum, as seen in Table-1. We detected therefore, signs of aggression to the esofagogastroduodenal mucosa more intense with the use of etoricoxib than with diclofenac, and the difference was statistically significant ($p<0.05$). Compared the scores of the groups treated with NSAIDs with the scores of the control group, it appeared that etoricoxib was four folds more aggressive in the esophagus, 3.5 folds in stomach and three folds in the duodenum. This contrasts with diclofenac, which was respectively 11, 12 and 10 folds more aggressive in esophagus, stomach and duodenum respectively, than that observed in the control group.

The histopathological findings seen in Figure-1, show discrete changes in the organs of animals in groups 1 (control) and 2 (etoricoxib), both considered normal. However, significant inflammatory reactions were observed in the esophagus, stomach and duodenum from animals treated with diclofenac.

Table 1- Values of the histological scores in the esophagus, stomach and duodenum of animals treated with saline, etoricoxib and diclofenac.

Groups	Esophagus	Estomach	Duodenum
1 (saline)	1	2	1
2 (etoricoxib)	4*	7*	3*
3 (diclofenac)	11	24	10

* $p<0.05$ compared with histological scores from organs of group 2 rats (etoricoxib).



Control: esophagus normal | Etoricoxib: esophagus with minor inflammatory reaction | Diclofenac: esofagitis

Control: normal estomach | Etoricoxib: estomach with minor edema | Diclofenac: gastric ulcer

Control: normal duodenum | Etoricoxib: normal duodenum | Diclofenac: inflammatory cells into duodenal mucosa

Figure 1 – Photomicropraphs of esophagus, stomach and duodenum, treated with saline, etoricoxib and diclofenac. HE, 100x.

DISCUSSION

Non steroid antiinflammatory drugs (NSAIDs) have been used for decades for the treatment of pain and inflammation. Conventional NSAIDs like diclofenac are very effective in treating pain; however, they have a poor tolerability profile as they are associated with an increased risk of damage to the gastrointestinal tract[6]. Cyclo-oxygenase-1 (COX-1) is the constitutive form and COX-2 is inducible. The products of COX-1 activity are involved in platelet function, regulation of renal haemodynamics and electrolyte balance, and protection of the GI mucosa. COX-2 is responsible for the

production of prostaglandins that mediate inflammation and pain, and it is primarily the inhibition of COX-2 that results in the analgesic and anti-inflammatory effects of NSAIDs[7]. COX-2-selective NSAIDs should, in theory, be as effective as nonselective NSAIDs, but lack the GI tolerability concerns associated with COX-1 inhibition. Consistent with this expectation, available COX-2-selective NSAIDs have similar efficacy to, but are better tolerated than conventional NSAIDs when used in the treatment of rheumatoid arthritis, osteoarthritis and acute pain[8,9].

O presente trabalho foi realizado no intuito de avaliar o grau de agressão à mucosa do esôfago, estômago e duodeno de ratos tratados com etoricoxibe. No presente estudo experimental foram demonstradas, além de alterações gástricas, lesões histológicas evidentes no esôfago e duodeno de ratos Wistar submetidos à administração de duas drogas antiinflamatórias. A relevância deste trabalho, portanto, consiste na avaliação integral da mucosa esofagogastroduodenal, não se restringindo apenas ao estômago.

This work was carried out in order to assess the degree of injury to the esophagus, stomach and duodenum mucosa of rats treated with etoricoxib. In this experimental study we demonstrated, besides gastric mucosa injury, lesions in the esophagus and duodenum of Wistar rats submitted to the administration of two anti-inflammatory drugs. The relevance of this work, therefore, consists in the full evaluation of the esofagogastroduodenal mucosa, not restricted to the stomach. We demonstrated that the duodenum is still affected by the inflammation induced by NSAIDs.

Some treatments have significant implications in NSAID induced esophageal complications including bleeding esophageal ulcers and esophagitis[10]. Although the efficacy of NSAIDs are undoubted, they induce esophageal and peptic ulceration causing disturbance in mucosal blood flow, and increased permeability and activation of neutrophils, resulting in their adherence to the vascular endothelium[11]. These adverse effects are often developed without symptoms and are of particular significance in the elderly people. The cytotoxic effects of NSAIDs are due not only to their inhibitory effects on cyclooxygenases, thereby inhibiting synthesis of prostaglandins which has been thought to play a central role in the gastrointestinal toxic effects of NSAIDs, but also to their direct cytotoxic effects on gastric mucosal lesions in rat[12]. Several epidemiologic studies have demonstrated that NSAIDs users have a higher risk of esophageal ulcers or esophagitis than non-NSAIDs users. Twenty percent of patients with acute gastrointestinal bleeding had esophageal ulcers, and 50% of patients with esophageal ulcers were associated with NSAIDs-use[13]. A study on the esophagogastroduodenoscopies conducted at an urban hospital, describes gastrointestinal reflux disease (GERD) and NSAIDs-ingestion as common causes of esophageal ulcers. Furthermore, midesophageal ulcers showed a greater tendency to hemorrhage compared to ulcers at the gastroesophageal junction, where esophageal ulcers are defined as a discrete break in the esophageal mucosa with a clearly circumscribed margin[14]. In a report describing patients with NSAIDs-induced esophageal

ulcers documented by endoscopy, the most common findings were anemia, retrosternal pain, and dysphagia. These NSAIDs-induced ulcers had characteristic endoscopic features that included a large, shallow, discrete ulcer in the midesophagus near the aortic arch with normal surrounding mucosa, suggesting that the injury resulted from mucosal contact with NSAIDs. In addition, 50% of patients with acute necrotizing esophagitis had taken NSAIDs[12].

Highly selective inhibitors of cyclooxygenase-2 (COX-2) have been developed and studies have shown a marked reduction in their ability to cause injury to the gastroduodenal mucosa. These agents are more selective in their ability to inhibit COX-2 than the traditional NSAIDs and they showed to develop gastroduodenal ulcers on a rate similar to placebo[15].

Etoricoxib has become available for use since the 90s. However, some doubts remain about their selective inhibition. One of these issues, for example, suggests that COX-2 can generate biologically important endogenous prostanoids. According to Mizuno et al, an increase in the expression of COX-2 in the mucosa may be necessary for healing of gastric ulcers[16].

Several studies were conducted in order to test the digestive damage caused by use of NSAIDs selective for COX-2. In 2000, the CLASS study, which compared the effects of celecoxib with ibuprofen and diclofenac (nonselective NSAIDs), showed a reduction of gastrointestinal effects with the new anti-inflammatory after six months of treatment[9]. However, this study was strongly criticized because it abbreviated analysis of the results for six months, when it was scheduled for twelve months in the original experimental design[17].

The etoricoxib is a COX-2-selective NSAID used in the treatment of rheumatoid arthritis, osteoarthritis, postoperative dental pain, chronic low back pain, acute gout and other inflammations. It is the COX-2 inhibitor least likely to interfere with the antiplatelet effect of aspirin, mediated via the irreversible inactivation of COX-1[18]. The incidence of upper GI perforations, ulcers and bleeds (PUBs) with etoricoxib was less than half that associated with traditional non-COX-selective NSAIDs[19]. Patients with rheumatoid arthritis, osteoarthritis or chronic low back pain were treated with once-daily etoricoxib 60 to 120mg (n = 3142) or a traditional non-COX-selective NSAID, such as diclofenac 150 mg/day. Etoricoxib was associated with fewer treatment discontinuations because of low NSAID-type gastrointestinal symptoms compared with traditional non-COX-selective NSAIDs. NSAID-type gastrointestinal symptoms were defined as acid reflux, dyspepsia, epigastric discomfort, heartburn, nausea and abdominal pain[5].

In our experimental study we used healthy rats. This fact may be a limitation, because the results may be different if used animals with chronic inflammatory diseases. The COX-2 caused gastric lesions in rats with induced arthritis as well as the conventional NSAIDs, supporting the hypothesis that the prostaglandin derived from COX-2 play an important role in maintaining the integrity of the gastric mucosa in arthritis rats[20]. Clinically, it should be important when these drugs are used to treat

rheumatoid arthritis. In conclusion, this experiment evidenced the advantage of etoricoxib compared to diclofenac. It was demonstrated in rats that the esofagogastroduodenal mucosal injury caused by etoricoxib was significantly lower than that caused by the use of diclofenac.

REFERENCES

1. Vane J R, Botting R M. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971;231:232-5
2. Vane JR, Botting RM. Mechanism of action of antiinflammatory drugs. Int J Tissue React. 1998;20:3-15.
3. Cannon CP, Curtis SP, Bolognese JA, Laine L; MEDAL Steering Committee. Clinical trial design and patient demographics of the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) study program: cardiovascular outcomes with etoricoxib versus diclofenac in patients with osteoarthritis and rheumatoid arthritis. Am Heart J. 2006;152:237-45.
4. Laine L, Curtis SP, Cryer B, Kaur A, Cannon CP; MEDAL Steering Committee. Assessment of upper gastrointestinal safety of etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: a randomized comparison. Lancet. 2007;369:465-73.
5. Laine L, Curtis SP, Cryer B, Kaur A, Cannon CP. Risk factors for NSAID-associated upper GI clinical events in a long-term prospective study of 34.701 arthritis patients. Aliment Pharmacol Ther. 2010;32:1240-8.
6. Gabriel SE, Jaakkimainen L, Bombadier C. Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs: a meta-analysis. Ann Intern Med. 1991;115:787-96.
7. Lipsky PE, Brooks P, Crofford LJ, et al. Unresolved issues in the role of cyclooxygenase-2 in normal physiologic processes and disease. Arch Intern Med. 2000;160: 913-20.
8. Bombardier C, Laine L, Reicin A, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N Engl J Med. 2000; 343:1520-8.
9. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Verburg KM, Geis GS. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. JAMA. 2000;284:1247-55.

10. Hawkey C J. Nonsteroidal anti-inflammatory drug gastropathy. *Gastroenterology.* 2000;119: 521-35.
11. Hawkey C J. Gastroduodenal problems associated with non-steroidal, anti-inflammatory drugs (NSAIDs). *Scand J Gastroenterol (Suppl).* 1993;200: 94-5.
12. Sugawa C, Takekuma Y, Lucas C E. Amamoto H. Bleeding esophageal ulcers caused by NSAIDs. *Surg Endosc.* 1997;11:143-6.
13. Wolfsen H C, Wang K K. Etiology and course of acute bleeding esophageal ulcers. *J Clin Gastroenterol.* 1992;14:342-6.
14. Higuchi D, Sugawa C, Shah S H, Tokioka S, Lucas C E. Etiology, treatment, and outcome of esophageal ulcers: a 10-year experience in an urban emergency hospital. *J GastrointestSurg.* 2003;7:836-42.
15. Wolfe, M. Michael, Lichtenstein, David R., Singh, Gurkirpal Gastrointestinal Toxicity of Nonsteroidal Antiinflammatory Drugs. N Engl J Med. 1999;340:1888-99.
16. Mizuno H, Sakamoto C, Matsuda K, et al. Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. Gastroenterology. 1997;112:387-97.
17. Jüni P, Rutjes A W S, Dieppe A. Are selective COX-2 inhibitors superior to traditional non-steroidal anti-inflammatory drugs? BMJ. 2002;324:1287-8.
18. Ouellet M, Riendeau D, Percival MD. A high level of cyclooxygenase-2 inhibitor selectivity is associated with a reduced interference of platelet cyclooxygenase-1 inactivation by aspirin. Proc Natl Acad Sci USA. 2001;98:14583-8.
19. Laine L, Curtis SP, Langman M, Jensen DM, Cryer B, Kaur A, Cannon CP. Lower gastrointestinal events in a double-blind trial of the cyclo-oxygenase-2 selective inhibitor etoricoxib and the traditional nonsteroidal anti-inflammatory drug diclofenac. Gastroenterology. 2008;135:1517-25.
20. Kato S, Ogawa Y, Kanatsu K, Okayama M, Watanabe T, Arakawa T, Takeuchi K. Ulcerogenic influence of selective cyclooxygenase-2 inhibitors in the rat stomach with adjuvant-induced arthritis. J Pharmacol Exp Ther. 2002 303: 503-9.