Comparative gastrointestinal toxicity of selective cyclooxygenase (COX-2) inhibitors

N Shafiq, S Malhotra & P Pandhi*
Department of Pharmacology
and
R Nada
Department of Pathology, PGIMER, Chandigarh, 160 012, India

Received 5 August 2004; revised 4 April 2005

Cyclooxygenase (COX-2) inhibitors were developed with the hope that they will cause fewer gastrointestinal adverse effects. Ability of selective as well as nonselective COX inhibitors to alter ischemia-reperfusion induced damage of gastric mucosa and hapten-induced colitis in rats has been compared. Celecoxib (10, 20 and 40 mg/kg) was significantly more potent at aggravating ischemia-reperfusion injury as compared to nimesulide. Similarly, celecoxib was found to maximally potentiate TNBS-induced colitis, followed by nimesulide and indomethacin. Celecoxib at its highest dose produced maximum deep histological injury. This paradoxical ulcer and colitis aggravating effect of selective COX-2 inhibitors may be explained by suppression of protective prostaglandins generated as a consequence of COX-2 induction in inflammatory states.

Keywords: COX-2, Celecoxib, Gastric-ulcer, Indomethacin, Ischemia-reperfusion, Nimesulide, NSAIDs.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are amongst the most widely used drugs as they have a particularly broad application. Chronic administration of NSAIDs produces gastro duodenal mucosal erosions in 35-60% patients, ulcerations in 10-25% and severe hemorrhages or perforation in about 1% of patients. Epidemiological studies have established that overall, NSAIDs enhance the risk of ulcer complications such as bleeding, perforation, hospitalization and death by approximately 3-10 fold. NSAIDs act by inhibition of cyclooxygenase (COX) enzyme. Inhibition of COX is also responsible for its gastrointestinal toxicity as it leads to a decrease in mucus and bicarbonate secretion, reduced mucosal blood flow, vascular injury, leukocyte accumulation and reduced cell turn over, all the factors that contribute to the genesis of mucosal cell damage.

Following the discovery of the second isoform of COX, called COX-2, it was demonstrated that COX-1 was constitutive and COX-2 expression could be induced by various inflammatory cytokines. It was then hypothesized that COX-2 specific inhibition could alleviate pain and inflammation without disrupting homeostatic functions like maintenance of gastric prostaglandins mediated by COX-1 derived prostanoids. However, this paradigm has recently been challenged in several studies. It was shown that within 40 min of oral challenge with acid there was a marked upregulation of COX-2 in the rat stomach. Subsequently, a crucial protective role for COX-2 in the so called 'adaptive cytoprotection' was demonstrated. COX-2 was shown to contribute to mucosal defense in stress ulcer and COX-2 inhibitors were shown to delay ulcer healing. Studies showing worsening of gastric ulcers or necrosis of small intestine and aggravation of ischemia reperfusion injury, exacerbation of DNBS (dinitro benzene sulfonic acid) induced colitis with COX-2 inhibitors led workers to question the promised gastrointestinal safety of COX-2 inhibitors.

Thus, the present study has been designed to make a comparative analysis of COX inhibitors of varying
selectivities (indomethacin, nimesulide and celecoxib), in two models of gastrointestinal injury: (i) ischemia-reperfusion induced gastric mucosal damage, and (ii) TNBS (tri nitro benzene sulfonic acid) induced colitis.

Materials and Methods

Male Wistar rats (150-200 g) were maintained under standard laboratory conditions. Chow and water was supplied ad libitum and a 12 hr light and 12 hr dark cycle was maintained. All experiments were approved by the Institute's ethics committee.

Indomethacin (nonselектив COX inhibitor); nimesulide (a partially selective COX-2 inhibitor) and celecoxib (a highly selective COX-2 inhibitor) was administered to the animals. Two protocols were followed for drug administration. For ischaemia reperfusion induced gastric mucosal damage, the drug/vehicle was administered, ip, 1 hr prior to the start of the experiment. For colitis experiments drug/vehicle was given by gavage twice a day for seven days. Indomethacin, nimesulide and celecoxib were administered in the following dose: indomethacin; 2.5, 5 and 10 mg/kg, nimesulide and celecoxib and celecoxib respectively were taken-2.5, 10 and 20 mg/kg. For the purpose of comparison following sets of doses of indomethacin, nimesulide and celecoxib respectively were taken-2.5, 10 and 10 mg/kg; 10, 20 and 40 mg/kg; 10, 20 and 40 mg/kg. These were considered as having equal anti-inflammatory activity based on previously assessed effect on formalin induced inflammation in rats. Equal volumes of vehicle (normal saline with 2 drops of Tween-80) was administered in the control animals. Six animals were taken in each group.

Gastric mucosal injury produced by ischaemia reperfusion—Animals were fasted overnight. Under pentobarbitalone (50 mg/kg, ip) anaesthesia, the abdomen was cut open. Following pylorus ligation, celiac artery was clamped and 1 ml of 0.1 N HCl was injected into the gastric lumen. Reperfusion was established 30 min later by removal of the clamp. After a 60 min reperfusion period, the stomach was excised and gross mucosal damage was assessed by calculation of lesion using 0-3 scoring system. The severity factor was defined according to the length of the lesion: 0=no lesion visible, 1=lesion ≤1 mm, 2=lesion 2-4 mm and 3=lesion >4 mm.

Lesion index was calculated as the total number of lesions multiplied by their respective severity factors. For histological study, a strip of the stomach wall parallel to the limiting ridge was processed using routine methods, stained with H & E and examined under light microscope. Grading of histological injury was done as follows:

- Grade 1: Superficial damage confined, to the surface epithelium.
- Grade 2: Damage extending beyond the region into the region of pits and glands. The length of the mucosal areas showing superficial and deep damage was determined and expressed as per cent of total length studied.

TNBS (tri nitro benzene sulfonic acid) induced colitis—Animals were given food and water ad libitum. TNBS [0.25 ml of 50%(v/v)] in alcohol was administered as enema. The animals were monitored at least thrice per day throughout the 7 days period and 7 days thereafter. Necropsy was performed as and when animals died. On the 14th day, the animals were sacrificed and inflammation was assessed by the following gross inflammatory score.

Macroscopic scoring of colonic damage

Ulcer—Normal appearance=0, focal hyperemia, no ulcers=1, ulceration without hyperemia or bowel wall thickening=2, ulceration with inflammation at one site=3, ulceration / inflammation at ≥ 2 sites=4, major sites of damage extending >1 cm along the length of the colon=5, when an area of damage extended for more than 5 cm of the length of the colon the score was increased by one for each extra cm of the length of colon involved=6-10.

Adhesions—No adhesions=0; minor adhesions (colon can be separated from other tissues by effort)=1; major adhesions=2.

Diarrhoea—Absent=0; present=1.

Thickness—The maximum thickness in mm(x) was added to the above score. The total score was calculated by summating the entire score.

Statistical analysis: The results are expressed as mean±SD. Differences between various treatment groups in ischemia reperfusion induced gastric mucosal injury and TNBS induced colitis were assessed by Wilcoxon’s sign rank test. P value <0.05 was considered significant.

Results

Gastric mucosal injury produced by ischaemia reperfusion—Indomethacin (5 and 10 mg/kg) and both nimesulide and celecoxib 10, 20 and 40 mg/kg compared to the respective control groups, showed significant increase in lesion index (Table 1).
Both nimesulide and celecoxib showed a greater increase in the lesion index than indomethacin (Table 1). Further, celecoxib showed a greater increase in lesion index as compared to nimesulide and the order of gastric mucosal damage was: celecoxib > nimesulide > indomethacin.

The histopathological examination of the tissue samples revealed that deep histological injury extending beyond the surface epithelium into the region of pits and glands (grade-2) was least in rats which were administered indomethacin 2.5 mg/kg. However, significant dose dependent, grade-2 damage was observed in the rest of the treatment groups. Again the order of damage was same as obtained above (Table 1).

TNBS (tri nitro benzene sulfonic acid) induced colitis—When compared to vehicle treated control, indomethacin at the dose of 10 mg/kg, nimesulide and celecoxib at the doses of 10, 20 and 40 mg/kg significantly increased the gross inflammatory score (Table 1).

Here again, celecoxib showed greater colonic damage compared to nimesulide and indomethacin.

It was also noted that while no deaths occurred in any of the groups receiving either vehicle or indomethacin, one animal died on day 10 in the nimesulide (40 mg/kg) and four animals died between days 8 and 12 in celecoxib (40 mg/kg) treated groups.

Discussion

The results demonstrate that at equivalent anti-inflammatory doses of the three agents used, pretreatment with celecoxib resulted in maximum gastric and colonic damage. The findings may appear paradoxical as there is ample evidence that selective inhibitors of COX-2 produced less gastric mucosal damage than conventional NSAIDs when administered acutely to healthy animals.17,19. Endoscopic studies have also demonstrated the absence of any effect on the gastric mucosa of the highly selective drugs.20,21.

In the present study celecoxib demonstrated an increase in gastric mucosal damage score in a dose dependent manner. Similar results (3-fold increase in mucosal injury) has earlier been reported with etodolac and other relatively COX-2 selective agents.22. Maricis, et al.13, have shown that prior treatment with selective COX-2 inhibitor, NS-398 and DFCI produced gastric mucosal injury similar to that
produced by indomethacin in ischemia–reperfusion induced mucosal lesions in rats.

The classical COX-2 hypothesis has downplayed the role of COX-2 expression in the gastrointestinal mucosa. While in normal gastric mucosa, COX-1 is the predominant isoenzyme, there is increasing evidence that detectable amounts of COX-2 mRNA and protein are both constitutively expressed and inducible in specific locations in the gastric mucosa,

Moreover, evidence is emerging for a beneficial role of prostaglandins generated by COX-2 in the stomach. It has been shown that marked induction of COX-2 mRNA occurs in the gastric mucosa after ischemia/reperfusion injury probably leading to aggravation of injury with the use of COX-2 inhibitors.

Upregulation of COX-2 mRNA is also observed in gastric mucosal erosions and ulcers and inhibition by specific COX-2 antagonist has been shown to delay healing.

Adaptive cytoprotection which refers to the reduced damage after a strongly injuring agent when a mild irritant is given previously, has been previously demonstrated to be blocked by treatment of NS-398, a COX-2 selective agent in rats.

Similar to the findings of gastric ulcer model, an exaggeration of colonic damage was observed in the groups pretreated with the three COX inhibitors with maximum damage occurring with celecoxib in TNBS induced colitis model. A number of agents capable of suppressing prostaglandin synthesis (when given at anti-inflammatory doses), cause such profound exacerbation of colonic damage that perforation often occurred. Experimental colitis was shown to be significantly more severe in COX-2 knockouts of wild type littermates.

In contrast to the present findings, celecoxib has been shown to reduce the severity of colitis induced by dinitro benzene sulfonic acid in rats. Nimesulide in the present study was shown to exacerbate colonic damage. However, Kankari et al. have shown that TNBS induced acute inflammatory edema was reduced by nimesulide. Nimesulide was administered as a single dose and mucosal lesion was not reduced in this study. Moreover, recently it was shown by Tanaka et al. that selective inhibition of COX-1 did not produce any intestinal damage in rats whereas concomitant administration of COX-1 and COX-2 inhibitors produced severe lesions.

The selective COX-2 inhibitors were shown to cause more gastrointestinal damage as compared to the non-selective agent, indomethacin. Several compounds with in vitro selectivities for COX-2 versus COX-1 (NS-397, nimesulide, DU-P697, etodolac) were found to significantly reduce inflammation only when given at doses that inhibited COX-1. Mice lacking the gene for COX-1 exhibit diminished inflammatory responses compared to wild type. The doses of the three drugs that were used for comparison in the present study brought about similar anti-inflammatory effect in the in vivo models of formalin induced inflammation in rats. Comparison of such doses for further experiments makes the findings more plausible.

Increasing number of clinical studies and case reports have shown that COX-2 inhibitors were not superior to non-selective inhibitors as far as their gastrointestinal sparing properties are concerned. Even the much widely known CLASS trial when more closely scrutinized, showed many discrepancies in the reporting of the better safety profile of COX-2 inhibitors.

It may be concluded from the present findings that COX-2 inhibition in pro-ulcerogenic settings such as ischemia-reperfusion, or in presence of colitis may cause serious damage. The implications of these findings should be tested by seeing the effect of COX-2 inhibitors in patients with pro-ulcerogenic states or inflammatory bowel disease. Till then caution needs to be exercised with the use of these agents in this subgroup of patients.

References

1. MS (Intercontinental – Medical statistics) publications.


