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Induced Gastric Mucosal Apoptosis in Helicobacter Pylori Positive Patients Taking Low Dose Aspirin: A 6-Month Case-control Study

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Background-Most studies showed that *Helicobacter pylori*(HP) infection increased the rate of gastric mucosal proliferation and apoptosis. Non-steroidal anti-inflammatory drugs (NSAIDs) is also known to affect apoptosis of gastric epithelial cells, but there is little information about interaction between Hp infection and NSAIDs administration. Aim-The aim of this study is to investigate the dynamic balance between apoptosis and proliferation and genetic changes in subjects started on aspirin. Methods-Serial biopsy specimens of the gastric antrum were obtained during endoscopy from 12 Hp positive subjects with eradication, 12 Hp positive subjects without eradication and 12 Hp negative subjects, all of whom were started on 100 mg of aspirin at time 0. Apoptosis and proliferation were measured by TUNEL and PCNA immunostaining. The expression of apoptosis associated proteins bcl-2, bax, and CPP-32 was also detected by immunohistochemical staining. Results-The apoptotic index (AI)was significantly increased in Hp positive patients without eradication after six month (from 12.17±3.83 to 17.58±7.82, P<0.05); whereas there was no significant changes in Hp positive patients 6 months after eradication and in Hp negative patients. Similarly there were significant increase in proliferation index (PI) and the ratio of AI/PI only in Hp positive subjects without eradication, accompanied by a marked increase in expression of apoptotic protein bax. The expression of bcl-2 and CPP32 remained unchanged in all groups. Conclusions-These results suggest that active Hp infection and aspirin intake increase apoptosis and proliferation of gastric epithelial cells, and upset the AI/PI balance. The additive effect is blocked by treatment of Hp. This may provide a molecular basis for eradication of Hp before initiation of aspirin treatment.

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Development of an Improved Formulation of Phosphatidylcholine (PC) - Associated Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

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Backgound: We have previously reported that: the gastric mucosa has hydrophobic barrier properties due to the presence of PC and other zwitterionic phospholipids within and coating the overlying mucus gel layer; and that NSAIDs have an ability to weaken this lining by chemically associating with PC (*Nature Med* 1:154,1995). Accordingly, we have developed a strategy to prevent this interaction,and as a consequence NSAID-induced topical injury, by pre-associating an NSAID with either purified or synthetic PC. Earlier pre-clinical and clinical studies demonstrated that aqueous suspensions of a number of PC-NSAIDs have both lower GI toxicity and enhanced therapeutic activity in comparison to the unmodified drugs (*Nature Med; Am J Gastro* 94:1818, 1999; *JPET* 277:2773,1996). In the present study we evaluated a new method to prepare PC-NSAIDs that has the advantage that the final formulation is both solid and cost-effective. *Methods:* PC-NSAIDs were prepared by admixing a lecithin oil containing 35% PC with one of the following NSAIDs: aspirin (ASA); ibuprofen (IBU); or indomethacin (INDO), until a uniform paste was formed. These solid formulations were then tested in acute rodent models of: ASA-induced gastric ulceration; IBU- and INDO - induced GI bleeding; and NSAID-induced analgesic activity (Randall-Sellito,*Arch Int Pharmacodyn* 111: 409, 1957). *Results:* It was demonstrated that gastric ulceration was significantly reduced by 70.3±3.5% by PC-ASA, and GI bleeding was significantly reduced by 42.3±6.4% and 82.0±8.5% by PC-IBU and PC-INDO respectively, in comparison to the values of rats administered the unmodified drugs.Consistent with these observations employing acute models of NSAID-induced injury, we also determined that PC-INDO induced less diarrhea and significantly fewer intestinal lesions and adhesions than unmodified INDO during a 4 day treatment period. The increased GI safety of the new PC-NSAID formulations was not attributable to a lower bioavailability of the NSAIDs when administered as a solid, as the analgesic activity of PC-ASA, PC-IBU and PC-INDO were all significantly enhanced in comparison to the unmodified drugs. *Conclusion:* These results indicate that the new cost-effective method to formulate PC-NSAIDs provides a GI safe, therapeutically superior product to treat chronic inflammatory disorders. Long-term clinical trials evaluating the GI safety of this new family of PC-NSAIDs are planned. (supported by NIH grants DK 53195 and DK 52740)

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Effects of Selective and Non-Selective COX-1 and COX-2 Inhibitors on Chronic Gastric Ulcer Healing

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Background. Prostaglandins (PGs) derived from the inducible cyclooxigenase COX-2, play an important role in gastric ulcer healing. The question is, whether the new NSAID, which inhibit predominantly COX-2, are really safe in the long-term use in conditions of a previously established chronic ulcer. Aim. To asses the different healing process and PGs levels, leukocyte infiltration and COX expression, using classical NSAID, in comparison with the new selective COX-2 inhibitor celecoxib (CLX), 0.35 mg/Kg. Materials and Methods. A chronic ulcer was induced in rats by injection of 5% acetic acid into the gastric subserosa. Animals were treated twice daily (i.g.) during 8 and 14 days with following: vehicle, piroxicam (PRX), 0.35 mg/Kg, metamizol (MET), 33 mg/Kg, and CLX, 1.8 mg/Kg. Macroscopic ulcer index (UI), myeloperoxidase activity (MPO) as an index of neutrophil infiltration and PGE_2 content, were measured at the ulcer site as well as in mucosa around the ulcer crater. Histology and immunoreactivity for COX-1 and COX-2 were also studied. Results. All NSAID delayed ulcer healing, although there where no significant differences in UI. MPO was significantly higher at the ulcer site than in the intact mucosa after treatments ($p<0.001$). PRX significantly increased MPO activity at the ulcer site vs sham and control ($p<0.01$). On the contrary CLX decreased the enzymatic activity ($p<0.05$). PGE_2 content increased significantly at the ulcer site ($p<0.01$) vs intact mucosa of the ulcerated control. All NSAID suppressed PGE_2 generation in ulcerated and non-ulcerated tissues. Histological examination showed no important differences between both drugs but confirmed high neutrophil infiltration in case of PRX. COX-1 was expressed predominantly in the neck region and bottom of gastric glands and there were not any differences between groups. COX-2 was expressed mainly in regions of maximal repair activity at the ulcer base and superficial mucous cells and mucous cells of the foveoles. COX-2 expression was markedly stronger with PRX and control than which CLX. Conclusions. Selective and non-selective NSAID delay ulcer healing, wich may be related with a descent in PGE2 content and different behaviour in COX-1 and COX-2 expression. Inflammatory responses in case of PRX may be also implicated.

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Does Gastric Acid Secretion Accelerate NSAID-Induced Tissue Peroxidation In Rat Stomach?

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BACKGROUND: Gastric mucosa was peroxidized during NSAID-induced gastric mucosal lesions formation. Recently, a monoclonal antibody for a tissue protein reacted with 4-hydroxynonenal (4HNE) has become available, which can indicate distribution of tissue peroxidation. Using this monoclonal antibody, we have previously reported that the deep part of the gastric mucosa is particularly peroxidized following NSAID treatment. The parietal cells which are particularly located in the deep part of the gastric mucosa, consume great amount of oxygen when they produce gastric juice. This high oxygen consumption may lead to enhanced production of oxygen radicals and thus increased tissue peroxidization in the deep part of gastric mucosa, but this possibility has not been tested at all. AIM: We thus aimed to elucidate the relationship between tissue peroxidation and acid secretion after the NSAID treatment. For this purpose, we investigated whether the pretreatment with rabeprazole, one of a proton pump inhibitor (PPI), affected the 4HNE generation induced by NSAID treatment or not. METHODS: Male Wistar rats were divided into the following 4 groups: 1) control group, 2) indomethacin group, 3) rabeprazole group, 4) rabeprazole and indomethacin group. Each rat in both the indomethacin group and the rabeprazole and indomethacin group was administered 30 mg/kg indomethacin per os after 24 hours fasting. Rats in both the rabeprazole group and the rabeprazole and indomethacin group was pretreated 30 min before the indomethacin administration with 10 mg/kg rabeprazole. Each rat stomach was removed at either 0, 15, 30, 60, 180 or 300 min after the NSAID treatment. These cryosections of these stomachs were immunohistochemically stained with both anti-4HNE antibody and anti-8(OH)dG antibody. RESULTS: 4HNE began to appear in the deep layer of the gastric mucosa at 30 min after the indomethacin treatment. The area of 4HNE time-sequentially extended to the surface layer. The formation of 8(OH)dG appeared from 180 min after the treatment in the same area as 4HNE. The pretreatment with rabeprazole significantly inhibited 4HNE generation and 8(OH)dG formation. CONCLUSION: The indomethacin treatment induced tissue peroxidation as well as DNA injury, especially in the deep part of, the gastric mucosa. The pretreatment with PPI significantly inhibited the NSAID treatment-induced tissue peroxidation. These results suggest that gastric acid secretion accelerates tissue peroxidation in gastric mucosa.

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Gastric Damage Induced By Different Doses Of Indomethacin In Rats Is Variably Affected By NOS Inhibitors.

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BACKGROUND/OBJECTIVES: Gastrointestinal damage is the major limitation to the use NSAIDs. Nitric oxide (NO) is a mediator of gastrointestinal mucosal defence, but it also contributes to mucosal injury in some circumstances. The role of NO in the NSAID-gastrointestinal damage was not defined. We investigated the effect of inhibitors of both inducible and constitutive NOS on the gastric damage (GD) induced by different doses of indomethacin (INDO). MATERIAL AND METHODS: GD was induced by intragastric instilation of INDO (2.5; 5; 20 and 40mg/Kg) and assessed 1, 3, 6, 12, 24 hs later. A lesion index (LI) was calculated as the sum of the lengths of all lesions. Different groups, received dexamethasone (1mg/Kg), L-NAME (100mg/Kg), N-Nitro-arginine (100mg/Kg), aminoguanidine (100mg/Kg), L-Arginine (1000mg/Kg), D-Arginine (1000mg/Kg) or L-Lisine (1000mg/Kg). After 1 hour, GD was induced by INDO (5 or 20mg/Kg) and 3 hs later the animals were killed and LI was calculated. RESULTS: INDO caused a significant and dose- dependent GD with maximal effect at a dose of 20 mg/Kg ($p<0.001$). GD peaked at 3 hs after injection of INDO (5 or 20mg/Kg) and returned to control levels at the end of 24 hs. Pretreatment with dexamethasone (LI = 12.0±5.2mm; N = 10), L-NAME (LI = 12.7±3.6mm; N = 10), N-Nitro-Arginine (LI = 12.3±6.3mm; N = 10), Aminoguanide (LI = 11.8±5.3mm; N = 10), or L-Arginine (LI = 6.0±3.1mm; N = 10) but not D-Arginine (LI = 21.4±6.1mmn; N = 7) or L-Lisine (LI = 29.4±11.5mm; N = 5) significantly inhibited ($P<0.05$) the INDO 20 mg/Kg induced damage (LI = 30.2±4.8mm; N = 16). In contrast, GD induced by INDO 5mg/Kg (LI = 7.8±2.2mm; N = 15) tended to increase by pretreatment by both L-NAME (LI = 11.4±3.2mm; N = 15) and N-Nitro-arginine (LI = 17±4.6mm; N = 9), but the differences did not attain statistical significance (p = 0,2 and p = 0,36), whereas the pretreatment with L-Arginine plus L-NAME (LI = 1.4±0.1mm; N = 10) reduced significantly ($p<0.05$) GD induced by INDO 5mg/Kg with L-NAME (11.4±3.2mm; N = 15). Aminoguanidine had no effect (LI = 6.5±2.2mm; N = 5), whereas L-Arginine (LI = 5.3±2.1mm; N = 10), but not D-Arginine (LI = 11.7±5.5mm; N = 5), tended to reduce GD induced by INDO 5mg/Kg (LI = 7.8±2.2mm; N = 15). CONCLUSIONS 1-NOS inhibitors had a different effects depending on the dose of the INDO. 2- The pretreatment with NOS substrate as well as inhibitors impaired gastric damage induced by INDO 20 mg/Kg. 3- The effect of L-Argine seems may be specific, since because D-Arginine and L-lisine do not cause it. Financial support= FAPESP, PRONEX.