LOCAL INJECTION OF TGF ß1 IN GASTRIC ULCERS ACCELERATES HEALING.

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Transforming growth factor-ß1 (TGFß1) is a multifunctional cytokine that is involved in wound repair. Exogenous application of TGFß1 accelerates tissue repair via induction of synthesis and production of extracellular matrix. Here we examine gastric ulcer healing in the rat after local injection of TGFß1. **Method:** Chronic gastric ulcers were induced in 30 Wistar rats by the application of 100% acetic acid to the serosal surface of the stomach. Immediately after ulcer induction and on day 2, TGFß1(50ng) or saline were locally injected into the subserosa. One group (control group) received no subserosal injection. Gastric blood flow was determined at the ulcer edge and base as percent of normal mucosa blood flow on day 11. Animals were sacrificed on day 11, the ulcer area was measured planimetrically, sections were embedded in paraffin and stained with H&E. Expression of TGFß1 was assessed by immunohistochemistry (polyclonal antibody, AB-101-NA, R&D Systems, UK) using the ABC method. **Results:** The application of TGFß1 lead to a significant acceleration of gastric ulcer healing (1,7 [SD1,6]mm^2 vs 3.7 [SD2,6]mm^2). Epithellum at the ulcer margin of not completely healed ulcers in the NaCl or control group was devoid of immunoreactive transforming growth factor ß1. Epithelium of histologically almost completely healed gastric ulcers in the TGFß1 treated group did show expression of immunoreactive transforming growth factor ß1. In all groups immunoreactive fibroblasts and immunostained extracellular matrix was observed in the ulcer bed. Gastric blood flow at the ulcer margin was significantly higher than at the ulcer crater but no significant difference was found in this flow between studied groups. TGFß1 treated animals did show smaller ulcers but excessive scarring was observed. **Conclusion:** Treatment of gastric ulcers by local injection of TGFß1 accelerates ulcer healing.

INFLUENCE OF HELICOBACTER PYLORI (Hp) COLONIZATION ON NITRIC OXIDE SYNTHASE (NOS) ACTIVITY AND LEVELS OF cGMP IN GASTRIC MUCOSA.

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Hp is recognized as a major cause of inflammatory illness of gastric mucosa. The physiopathological mechanisms of this inflammatory process are unclear, but the evidences about the implication of oxidative stress are increasing. Then, the raise of NOS activity in leukocytes can play a role in the lesive effect of Hp. On the other hand, nitric oxide produced by constitutive NOS (NOSc) has a protective effect on gastric mucosa mediated by cGMP. In this way, the decrease of cGMP levels in mucosa can induce an imbalance of homeostatic mechanisms in stomach. In this sense, we are studying the NOS activity and cGMP levels in gastric biopses of 77 outpatients of Gastroenterology Service of "Virgen Macarena" hospital. In this sample, 28 patients were women and 49 were men, 48 were Hp positives and 29 Hp negatives, 20 with duodenal ulcer, 6 with gastric ulcer, 26 with gastritis, 13 without lessions and 7 with NSAID-induced lessions. 15 patients diagnostized of gastritis had not received any treatment previously, 7 patients had received treatment with H2-antagonists and other 4 with omeprazol. 9 patients with duodenal ulcer had not received treatment previously, 7 had been treated with H2-antagonists and 4 with omeprazol. The results obtained show: 1) A drastic and significant decrease of NOSc activity in gastric mucosa of patients with NSAID-induced gastropathy (2.75 +/- 1.02 pmol.g^{-1}.min^{-1}) versus normal mucosa (17.21 +/- 4.54 pmol.g^{-1}.min^{-1}) ($p<0.02$). Similar results are obtained with gastric mucosal levels of cGMP (22,08 +/- 4.15 pmol.g of $tissue^{-1}$ in patients treated with NSAID versus 100.55 +/- 27.35 pmol.g of $tissue^{-1}$ in normal mucosa) ($p<0.05$). 2) In patients with duodenal ulcer without previous treatment it is observed a reduction in NOSc but it is not statistically significant, it is also observed a increase of inductible NOS (NOSi) (62,46 +/- 16,27 pmol.g of $tissue^{-1}$.min^{-1} versus 20.89 +/- 3.71 pmol .g of $tissue^{-1}$.min^{-1} in normal mucosa) ($p<0.05$). 3) In patients with gastritis it is observed a decreased activity of NOSc and a increase of NOSi, but these modifications are not significant.
According to the results obtained we can affirm that modifications in NOS activity are correlated with the affectation grade of gastric mucosa. Moreover, the NSAID administration induced the biggest decrease of NOSc activity. Then, it is possible that the change of NOSc activity together with the increase of oxidative mechanisms can be in the basis of gastrolesive processes induced by Hp or NSAID administration.

● INVOLVEMENT OF INDIGENOUS BACTERIA AND ENDOTOXIN IN NITRIC OXIDE SYNTHASE INDUCTION AND INTESTINAL INJURY PROVOKED BY INDOMETHACIN.

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Gut bacteria have been implicated in the chronic intestinal damage caused by non-steroidal anti-inflammatory drugs such as indomethacin. Induction of a calcium-independent nitric oxide synthase (iNOS) has also recently been proposed to be involved in the chronic intestinal microvascular injury provoked by indomethacin (*Br. J. Pharmac., 116, 2286*). The role of local gut bacteria in such intestinal damage has been further explored by histology and using the broad-spectrum antibiotic, ampicillin, metronidazole and polymixin B which binds to and inactivates endotoxin.

Indomethacin (10 mg kg^{-1} s.c.) caused a dose- and time-dependent increase in macroscopic injury in the jejunum, with a score (1-5 scale) after 24h of 3.25±0.25 (n=10). After 15-18h, the epithelial barrier was disrupted, with histological evidence of bacterial invasion of the underlying tissue, predominantly with gram-negative staining organisms. Treatment with ampicillin (200 mg kg^{-1} day^{-1}) or metronidazole (200 mg kg^{-1} day^{-1}) abolished the appearance of macroscopic damage (score 0.2±0.2 and 0.1±0.1 respectively n=6). Indomethacin substantially increased the low basal level of jejunal iNOS activity, assayed as the conversion of ^{14}C-L-arginine to citrulline in the presence of EGTA (1mM), from 13±4 to 132±30 pmol min^{-1} mg^{-1} protein determined after 24h. This iNOS activity was inhibited (98±1 and 95±5%, n=5 for each, $P<0.001$) by pretreament with ampicillin or metronidazole. Treatment with polymixin B (3 mg kg^{-1} s.c.) likewise abolished the macroscopic damage, the increase in plasma leakage and the expression of jejunal iNOS determined after 24h (n=4, $P<0.01$).

This findings confirm the involvement of gut bacteria, in the small-intestinal damage caused by indomethacin. Moreover, the associated expression of iNOS, which is implicated in microvascular injury, is likewise abolished by the anti-bacterial agents. The inhibitory effects of polymixin B further suggest that the local release of endotoxin, perhaps following translocation of the luminal bacterial into the mucosa, that leads to iNOS induction, are important events in the pathogenesis of such intestinal injury.

● NON-STEROIDAL ANTI-INFLAMMATORY DRUGS INDUCE NITRIC OXIDE SYNTHASE AND MICROVASCULAR INJURY IN RAT JEJUNUM: ROLE OF PEROXYNITRITE

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Non-steroidal anti-inflammatory drugs (NSAIDs) provoke the chronic development of lesions in the rat small intestine. Induction of calcium-independent nitric oxide synthase (iNOS) in the intestine, which is associated with microvascular injury, follows subcutaneous injection of indomethacin (*Br. J. Pharm., 116, 2286*). We have now further explored the involvement of iNOS in the intestinal damage caused by such agents, and the cytotoxic role of peroxynitrite, formed from superoxide and excessive NO.

Administration of indomethacin (10 mg kg^{-1}, p.o.) caused a time-dependent increase in leakage of radiolabelled human serum albumen and in iNOS activity in the jejunum, determined by the conversion of ^{14}C-L-arginine to citrulline in the presence of EGTA (1mM), commencing 18h after challenge. A single oral dose of indomethacin, diclofenac or flurbiprofen (10, 40 and 40 mg kg^{-1} respectively) increased jejunal plasma leakage by Δ 258±15, 166±6 and 183±14µl g^{-1} tissue respectively ($P<0.001$; n=4), determined 24h later. Administration of the systemically acting conjugate of superoxide dismutase with polyethylene glycol, SOD-PEG (2500 kg^{-1} i.v.), 15h following indomethacin, reduced the 24h plasma leakage by 61±8% (n=6, $P<0.01$). Jejunal iNOS activity substantially increased from a basal value of 14±1 to 109±20, 90±10 and 83±1 pmol min^{-1} mg protein respectively, ($P<0.01$) 24h following these doses of indomethacin, diclofenac and flurbiprofen. Western blots of homogenates of jejunal tissue obtained 24h after indomethacin challenge, incubated with iNOS antibody to mouse macrophage, displayed a single band corresponding to 130kDa, indicative of the iNOS isoform.

These data demonstrate that oral administration of these NSAIDs leads to jejunal plasma leakage associated with expression of iNOS, determined both by radioassay and immunoblotting. Since inhibition of iNOS is now known to abolish this jejunal plasma leakage, the observation that SOD-PEG can likewise attenuate the microvascular damage supports the involvement of peroxynitrite, in the chronic intestinal injury provoked by NSAIDs.