GASTRIC ANTISECRETORY DRUGS INDUCE LEUKOCYTE-ENDOTHELIAL CELL INTERACTIONS THROUGH GASTRIN RELEASE AND ACTIVATION OF CCK-2 RECEPTORS *

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Abbreviations:
PPI, proton pump inhibitor
FMLP, formyl-methionyl-leucyl-phenylalanin
PMA, phorbol 12-myristate-13-acetate.

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ABSTRACT

Antisecretory drugs are effective antiulcer agents but its chronic use generates hypergastrinemia and accelerates the development of atrophic gastritis in H. pylori-positive patients. We have recently shown that gastrin exerts a proinflammatory effect in rats through CCK-2 receptor activation that contributes to the inflammation induced by Helicobacter pylori. The present study was designed to examine whether gastrin hypersecretion in response to treatment with antisecretory drugs induces an inflammatory response that could promote mucosal atrophy. The effects of omeprazole or famotidine on leukocyte/endothelial cell interactions in vivo were analyzed in rat mesenteric venules using intravital microscopy. Administration of a single dose of omeprazole or famotidine acutely increased gastrinemia and leukocyte rolling and adhesion, but not emigration into the interstitium. Daily treatment with omeprazole for a short period (3 days) induced a similar response, but when this treatment was extended to fourteen days and a steady hypergastrinemic state was established, increased leukocyte rolling, adhesion and emigration was observed. Pretreatment with the CCK-2 receptor antagonist proglumide prevented these inflammatory events in all cases. Leukocytes from rats treated with omeprazole showed increased expression of CD11b/CD18 initially in granulocytes (3 day protocol) and later in monocytes and lymphocytes (14 day protocol). These changes were not observed in animals pretreated with proglumide and were not reproduced by incubation of leukocytes from untreated animals in vitro with gastrin. Thus, hypergastrinemia induced by chronic treatment with antisecretory drugs may promote inflammation, which could partly explain their worsening effect in corpus gastritis observed in H. pylori infected patients.
INTRODUCTION

Gastrin is a gastrointestinal hormone that plays an essential role regulating gastric acid secretion and promoting cellular growth. Its release from antral G cells is closely controlled by neuroendocrine mechanisms that are highly sensitive to local changes. Luminal acidity exerts a negative feedback on gastrin secretion through the release of somatostatin from D cells. When acid secretion is pharmacologically inhibited, somatostatin secretion decreases and, consequently, G cells become unrestrained and provoke hypergastrinemia (Walsh, 1994). This neuroendocrine secretory circuit is also sensitive to local inflammation. Helicobacter pylori infection and the associated gastritis reduce somatostatin and increase gastrin synthesis (Blaser and Atherton, 2004), while G cell activity can be directly stimulated by proinflammatory cytokines (Weigert et al., 1996). On the other hand, accumulating evidence suggests that these neuroendocrine secretions exert immunomodulatory effects themselves. We have recently demonstrated that gastrin has a direct proinflammatory effect in rats through the activation of its CCK-2 receptors, and that hypergastrinemia induced by Helicobacter pylori components contributes to the inflammation induced by these bacterial products (Alvarez et al., 2006). On the contrary, somatostatin displays anti-inflammatory properties (Karalis et al., 1994), and recent evidences suggest that the equilibrium between gastrin and somatostatin release is important to define the host lymphocytic response to Helicobacter infection (Takaishi et al., 2005; Zavros et al., 2003).

Inhibition of gastric acid secretion with proton pump inhibitors (PPIs) has been proved very effective to induce healing and symptom relief in peptic ulcer and gastroesophageal reflux disease. However, many clinical studies do support an accelerating effect of PPIs on the development of atrophic gastritis in H. pylori-
positive patients. This effect is usually associated to a reduction in antral gastritis and it was primarily assumed that both changes were the consequence of an alteration in the pattern of *Helicobacter pylori* colonization (Logan et al., 1995). The increased corpus pH would be beneficial for bacterial survival and promote the migration of the germ from the antrum to the corpus. However, latter studies have shown aggravated corpus gastritis with no increase (Kuipers et al., 1995; Meining et al., 1997), or even reduction (Stolte et al., 1998; Schenk et al., 2000; Moayyedi et al., 2000), in *Helicobacter* density in corpus. These evidences question the initial hypothesis and leave the mechanism responsible for the corpus deterioration unresolved.

High serum levels of gastrin before PPI therapy in *H. pylori*-infected patients seems to predispose to an accelerated progression in gastric atrophy during treatment (Eissele et al., 1997), and most clinical studies showed a strong correlation between the degree of atrophic gastritis and gastrinemia (Fox and Wang, 2007). The development of mucosal atrophy is the result of a chronic inflammatory process that finally destroys the normal gland architecture. Our hypothesis is that gastrin hypersecretion in response to treatment with antisecretory drugs may contribute to mucosal atrophy by promoting the inflammatory process. Thus, the aim of the present study is to analyze whether antisecretory drugs induce inflammation and the involvement of gastrin hypersecretion in such a response.
METHODS

Intravital microscopy

Leukocyte-endothelial cell interactions were evaluated in fasted male Sprague-Dawley rats (200-250 g), the details of the experimental preparation having been described previously (Alvarez et al., 2002). In brief, rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.). A midline abdominal incision was made and a segment of the midjejunum exteriorized and placed over a transparent pedestal for tissue transillumination. A selected loop of the exposed mesentery was continuously superfused with bicarbonate buffer saline (pH 7.4, 37 ºC, 2 ml min⁻¹) and observed through an orthostatic microscope equipped with a video camera. Images were captured on videotape for playback analysis (final magnification of the video screen was x1300).

The mesentery was left to stabilize for a period of 30 min and images of 3 unbranched mesenteric venules (with diameters between 25 and 40 µm), were recorded for a period of 5 min per venule. The numbers of rolling, adherent and emigrated leukocytes were determined off-line during playback analysis of videotaped images. Rolling leukocyte flux was assessed by counting the number of leukocytes passing a reference point in the vessel per min. Leukocyte rolling velocity was calculated by measuring the time required for a leukocyte to traverse a distance of 100 µm along the length of the venule and was expressed as µm/s. A leukocyte was considered to be adherent to the venular endothelium if it remained stationary for a period equal to or exceeding 30 s. Adherent cells were expressed as the number of white blood cells per 100 µm of venule. Leukocyte emigration was evaluated as the total number of interstitial leukocytes per field. Systemic arterial blood pressure, venular diameters
and centerline red blood cell velocity were evaluated on-line and venular blood flow and venular wall shear rate ($\gamma$) were calculated as previously described (Calatayud et al., 1999).

At the end of the experiment, the stomach of some rats was removed, fixed with paraformaldehyde (4% in PBS pH 7.4) and embedded in paraffin. In some cases, a portal blood sample was collected in citrate to analyze the expression of adhesion molecules in circulating leukocytes and measure plasma gastrin concentration by radioimmunoassay (RIA).

**Protocols**

Animals received the proton pump inhibitor (omeprazole 40 mg/kg, p.o.), the H2 receptor antagonist (famotidine 30 mg/kg, p.o.) or their vehicle (carboxymethylcellulose 0.2%) and were anesthetized four hours later. The mesentery was then exposed and left to stabilize. Videotape recordings and haemodynamic measurements were done 5h after drug administration. The doses used were taken from experimental studies analyzing their potency as antisecretory drugs. We chose the doses of omeprazole and famotidine inducing a maximal antisecretory effect in rats, which turned out to be much higher than those necessary to inhibit gastric acid secretion in humans. Both treatments cause significant, and quantitatively similar, increases in gastrinemia that peak five hours after dosing (Decktor et al., 1989).

A second group of rats was administered with omeprazole during three consecutive days (40 mg/kg/day, p.o.) and the intravital microscopy experiments were performed 24 hours after the last dose. In order to analyze the evolution of gastrinemia on the
day previous to the experiment, a portal blood sample was obtained from some animals receiving the same treatment 8, 12 or 24 hours after the last dose of omeprazole or vehicle.

In the third protocol, animals received vehicle or omeprazole (10 or 40 mg/kg) or famotidine (30 mg/kg) daily during fourteen consecutive days and the experiment performed as before, 24 hours after the last dose.

The involvement of gastrin in the effects induced by these agents was tested by pre-treating some rats with proglumide (30 mg/kg, i.p.) every time that an antisecretory drug was administered. This dose of proglumide was effective to prevent the proinflammatory effect of exogenously administered gastrin without affecting the effects induced by a common inflammatory mediator like PAF (platelet activating factor, $10^{-7}$M) (Alvarez et al., 2006).

**Immunohistochemical studies**

Leukocyte infiltration in the gastric mucosa was analyzed by detecting the common leukocyte antigen CD45 in the leukocyte surface by immunohistochemistry. Sections of the gastric corpus (5 µm) were deparaffined, hydrated and processed for antigen retrieval with alpha-chymotrypsin (Sigma Chemical CO). After blocking (10% goat serum, 1% BSA), specimens were incubated with a mouse monoclonal [MRC OX-1] anti-CD45 antibody (Abcam, 1:100, 4°C, overnight). A rabbit anti-mouse HRP conjugate (Dako Cytomation, 1:100) was used as secondary antibody and was incubated for 1 hour at room temperature. Finally, tissues were incubated with DAB Enhanced Liquid substrate System for Immunohistochemistry (Sigma Chemical CO) and counterstained with hematoxylin. Appropriate negative control experiments excluding the primary and/or the secondary antibodies were performed and no staining
was detected. Leukocyte infiltration was measured by counting the number of positive cells per field (2.0x0.8 mm$^2$ grid).

**Flow Cytometry Analysis**

The expression of adhesion molecules was analyzed in circulating leukocytes from portal blood of animals treated during 3 or 14 days with omeprazole (40 mg/kg/day, p.o.) with or without cotreatment with proglumide. In a second set of experiments, leukocyte stimulation assays *in vitro* were carried out to check whether gastrin exert a direct effect on the expression of these adhesion molecules. In this case, the analysis was performed in portal whole blood from untreated animals incubated for 45 minutes at 37°C with vehicle, gastrin (10$^{-11}$-10$^{-9}$ M), formyl-methionyl-leucyl-phenylalanin (FMLP, 10$^{-7}$ M) or phorbol 12-myristate-13-acetate (PMA, 10$^{-7}$ M). The gastrin concentrations used correspond to those inducing leukocyte-endothelial cell interactions in rat mesentery (Alvarez et al., 2006).

For the flow cytometry analysis, duplicated samples of citrated venous whole-blood (40 µl) were transferred to polypropylene centrifuge tubes and incubated for 30 min in ice with saturating amounts (10 µl) of FITC-labeled antibodies (Serotec) against the adhesion molecules L-selectin (clone OX-85) and Mac-1 (CD11b/CD18, clone ED8), the alpha subunits of β2 integrins CD11a (clone WT.1) and CD11c (clone 8A2), the common β2 subunit of these integrins (CD18, clone WT.3) and the alpha 4 chain of VLA4 (CD49, clone TA-2). Appropriate negative control antibodies were used to verify the staining specificity. Removal of red blood cells and fixation of leukocytes was performed through an automated lysing procedure with an EPICS Q-PREP system (Coulter Electronics). Neutrophils, monocytes, and lymphocytes were identified for analysis by forward- and right-angle light scatter and gates were set to
exclude other cell types and debris. Ten thousand events were counted per sample.
All the analyses were performed with an EPICS XL-MCL Flow Cytometer (Coulter Electronics).

**Materials**

Omeprazole, proglumide, famotidine, FMLP and PMA were all obtained from Sigma Chemical. Pentobarbital was from B. Braun Medical SA (Rubi, Barcelona, Spain). The antibodies were from Serotec (Oxford, UK), Abcam (Cambridge, UK) or Dako Cytomation (Glostrup, Denmark). Gastrin RIA kit was purchased from IBL Hamburg, Germany.

**Statistical Analysis**

All values are mean ± sem. Data within groups were compared using the Student’s t-test or a one-way ANOVA followed by a Newman-Keuls post hoc test. The differences were considered significant when the P value was < 0.05.
RESULTS

Administration of a single dose of omeprazole (40 mg/kg) or famotidine (30 mg/kg) induced, 5 hours later, significant increases in the number of rolling leukocytes with a decrease of their rolling velocity in mesenteric venules. This was associated to significant leukocyte adhesion but not leukocyte emigration into the extravascular tissue (Figure 1). Similarly, rats receiving this dose of omeprazole daily during three consecutive days, showed increased leukocyte rolling, reduced rolling velocity and increased adhesion 24 hours after the last dose, while no significant changes in leukocyte emigration were detected. When the omeprazole treatment was extended to 14 days and the leukocyte-endothelial interactions evaluated 24 hours later, rats receiving 10 or 40 mg/kg/day of omeprazole displayed a dose-dependent increase in leukocyte rolling and adhesion, but also in emigration (Figure 2). Chronic famotidine treatment (30 mg/kg/day, 14 days) caused a response equivalent to that observed in animals receiving the lower dose of omeprazole, with high levels of leukocyte rolling and adhesion and a slight trend to increased emigration. The haemodynamic parameters (shear rate of the vessels and mean arterial pressure) were comparable between all groups.

The situation observed in the mesentery of rats treated chronically with omeprazole (40 mg/kg/day, 14 days) was in keeping with the histological findings in the gastric corpus, where a significant increase in leukocyte infiltration was detected by immunohistochemical staining of the common leukocyte antigen CD45 (115±26 positive cells / field in the omeprazole group vs. 62±11 positive cells / field in the control group, P<0.05).

Analysis of the gastrin concentration in portal blood at the end of the intravital experiments revealed that a single dose of omeprazole (40 mg/kg) induced a three-
fold increase with respect to the control level 5 hours after dosing. In the same circumstances, famotidine (30 mg/kg) increased gastrin levels 2.5 times (Table 1). Rats receiving omeprazole (40 mg/kg/day) during three consecutive days showed normal gastrinemia 24 h after the last dose when the intravital experiment was performed (Table 1). However, the animals following this three-day regime presented hypergastrinemia for the most part of the day previous to the experiment since gastrin levels 8 hours after the third dose of omeprazole were higher than those observed 5 hours after a single dose and remained elevated twelve hours after dosing (Figure 3).

When the antisecretory treatment was extended to 14 days, both omeprazole (40 mg/kg/day) and famotidine (40 mg/kg/day) induced a significant increase in the gastrin levels detected 24 hours after the last dose, although the effect was higher with the PPI (Table 1).

Treatment with the CCK-2 receptor antagonist proglumide (30 mg/kg) prior to each dose of antisecretory agent prevented the increases in leukocyte-endothelial cell interactions induced by these drugs in every treatment protocol assayed (Figure 4). Flow cytometric detection of adhesion molecules in leukocytes from portal blood samples revealed that omeprazole treatment during three days (40 mg/kg/day) induced a significant reduction in L-selectin expression in granulocytes, monocytes and lymphocytes. A significantly increased expression of the adhesion molecule CD11b/CD18 was detected specifically in granulocytes. Leukocytes from rats cotreated with proglumide and omeprazole showed normal L-selectin and CD11b/CD18 expression (Figure 5). Omeprazole treatment did not affect the expression of the α integrin subunits CD11a, CD11c and CD49, nor the expression of the β2 integrin subunit CD18 (Table 2).
Granulocytes from rats treated for 14 consecutive days with the same dose of omeprazole presented normal expression of L-selectin and CD11b/CD18. Conversely, monocytes and lymphocytes in these blood samples showed reduced L-selectin and increased CD11b/CD18 in the cellular surface. These changes were not observed in animals cotreated with proglumide and omeprazole (Figure 6). As occurred in the three-day protocol, the expression of CD11a, CD11c, CD18 or CD49 was not affected by any treatment (Table 3).

In vitro treatment with different concentrations of gastrin capable of inducing leukocyte-endothelial cell interactions in vivo (Alvarez et al., 2006), did not modify the expression of the integrin CD11b/CD18 in either granulocytes, monocytes or lymphocytes (Figure 7).
DISCUSSION

The present study demonstrates that treatment with antisecretory drugs induce inflammatory events in the rat mesentery through the release of gastrin and the consequent activation of CCK-2 receptors.

We have analyzed the acute effects on leukocyte-endothelium interactions of two treatments inducing a maximal inhibition of acid secretion through different mechanisms, proton pump blocking with omeprazole (40 mg/kg) or H2-receptor antagonism with famotidine (30 mg/kg) (Decktor et al., 1989), and the effects were very similar: increased number of rolling and adherent leukocytes, with little effect on leukocyte emigration. When we analyzed the influence of chronic antisecretory treatments, augmented rolling and adhesion, and a slight non-significant increase in emigration were observed in animals receiving this dose of omeprazole during three days or rats treated during fourteen days with famotidine or a lower dose of omeprazole (10 mg/kg). However, a complete inflammatory response, with increased rolling, adhesion and significant emigration was observed in rats receiving the full inhibitory dose of omeprazole for fourteen days. Moreover, we observed that this chronic treatment with the PPI induced gastritis since augmented leukocyte infiltration was observed in the gastric corpus of these animals.

We recently shown that exogenous gastrin exerts a proinflammatory action through CCK-2 receptors. These receptors were detected in mesenteric macrophages and PMNs, and two different antagonists (proglumide and L-365,260) completely prevented gastrin effects (Alvarez et al., 2006). In the present study, pretreatment with proglumide prevented the inflammatory events in every protocol used, indicating that they are mediated by gastrin. In fact, we observed a relationship between the
grade of the inflammatory changes and the alterations in gastrin levels. In the three-day protocol, gastrinemia was normal 24h after the last administration. Previous studies showed that gastrin release after a single dose of omeprazole peaks at 5h and reverts to control levels 12h after dosing (Decktor et al., 1989). However, in animals treated during three days we detected a 5.4 fold increase over control values 12h after the last dose. Thus, this protocol generates an intermediate situation in which, animals present high gastrin levels the major part of the day but constant hypergastrinemia is not yet established. In this setting, gastrin hypersecretion after dosing seems to induce an inflammatory response that, once initiated, remains longer than the hormonal stimuli, and what we see at 24h would be the remnants of the inflammation evoked initially by gastrin and prevented by proglumide. Rats receiving this dose of omeprazole or famotidine during fourteen days develop constant hypergastrinemia, but gastrin levels were higher in the PPI-treated group. It is just in these rats where we observe increased leukocyte emigration. Thus, the present results confirm the proinflammatory action of gastrin, but also indicate that transient elevations of gastrinemia have no serious consequences. They induce the initial steps of an inflammatory response but not the formation of an inflammatory focus since leukocytes do not invade the interstitium. However, when steady high gastrin plasma levels are present, a complete inflammatory response takes place.

The leukocyte-endothelial interactions are mediated by several molecules expressed by endothelial cells and leukocytes. L-selectin is constitutively expressed in most leukocytes and it is rapidly shed from surface upon cellular activation. Selectins are fundamental mediators of leukocyte rolling, while adhesion and migration require leukocyte integrins (Liu et al., 2004). Treatment with omeprazole for three days induced a reduction of L-selectin in PMNs, monocytes and lymphocytes, but an
increased expression of the integrin CD11b/CD18 specifically in PMNs. When the treatment is extended to fourteen days, PMNs seemed to be in a resting state while monocytes and lymphocytes expressed higher levels of CD11b/CD18 and reduced L-selectin. The increased integrin expression in PMNs after three days of treatment point to granulocytes as the main protagonists in this early period, as corresponds to an acute inflammatory state. The later activation of monocytes and lymphocytes reflects a chronic inflammatory process. Thus, the pattern of expression of leukocyte adhesion molecules gives supporting evidence of the presence of an evolving inflammatory reaction in vivo.

Leukocytes from animals co-treated with omeprazole and proglumide did not show any alteration in the expression of these adhesion molecules, which points to gastrin as the ethyologic factor for these differences. However, the sequential changes in the cell type affected argue against a direct molecular effect of gastrin. They rather seem to be part of the complete response occurring in vivo, a view reinforced by the unaltered expression of these molecules on leukocytes treated ex vivo with gastrin.

These data indicate that gastrin must be activating its receptor in some structure only present in vivo. Gastrin stimulates gastric enterochromaffin-like cells to release histamine (Walsh, 1994), which is a common mediator of acute inflammatory reactions. The ability of histamine to induce leukocyte recruitment was initially attributed to H₁ receptor activation (Asako et al., 1994), but later studies have shown that the other receptor types (H₂-4) may also modulate the immune function (Akdis and Simons, 2006). We observed that the proinflammatory effect induced by gastrin superfusion in the mesentery occurs without degranulation of mastocytes, the main source of histamine in this tissue, and after H₁ receptor blockade (Alvarez et al.,
2006). Both results suggest that the observed effects are at least partially independent of endogenous histamine. However, we cannot discard that histamine released in the gastric mucosa in response to gastrin could still induce some inflammatory events through the H₃-4 receptors (Akdis and Simons, 2006; Zhang et al., 2007). On the other hand, our previous results point to macrophages as one likely target for gastrin. The activation of these resident cells would trigger the initial signal for leukocytes to interact with the venular endothelium and start the inflammatory process. Once initiated, and provided that the original stimulus continues, the inflammation would take off and follow its own kinetics. Gastrin may also act on endothelial cells where it seems to modify the expression of adhesion molecules and increase chemokine secretion (Lefranc et al., 2004; Clarke et al., 2006). We also detected CCK-B receptors in mesenteric PMNs, whereas others reported its presence in mononuclear cells (Sacerdote et al., 1991; Schmitz et al., 2001). Although gastrin did not affect the expression of adhesion molecules in leukocytes in our in vitro experiments, it is possible that plasma gastrin could modify their behavior once they are activated by the ongoing inflammatory process.

Previous reports describing the modulation by gastrin of leukocyte function showed a parallelism between the effects observed in human and rat cells (Sacerdote et al., 1988). Thus, our results could explain why chronic treatment with antisecretory drugs induce an aggravating effect on H. pylori induced gastritis in humans (Fox and Wang, 2007) and animals (Takaishi et al., 2005). This deleterious effect has been mainly observed with PPIs, because they are the principal drugs used chronically to treat GERD. The few studies analyzing the effects of anti-H2 agents showed analogous changes albeit of lower intensity (Meining et al., 1997; Meining et al., 1998). This indicates that this adverse event is not related with the mechanism of action of these drugs.
drugs but with their common hyposecretory effects, and the same is true for the hypergastrinemic response. Furthermore, PPIs seem to be more powerful than anti-H2 agents in all of these actions, in patients and in the present study, which further supports the link between these three effects.

Recent studies indicate that PPIs may reduce leukocyte-endothelial cell interactions in vitro (Yoshida et al., 2000; Handa et al., 2006), an effect observed with drug concentrations probably reached, at least transiently, in our rats (Lee et al., 2007). However, our results suggest that, if these anti-inflammatory actions are taking place in vivo, they are clearly overwhelmed by the pro-inflammatory effect derived from hypergastrinemia.

The presently reported inflammatory events contrast with the significant efficacy of antisecretory drugs to reduce GERD symptoms and cure peptic ulcers in humans. Their therapeutic value resides in their high efficiency increasing luminal pH, as was summarized in the old axiom of “no acid, no ulcer”. This potent effect could conceal the proinflammatory actions, which could possibly arise under the influence of an acid-independent inflammatory stimulus such as H. pylori. However, we observed that omeprazole treatment induces gastritis in rats without H. pylori infection. This difference could be due to the high doses used in the present study to accelerate the changes induced more gradually in humans. Alternatively, rodents could be more sensitive than humans to the proinflammatory effect of gastrin as occurs with its growth promoting action (Watson et al., 2006). Finally, the proinflammatory effect of gastrin may be facilitated by the overgrowing of other microorganisms in response to the increased pH (Zavros et al., 2002).
To conclude, this study confirms the proinflammatory activity of gastrin and adds to the growing evidence indicating that this hormone has varied molecular and functional consequences beyond stimulation of gastric acid secretion or cellular growth promotion (Dockray et al., 2005). Further research is required in order to define the relevance of this effect of gastrin in patients suffering hypergastrinemia as a consequence of *H. pylori* infection, antisecretory treatments or both.
REFERENCES


ranitidine and lansoprazole in patients with duodenal ulcer disease.  


FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. Leukocyte/endothelial cell interactions induced acutely by two different antisecretory drugs. Rats were treated with omeprazole (40 mg/kg, p.o.), famotidine (30 mg/kg, p.o.) or vehicle and their effects on leukocyte rolling flux (A), rolling velocity (B), adhesion (C) and emigration (D) were analyzed in mesenteric postcapillary venules 5 hours after drug/vehicle administration. *P<0.05, **P<0.01 and ***P<0.001 vs respective control (omeprazole vehicle: n=5, omeprazole: n=6, famotidine vehicle: n=5, famotidine: n=5).

Figure 2. Leukocyte/endothelial cell interactions induced by repeated daily treatment with omeprazole or famotidine. Rats were treated with omeprazole 10 mg/kg during 14 consecutive days or with omeprazole 40 mg/kg during 3 or 14 consecutive days or with famotidine 30 mg/kg during 14 consecutive days and their effects on leukocyte rolling flux (A), rolling velocity (B), adhesion (C) and emigration (D) were analyzed in mesenteric postcapillary venules 24 hours after the last drug/vehicle administration. *P<0.05, **P<0.01 and ***P<0.001 vs respective control (omeprazole vehicle 3 days: n=5, omeprazole 3 days: n=5, omeprazole vehicle 14 days: n=14, omeprazole 10 mg/kg 14 days: n=5, omeprazole 40 mg/kg 14 days: n=7, famotidine vehicle 14 days: n=3, famotidine 30 mg/kg 14 days: n=5).

Figure 3. Gastrin concentration in plasma from portal blood.
Results correspond to pg/ml. Blood samples were taken from rats 8, 12 and 24 hours after treatment with omeprazole (40 mg/kg, p.o.) or their vehicle. P<0.001 vs value in respective vehicle-treated rats.
Figure 4. Leukocyte/endothelial cell interactions induced by different treatments with antisecretory drugs and effect of a CCK-2 receptor antagonist. Leukocyte rolling flux (A), rolling velocity (B), adhesion (C) and emigration (D) were analyzed in mesenteric postcapillary venules of rats were treated with: a) a single dose of omeprazole (40 mg/kg p.o., O) or famotidine (30 mg/kg p.o., ▼) 5 hours before; b) omeprazole (40 mg/kg, p.o.) during 3 consecutive days (O); c) omeprazole (10 mg/kg, p.o.) during 14 consecutive days (●); d) omeprazole (40 mg/kg, p.o.) during 14 consecutive days (●). In protocols b, c and d the effects on leukocyte/endothelial cell interactions were analyzed 24 hours after the last dose. Some animals were treated with proglumide (30 mg/kg, i.p.) 30 min before each dose of antisecretory drug. *P<0.05, **P<0.01 and ***P<0.001 vs respective proglumide treated group (n≥5 in each group).

Figure 5. Expression of (A) L-selectin and (B) CD11b/CD18 in leukocytes from animals treated with omeprazole (40 mg/kg, p.o.) or its vehicle during 3 consecutive days. Some animals were treated with proglumide (30 mg/kg, i.p.) 30 min before each dose of omeprazole. Portal blood samples were obtained 24 hours after the last drug/vehicle administration and the expression of adhesion molecules was analyzed by flow cytometry. *P<0.05 vs respective control; + P<0.05 vs respective omeprazole treated group.

Figure 6. Expression of (A) L-selectin and (B) CD11b/CD18 in leukocytes from animals treated with omeprazole (40 mg/kg, p.o.) or its vehicle during 14 consecutive days. Some animals were treated with proglumide (30 mg/kg, i.p.) 30
min before each dose of omeprazole. Portal blood samples were obtained 24 hours after the last drug/vehicle administration and the expression of adhesion molecules was analyzed by flow cytometry. *P<0.05 vs respective control; + P<0.05 vs respective omeprazole treated group.

Figure 7. CD11b/CD18 expression in leukocytes from untreated animals incubated in vitro with different concentrations of gastrin (10^{-11}-10^{-9} M) or positive controls (FMLP 10^{-7} M, PMA 10^{-7} M). The expression of adhesion molecules was analyzed by flow cytometry and results are expressed as percentage of values obtained in samples treated with vehicle.
Table 1. Gastrin concentration in plasma from portal blood.

Results correspond to pg/ml. Blood samples were taken from rats: (A) 5 hours after treatment with omeprazole (40 mg/kg, p.o.), famotidine (30 mg/kg, p.o.) or their vehicle; (B) 24 h after the last dose of a three-day treatment with omeprazole (40 mg/kg/day, p.o.) or its vehicle; (C) 24 h after the last dose of a fourteen-day treatment with omeprazole (40 mg/kg, p.o.), famotidine (30 mg/kg, p.o.) or their vehicle. P<0.001 vs value in respective vehicle-treated rats.

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**Table 2.** Effect of treatment with omeprazole during three days on the expression of leukocyte adhesion molecules.

Results correspond to mean values of fluorescence emitted by lymphocytes, monocytes and polymorphonuclear leukocytes in blood samples from control rats or rats treated with omeprazole (40 mg/kg/day, 3 days) with or without pretreatment with proglumide (30 mg/kg/day, 3 days). Omeprazole treatment did not induce any significant change in the expression of the adhesion molecules analyzed. Lymphocytes and PMN were negative for CD11c and CD49 expression, respectively.

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<th>CD 18</th>
<th></th>
<th>CD11a</th>
<th></th>
<th>CD11c</th>
<th></th>
<th>CD49</th>
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<td>LYMPH</td>
<td>MON</td>
<td>PMN</td>
<td></td>
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<td>MON</td>
<td>PMN</td>
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<tr>
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<td>10,2±1,3</td>
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<td>17,5±1,6</td>
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<td>10,6±0,7</td>
<td>5,9±0,2</td>
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</tbody>
</table>
Table 3. Effect of chronic omeprazole treatment (14 days) on the expression of leukocyte adhesion molecules.

Results correspond to mean values of fluorescence emitted by lymphocytes, monocytes and polymorphonuclear leukocytes in blood samples from control rats or rats treated with omeprazole (40 mg/kg/day, 14 days) with or without pretreatment with proglumide (30 mg/kg/day, 14 days). Omeprazole treatment did not induce any significant change in the expression of the adhesion molecules analyzed. Lymphocytes and PMN were negative for CD11c and CD49 expression, respectively.

<table>
<thead>
<tr>
<th></th>
<th>CD 18</th>
<th>CD11a</th>
<th>CD11c</th>
<th>CD49</th>
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<tbody>
<tr>
<td></td>
<td>LYMPH</td>
<td>MON</td>
<td>PMN</td>
<td>LYMPH</td>
</tr>
<tr>
<td>C</td>
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<td>12,9±1,3</td>
<td>14,0±1,6</td>
<td>10,5±0,4</td>
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</tbody>
</table>
**FIGURE 3**

![Graph showing gastrin levels over time for different treatments](image)

- **Vehicle**
- **Omeprazole 40 mg/kg**

Gastrin levels (pg/ml) at 8 hours, 12 hours, and 24 hours.
FIGURE 4

A

% of control value

vehicle proglumide

*** ** *

B

% of control value

vehicle proglumide

*** ** *

C

% of control value

vehicle proglumide

Ome 40 mg/kg, 5 hours
Ome 40 mg/kg/day, 3 days
Ome 10 mg/kg/day, 14 days
Ome 40 mg/kg/day, 14 days
Famot 30 mg/kg, 5 hours

D

% of control value

vehicle proglumide

*
FIGURE 5

A

B

Mean fluorescence

Control
Omeprazole
Progl + Ome

PMN
MON
LYMPH

Mean fluorescence

PMN
MON
LYMPH