Gastric Antisecretory Drugs Induce Leukocyte-Endothelial Cell Interactions through Gastrin Release and Activation of CCK-2 Receptors

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ABSTRACT

Antisecretory drugs are effective antulcer agents, but its chronic use generates hypergastrinemia and accelerates the development of atrophic gastritis in Helicobacter 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 H. 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 14 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 they 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.

Gastrin is a gastrointestinal hormone that plays an essential role in 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; 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), whereas 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 H. pylori components contributes to the inflammation induced by these bacterial products (Álvarez 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 (Zavros et al., 2003; Takaishi et al., 2005).

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 *H. 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, later studies have shown aggravated corpus gastritis with no increase (Kuipers et al., 1995; Meining et al., 1997), or even reduction (Stolte et al., 1998; Moayyedi et al., 2000; Schenk 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 (Eissle et al., 1997), and most clinical studies showed a strong correlation between the degree of atrophic gastritis and gastremia (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.

**Materials and 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 was exteriorized and placed over a transparent pedestal for tissue transillumination. A selected loop of the exposed mesentery was continuously superfused with 2 ml min⁻¹ bicarbonate buffer saline, pH 7.4, at 37°C, and it was 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 1300-X).

The mesentery was left to stabilize for a period of 30 min, and images of three 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 minute. 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 micrometers per second. 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 online, and venular blood flow and venular wall shear rate (γ) were calculated as described previously (Calatayud et al., 1999).

At the end of the experiment, the stomach of some rats was removed, fixed with paraformaldehyde (4% in phosphate-buffered saline, 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 to measure plasma gastrin concentration by radioimmunoassay.

**Protocols.** In the first protocol, 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 they were anesthetized 4 h later. The mesentery was then exposed and left to stabilize. Videotape recordings and hemodynamic measurements were done 5 h 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 peaked 5 h after dosing (Decktor et al., 1989).

In the second protocol, rats were administered omeprazole during three consecutive days (40 mg/kg/day p.o.), and the intravital microscopy studies were performed 24 h after the last dose. 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 h 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 14 consecutive days, and the experiment was performed 24 h after the last dose.

The involvement of gastrin in the effects induced by these agents was tested by pretreating 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 such as 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 deparaffinized, hydrated, and processed for antigen retrieval with α-cromyotropsin (Sigma-Aldrich, St. Louis, MO). After blocking (10% goat serum and 1% bovine serum albumin), specimens were incubated with a mouse monoclonal (MRC OX-1) anti-CD45 antibody (1:100; 4°C overnight; Abcam plc, Cambridge, UK). A rabbit anti-mouse horseradish peroxidase conjugate (1:100; Dako Denmark A/S, Glostrup, Denmark) was used as secondary antibody, and it was incubated for 1 h at room temperature. Finally, tissues were incubated with DAB Enhanced Liquid substrate System for Immunohistochemistry (Sigma-Aldrich) 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 (2.0 × 0.8 mm² 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 exerted 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 min at 37°C with vehicle, gastrin (10⁻¹¹–10⁻⁹ M), N-formyl-methionyl-leucyl-phenylalanine (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 the samples were incubated for 30 min in ice with saturating amounts (10 μl) of fluorescein isothiocyanate-labeled an-
differences were considered significant when the analysis of variance followed by a Newman-Keuls post hoc test. The groups were compared using the Student’s t-test.

Materials. Omeprazole, proglumide, famotidine, fMLP, and PMA were all obtained from Sigma-Aldrich. Pentobarbital was from B. Braun Medical SA (Rubi, Barcelona, Spain). The antibodies were from Serotec, Abcam plc, or Dako Denmark A/S. Gastrin radioimmunoassay kit was purchased from IBL Hamburg (Hamburg, Germany).

Statistical Analysis. All values are mean ± S.E.M. Data within groups were compared using the Student’s t test or a one-way analysis of variance 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 h 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 to leukocyte emigration into the extravascular tissue (Fig. 1, A–D). Likewise, rats receiving this dose of omeprazole daily during three consecutive days showed increased leukocyte rolling, reduced rolling velocity, and increased adhesion 24 h after the last dose, whereas no significant changes in leukocyte emigration were detected. When the omeprazole treatment was extended to 14 days and the leukocyte-endothelial interactions were evaluated 24 h later, rats receiving 10 or 40 mg/kg/day omeprazole displayed a dose-dependent increase in leukocyte rolling and adhesion and also in emigration (Fig. 2, A–D). Chronic famotidine treatment (30 mg/kg/day for 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 hemodynamic 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 for 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 versus 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 3-fold increase with respect to the control level 5 h 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 3-day regime presented hypergastrinemia for the most part of the day previous to the experiment, because gastrin levels 8 h after the third dose of omeprazole were higher than those observed 5 h after a single dose, and levels remained elevated 12 h after dosing (Fig. 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 h 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) before each dose of antisecretory agent prevented the increases in leukocyte-endothelial cell interactions induced by these drugs in every treatment protocol assayed (Fig. 4, A–D).

![Fig. 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 h after drug/vehicle administration. * P < 0.05; ** P < 0.01; and *** P < 0.001 versus respective control (omeprazole vehicle, n = 5; omeprazole, n = 6; famotidine vehicle, n = 5; famotidine, n = 5).]
Flow cytometric detection of adhesion molecules in leukocytes from portal blood samples revealed that omeprazole treatment during 3 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 in mesenteric macrophages and PMNs, and lymphocytes in these blood samples showed reduced L-selectin and increased 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 (Fig. 6, A and B). As occurred in the 3-day protocol, the expression of CD11a, CD11c, CD18, or CD49, nor did it affect the expression of the 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.

Discussion

The present study demonstrates that treatment with antisecretory drugs induces 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 that induce 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) (Dector et al., 1989); 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 nonsignificant increase in emigration, were observed in animals receiving this dose of omeprazole during 3 days or in rats treated during 14 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 14 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

**TABLE 1**

Gastrin concentration in plasma from portal blood

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vehicle</th>
<th>Omepr 40 mg/kg</th>
<th>Famotidine, 30 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 5 h</td>
<td>208 ± 19</td>
<td>651 ± 99 a</td>
<td>525 ± 82 a</td>
</tr>
<tr>
<td>B 3 days</td>
<td>184 ± 20</td>
<td>178 ± 34</td>
<td></td>
</tr>
<tr>
<td>C 14 days</td>
<td>224 ± 27</td>
<td>409 ± 30 a</td>
<td>339 ± 49 a</td>
</tr>
</tbody>
</table>

a P < 0.001 vs. value in respective vehicle-treated rats.

![Fig. 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, 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 h after the last drug/vehicle administration. **, P < 0.05; ***, P < 0.01; and ****, P < 0.001 versus 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; and famotidine 30 mg/kg 14 days, n = 5).](image)

![Fig. 3. Gastrin concentration in plasma from portal blood. Results correspond to picograms per milliliter. Blood samples were taken from rats 8, 12 and 24 h after the last dose of a 3-day treatment with omeprazole (40 mg/kg/day p.o.) or its vehicle. P < 0.001 versus value in respective vehicle-treated rats.](image)
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 relation-ship between the grade of the inflammatory changes and the alterations in gastrin levels. In the 3-day protocol, gastrinemia was normal 24 h after the last administration. Previous studies showed that gastrin release after a single dose of omeprazole peaks at 5 h and reverts to control levels 12 h after dosing (Decktor et al., 1989). However, in animals treated during 3 days, we detected a 5.4-fold increase over control values 12 h 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 24 h would be the remnants of the inflammation evoked initially by gastrin and prevented by proglumide. Rats receiving this dose of omeprazole or famotidine during 14 days develop

**TABLE 2**

Effect of treatment with omeprazole during 3 days on the expression of leukocyte adhesion molecules

<table>
<thead>
<tr>
<th></th>
<th>CD18</th>
<th>CD11a</th>
<th>CD11c</th>
<th>CD49</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lymph</td>
<td>Mon</td>
<td>PMNs</td>
<td>Lymph</td>
</tr>
<tr>
<td>Control</td>
<td>18.7 ± 1.6</td>
<td>20.6 ± 2.6</td>
<td>16.6 ± 2.7</td>
<td>9.5 ± 0.6</td>
</tr>
<tr>
<td>Ome</td>
<td>18.2 ± 1.3</td>
<td>18.7 ± 1.3</td>
<td>17.5 ± 1.6</td>
<td>9.4 ± 0.4</td>
</tr>
<tr>
<td>Progl + Ome</td>
<td>18.7 ± 2.2</td>
<td>20.9 ± 2.8</td>
<td>16.8 ± 1.7</td>
<td>9.5 ± 0.4</td>
</tr>
</tbody>
</table>

**Fig. 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 1) a single dose of omeprazole (40 mg/kg p.o.); 2) famotidine (30 mg/kg p.o.); 3) omeprazole (40 mg/kg p.o.); or its vehicle during three consecutive days (C); 3) omeprazole (10 mg/kg p.o.) during 14 consecutive days (E); and 4) omeprazole (40 mg/kg p.o.) during 14 consecutive days (F). Some animals were treated with proglumide (30 mg/kg i.p.) 30 min before each dose of anti-secretory drug. *, *P < 0.05; **, **P < 0.01; and ***, ***, **P < 0.001 versus respective proglumide treated group (n ≥5 in each group).

**Fig. 5.** Expression of L-selectin (A) and CD11b/CD18 (B) in leukocytes from animals treated with omeprazole (40 mg/kg p.o.) or its vehicle during three 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 h after the last drug/vehicle administration, and the expression of adhesion molecules was analyzed by flow cytometry. *, *P < 0.05 versus respective control; **, **P < 0.05 versus respective omeprazole-treated group.
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 they 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, because 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, whereas adhesion and migration require leukocyte integrins (Liu et al., 2004). Treatment with omeprazole for 3 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 14 days, PMNs seemed to be in a resting state, whereas monocytes and lymphocytes expressed higher levels of CD11b/CD18 and reduced L-selectin. The increased integrin expression in PMNs after 3 days of treatment points 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 cotreated with omeprazole and proglumide did not show any alteration in the expression of these adhesion molecules, which points to gastrin as the etiologic factor for these differences. However, the sequential changes in the cell type affected argue against a direct molecular effect of gastrin. Rather, they 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

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**TABLE 3**

Effect of chronic omeprazole treatment (14 days) on the expression of leukocyte adhesion molecules

<table>
<thead>
<tr>
<th>CD18</th>
<th>CD11a</th>
<th>CD11c</th>
<th>CD49</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lymph</td>
<td>Mon</td>
<td>PMNs</td>
</tr>
<tr>
<td>Control</td>
<td>15.7 ± 1.8</td>
<td>12.4 ± 0.9</td>
<td>15.2 ± 2.7</td>
</tr>
<tr>
<td>Ome</td>
<td>14.7 ± 1.4</td>
<td>13.9 ± 2.0</td>
<td>14.2 ± 2.8</td>
</tr>
<tr>
<td>Progl + ome</td>
<td>15.7 ± 1.2</td>
<td>12.9 ± 1.3</td>
<td>14.0 ± 1.6</td>
</tr>
</tbody>
</table>

**Fig. 6.** Expression of L-selectin (A) and CD11b/CD18 (B) 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 h after the last drug/vehicle administration, and the expression of adhesion molecules was analyzed by flow cytometry. *P < 0.05 versus respective control; +, P < 0.05 versus respective omeprazole-treated group.

**Fig. 7.** CD11b/CD18 expression in leukocytes from untreated animals incubated in vitro with different concentrations of gastrin (10<sup>-11</sup>–10<sup>-9</sup> M) or positive controls (fMLP at 10<sup>-7</sup> M; PMA at 10<sup>-7</sup> 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.
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 H₂ receptor activation (Asakura et al., 1994), but later studies have shown that the other receptor types (H₃–₄) 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). Conversely, 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 antiseptic drugs induces an aggravating effect on H₂-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 gastroesophageal reflux disease. The few studies analyzing the effects of anti-H₂ agents showed analogous changes albeit of lower intensity (Meining et al., 1997, 1998). This indicates that this adverse event is not related to the mechanism of action of these drugs but to 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 proinflammatory effect derived from hypergastrinemia. The presently reported inflammatory events contrast with the significant efficacy of antiseptic drugs to reduce gas-

troesophageal reflux disease 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 overgrowth 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 to define the relevance of this effect of gastrin in patients suffering hypergastrinemia as a consequence of H. pylori infection, antisecretory treatments or both.

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