Efficacy of an inhibitor of adhesion molecule expression (GI270384X) in the treatment of experimental colitis

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Departments of 1Gastroenterology and 2Pathology, Hospital Clínic Barcelona, CIBER-EHD, and 3Institut d’Investigacions Biomèdiques de Barcelona, Consejo Superior de Investigaciones Científicas, Barcelona, Spain; and 4Neurology and Gastrointestinal Centre of Excellence for Drug Discovery, GlaxoSmithKline R&D Ltd., Harlow, United Kingdom

Submitted 1 February 2007; accepted in final form 25 July 2007

Panés J, Aceituno M, Gil F, Miquel R, Piqué JM, Salas A, McLean P. Efficacy of an inhibitor of adhesion molecule expression (GI270384X) in the treatment of experimental colitis. Am J Physiol Gastrointest Liver Physiol 293: G739–G748, 2007. First published July 26, 2007; doi:10.1152/ajpgi.00059.2007.—Modulation of adhesion molecule expression or function is regarded as a promising therapy for inflammatory conditions. This study evaluates the effects of an inhibitor of adhesion molecule expression (GI270384X) in two experimental models of colitis. Colitis of different severity was induced in C57BL/6J mice by administering 1, 2, or 3% dextran sulfate sodium (DSS). GI270384X (3, 10, or 25 mg·kg−1·day−1) was administered as pretreatment or started 3 days after colitis induction. In IL-10-deficient mice, the highest dose was given for 2 wk. The clinical course of colitis, pathological changes, serum inflammatory biomarkers, expression of adhesion molecules, and leukocyte-endothelial cell interactions in colonic venules were measured in mice treated with vehicle or with active drug. In the most severe forms of colitis (2% and 3% DSS and IL-10-deficient mice), the magnitude of colonic inflammation was not modified by treatment with GI270384X. In a less severe form of colitis (1% DSS), GI270384X treatment dose dependently ameliorated the clinical signs of colitis, colonic pathological changes, and serum levels of biomarkers (IL-6 and serum amyloid A). Administration of 25 mg·kg−1·day−1 GI270384X abrogated upregulation of ICAM-1 in the inflamed colon but had no effect on VCAM-1 or E-selectin expression. This was associated with a significant reduction in number of rolling and firmly adherent leukocytes in colonic venules. These results indicate that GI270384X is effective in the treatment of experimental colitis of moderate severity. Reduced adhesion molecule expression and leukocyte recruitment to the inflamed intestine contribute to this beneficial effect.

endothelium; ICAM-1; leukocyte

ONE OF THE KEY ASPECTS IN the pathogenesis of inflammatory bowel diseases (IBD) that contributes to perpetuate and amplify the inflammatory damage is the recruitment of inflammatory cells into the inflamed intestine. The process of leukocyte emigration involves several steps. Leukocytes initially roll along the vessel wall and then become activated by local factors generated by the endothelium, leading to their firm adhesion, and finally transmigrate across the endothelium. Initial rolling interactions are mediated by selectins and their ligands, whereas firm adhesion of circulating leukocytes to intestinal endothelium depends on the coordinated expression of adhesion molecules on endothelial cells, including vascular cell adhesion molecule-1 (VCAM-1), mucosal adressin cell adhesion molecule-1, and intercellular adhesion molecule-1 (ICAM-1), along with their respective integrin counterreceptors expressed on the surface of circulating leukocytes (24). The fundamental importance of increased cell adhesion molecule expression in IBD is supported by reports demonstrating that leukocyte recruitment and mucosal damage in experimental IBD are blocked by immunoneutralization of these adhesion molecules (11, 30, 34). It has been recently shown that immunoneutralization of α4-integrins, the receptors for VCAM-1 and mucosal adressin cell adhesion molecule-1, is effective in the treatment of human Crohn disease (19), and a more selective anti-α4β7-antibody is effective for induction of remission in active ulcerative colitis (8).

So far, blockade of adhesion molecule function has been based on administration of blocking antibodies. Although effective, this strategy is associated with significant adverse events related to the immunogenicity of the injected protein, including anaphylactic reactions and loss of response (28). Therefore, modulation of the process of leukocyte recruitment by small molecules that interfere with adhesion molecule expression as opposed to the use of monoclonal antibodies (MAbs) that block adhesion molecule function seems a promising alternative therapeutic approach. GI270384X is a methyl-capped polyethylene glycol derivative of (E)-4-1,3-bis-(cyclohexylmethyl)-1,2,3,6-tetrahydro-2,6-dioxo-9H-purin-8-yl, a novel and potent inhibitor of cytokine-induced ICAM-1 and E-selectin expression that inhibits TNF-induced neutrophil adhesion to human umbilical vein endothelial cells with an IC50 of 30 nM (14).

The overall objective of this study was to assess the potential efficacy of GI270384X in experimental colitis and determine its molecular and cellular mechanisms of action. The particular objectives included 1) to determine the effects of GI270384X on the course of colitis in two different experimental models and the potential relationship of this effect with colitis severity, 2) to assess the in vivo effects of the drug on expression of various endothelial adhesion molecules, and 3) to determine whether potential changes in adhesion molecule expression influence the process of leukocyte recruitment into the inflamed intestine.

MATERIALS AND METHODS

Animal Models of Colitis

Dextran sulfate sodium-induced colitis. Male C57BL/6J mice weighing 28–30 g were maintained under conventional housing conditions.

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Experimental colitis was induced in mice by giving 1, 2, or 3% (wt/vol) dextran sulfate sodium (DSS; 40 kDa; ICN Biomedicals) in drinking water ad libitum during the 8 days of the experimental period. Control animals received drinking water without DSS. The volume of drinking water was monitored in control and colitic mice in all treatment groups. Body weight, stool consistency (score: 0, normal stools; 1, soft stools; and 2, liquid stools), and rectal bleeding [score: 0, fecal occult blood test negative (HemoFEC; Boehringer Mannheim, Barcelona, Spain); 1, fecal occult blood test positive; and 2, visible rectal bleeding] were assessed daily. The disease activity index (DAI) was determined as a combination of the above-mentioned parameters according to the scoring criteria previously described (21).

Mice were killed on day 8 after the onset of colitis induction by an overdose of subcutaneous ketamine (Ketolar; Parke-Davies, Morris Plains, NJ) and xylazine (Sigma, St. Louis, MO). The colon was weighed, and its length was measured after exclusion of the cecum.

The volume of drinking water was monitored in control and colitic mice in all treatment groups. Animals were studied between 80 and 120 days of age. At weaning, mice were transferred to housing with conventional conditions.

### Assessment of Endothelial Adhesion Molecule Expression In Vivo

Mabs. The Mabs used to quantify endothelial expression were YN1/1.7.4, a rat IgG2a directed against mouse ICAM-1 (16); MK1.91, a rat IgG1 directed against mouse VCAM-1 (10); RME-1, a murine IgG directed against mouse E-selectin (38); and UPC-10, a nonbinding IgG (4). The binding Mabs directed against ICAM-1, VCAM-1, and E-selectin were labeled with 125I, whereas the nonbinding Mab UPC-10 was labeled with 131I (Amersham Iberica, Madrid, Spain). Radioiodination of the Mabs was performed by the iodogen method as previously described (9). Labeled Mabs were stored at 4°C and used within 3 wk after the labeling procedure. The specific activity of labeled Mabs is 20-0.5 mCi/ng.

**Endothelial expression of ICAM-1, VCAM-1, and E-selectin.** Animals were anesthetized with ketamine (150 mg/kg body wt sc) and xylazine (7.5 mg/kg body wt sc), and the left carotid artery and the left jugular vein were cannulated with PE-10 tubing (Portex, Hythe, UK). For assessment of endothelial expression of ICAM-1, VCAM-1, and E-selectin, a mixture of 10 μg of 125I-labeled YN1/1.7.4 and 40 μg of unlabeled YN1/1.7.4, 10 μg 125I-labeled MK1.91 and 20 μg of unlabeled MK1.91, and 10 μg of 125I-labeled RME-1 without additional unlabeled RME-1, were administered, respectively. In all cases 10 μg of 131I-labeled UPC-10 were added to the injection mixture. Doses of anti-ICAM-1, anti-VCAM-1, and anti-E-selectin Mabs proved to be saturating in previous assays (12, 17, 25, 26). The mixture of binding and nonbinding Mabs was administered through the jugular vein catheter. Blood samples were obtained through the carotid artery catheter 5 min after injection of the Mab mixture. Thereafter, the animals were heparinized (1 mg/kg iv sodium heparin) and rapidly exsanguinated. Entire organs were then harvested and weighed.

**Measurement of Myeloperoxidase Activity**

Distal colon samples (~20 mg) were snap-frozen in liquid nitrogen and stored at −80°C for later assay of myeloperoxidase (MPO) activity. MPO activity was photometrically determined by employing 3,3′,5,5′-tetramethylbenzidine as a substrate (36). Samples were homogenized with 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer (pH 6.0). Homogenates were then disrupted for 30 s by using a Labsonic sonicator (B. Braun, Melsungen, Germany) and subsequently snap-frozen in dry ice and thawed on three consecutive occasions before a final 30-s sonication. Samples were incubated at 60°C for 2 h and then spun down at 4,000 g for 12 min. Supernatants were collected for MPO assay. Enzyme activity is assessed photometrically at 630 nm. The assay mixture consists of 20 μl of supernatant, 10 μl of tetramethylbenzidine (final concentration of 1.6 mM) dissolved in DMSO, and 70 μl of H2O2 (final concentration of 3.0 mM) diluted in 80 mM phosphate buffer, pH 5.4. An enzyme unit is defined as the amount of enzyme that produces an increase of 1 absorbance unit per minute.

**Biomarkers of Colitis Severity**

The day of study termination and after induction of anesthesia, 2 ml of blood were obtained by cardiac puncture under aspetic conditions and spun at 1,500 g for 10 min at 4°C; the supernatant serum was frozen at −80°C for later assay of serum amyloid A (SAA) protein with a quantitative colorimetric commercial kit (Biosource International, Nivelles, Belgium) and IL-6 with a commercially available ELISA kit (Dialclone, Besançon, France).

### In Vivo Assessment of Leukocyte-Endothelial Cell Interactions in Colonic Venules

Leukocyte-endothelial cell interactions in colonic submucosal and lamina propria venules were assessed by intravital microscopy. Mice were anesthetized with ketamine (150 mg/kg sc) and xylazine (7.5 mg/kg sc), and the left jugular vein was cannulated. Throughout the experiments, rectal temperature was monitored with an electrothermometer and maintained between 36.5 and 37.5°C with an infrared heat lamp. The abdomen was opened via a midline incision, and...
a segment of the distal colon was chosen for microscopy examination, exteriorized, and covered with a cotton gauze soaked with bicarbonate buffer. Mice were then placed on an adjustable microscope stage, and the colon was extended over a nonautofluorescent cover slip that allows observation of a 2-cm² segment of tissue. An inverted microscope (Diaphot 300; Nikon, Tokyo, Japan) with a CF Fluor ×40 objective lens (Nikon) was used. A charge-coupled device camera (model XC-77; Hamamatsu Photonics, Hamamatsu, Japan) with a C2400 charge-coupled device camera control unit and a C2400-68 intensifier head (Hamamatsu Photonics), mounted on the microscope, projected the image onto a monitor (Trinitron XK-14CP1; Sony, Tokyo, Japan), and the images were recorded with a video cassette recorder (SR-S368E; JVC, Tokyo, Japan) for off-line analysis. A video date-time generator (Panasonic digital AV mixer WJ-AVE55; Matsushita Communication Industrial, Tokyo, Japan) displayed these parameters on recorded and live images. Leukocytes were in vivo labeled by intravenous injection of rhodamine-6G (Molecular Probes, Matsushita Communication Industrial, Tokyo, Japan) displayed these submucosal and lamina propria venules with internal diameters ranging between 25 and 40 μm were selected for observation. Venular internal diameter was measured on-line by a video caliper (Microcirculation Research Institute, Texas A&M University, College Station, TX). The flux of rolling leukocytes, leukocyte rolling velocity, number of adherent leukocytes, venular blood flow, and venular wall shear rate (γ) were determined off-line after playback of videotapes. Rolling leukocytes are defined as those white blood cells that move at a velocity less than that of free-flowing leukocytes in the same vessel. The flux of rolling leukocytes was measured as the number of rolling leukocytes that pass a fixed point within a small (10 μm) viewing area of the vessel in a 1-min period. Leukocyte rolling velocity was calculated as the mean of 10 rolling leukocyte velocities (expressed in μm/s). Leukocytes were considered adherent to venular endothelium when stationary for 30 s or longer and expressed as number per 100-μm length of venule. Venular blood flow was estimated from the mean of the velocity of three free-flowing leukocytes (Idv), using the empirical relationship of venular blood flow = Ivν/1.6 (7). Venular wall shear was calculated, assuming cylindrical geometry, using the newtonian definition γ = 8 (Vbf/internal diameter) (18). In each animal, three to six random venules were examined, and results were calculated as the mean of each parameter in all venules examined.

Treatment Groups

Study 1. Study 1 was concerned with GI270384X in the treatment of DSS-induced colitis of diverse severity.

Severe colitis was induced in different groups of mice by administering 3% DSS continuously in drinking water. To assess the effects of treatment with GI270384X on clinical, biochemical, and histological parameters of colitis, four groups of mice (n = 8 per group) were studied: one control group (no colitis), one group of colitic mice treated with vehicle, one group of colitic mice treated with 25 mg·kg⁻¹·day⁻¹ per os (po) of GI270384X, administered by gavage, starting before the induction of colitis and maintained during the 8 days of the study (preventive), and one group of colitic mice treated with 25 mg·kg⁻¹·day⁻¹ po GI270384X, starting at day 3 after the beginning of colitis induction and during the following 5 days, until termination of the study at day 8 (therapeutic).

In the IL-10⁻/⁻ model of colitis, three groups of animals were studied: 1) wild type (n = 8), 2) IL-10⁻/⁻ mice treated with vehicle (1% methylcellulose) per os (n = 8), and 3) IL-10⁻/⁻ mice treated with 25 mg·kg⁻¹·day⁻¹ po GI270384X (n = 8). Only the dose of 25 mg·kg⁻¹·day⁻¹ GI270384X was studied because results from the first part of this study (DSS model) showed consistent beneficial effect of this dose, whereas the dose of 10 mg·kg⁻¹·day⁻¹ achieved only partial amelioration of colitis severity.

Study 2: dose-response effect. After we observed a beneficial effect of treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X in 1% DSS-induced colitis, the efficacy of lower doses of the drug (3 and 10 mg·kg⁻¹·day⁻¹ po) was assessed in additional groups of mice, determining clinical, biochemical, and pathological parameters of colitis severity in all groups (n = 9 or 10 per group).

Study 3: adhesion molecule expression. Adhesion molecule expression studies (radioiodinated antibody technique) were carried out in groups of control noncolitic animals and in mice with colitis induced by 1, 2, or 3% DSS, treated with vehicle or 25 mg·kg⁻¹·day⁻¹ GI270384X from day 3 up to the end of the study at day 8 (n = 8 per group), as well as in IL-10⁻/⁻ mice treated with vehicle or 25 mg·kg⁻¹·day⁻¹ GI270384X during the 2-wk period (n = 7 or 8 per group). Because adhesion molecule expression was quantified by the radioiodinated antibody method, different groups of animals were used for determination of ICAM-1, VCAM-1, and E-selectin expression in the DSS model.

Study 4: leukocyte recruitment. Similar to adhesion molecule expression experiments, intravital microscopy studies were carried out in groups of control noncolitic animals and in mice with colitis induced by 1% DSS, treated with vehicle or 25 mg·kg⁻¹·day⁻¹ GI270384X from day 3 up to the end of the study at day 8 (n = 7–10 per group).

Statistical Analyses

Data were analyzed by standard statistical methods, i.e., ANOVA with the Bonferroni (post hoc) test and Student’s paired or unpaired t-test, when appropriate. All values are expressed as means ± SE. Statistical significance was set at P < 0.05.

RESULTS

Study 1: Effects of GI270384X on Colitis and Relationship With Severity

Administration of 1, 2, or 3% DSS in drinking water resulted in induction of colitis of increasing severity with a corresponding reduction of body weight at day 8 of −10.1 ± 1.4%, −19.9 ± 2.6%, and −27.7 ± 1.9% and DAI scores of 2.84 ± 0.38, 3.81 ± 0.13, and 4.0 ± 0.0, respectively.

Development of colitis in response to DSS administration was also associated with an increase in colon weight, a shortening of the colon length, and an increase in colon weight-to-length ratio (Table 1). In the most severe form of colitis induced by 3% DSS, administration of 25 mg·kg⁻¹·day⁻¹ GI270384X starting at day 3 after the beginning of colitis induction or as pretreatment starting 1 day before induction of colitis did not modify the changes in body weight (Fig. 1A), changes in DAI score (Fig. 1B), or pathological changes in the colon (Table 1) relative to the vehicle-treated group.
In moderately severe colitis induced by 2% DSS, treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X from days 3 to 8 after the induction of colitis also did not significantly affect the clinical course of colitis. Compared with that shown in vehicle-treated animals, the loss in body weight (−19.9 ± 2.6% vs. −21.6 ± 1.8%) and the increase of the DAI score (3.81 ± 0.13 vs. 3.94 ± 0.06) at day 8 were not significantly modified. Inflammatory changes in the colon at study termination were also similar in vehicle and GI270384X-treated animals in this colitic group (Table 1).

In a less severe form of colitis induced by 1% DSS, a significant beneficial effect of treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X from day 3 until study completion at day 8 was observed. At the end of the study, reduction in body weight normally associated with development of colitis was significantly attenuated by treatment with GI270384X (Fig. 2A), and a significant reduction in the DAI score was also observed (Fig. 2B). Colonic pathological changes associated with the development of colitis, particularly shortening of colon length, and the increase in the weight-to-length ratio of the colon in DSS-induced colitis of different severity in animals treated with vehicle or GI270384X 25 mg·kg⁻¹·day⁻¹ from day 3 to 8 after colitis induction or as pretreatment

### Table 1. Pathological changes of the colon in DSS-induced colitis of different severity in animals treated with vehicle or GI270384X 25 mg·kg⁻¹·day⁻¹ from day 3 to 8 after colitis induction or as pretreatment

<table>
<thead>
<tr>
<th></th>
<th>Control (0% DSS)</th>
<th>Vehicle</th>
<th>GI270384X (pretreatment)</th>
<th>Vehicle</th>
<th>GI270384X</th>
<th>Vehicle</th>
<th>GI270384X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon weight, mg</td>
<td>163±11</td>
<td>186±10</td>
<td>214±10</td>
<td>166±7</td>
<td>220±9</td>
<td>211±12</td>
<td>222±8</td>
</tr>
<tr>
<td>Colon length, mm</td>
<td>77.3±2.7</td>
<td>56.4±2.5</td>
<td>63.6±1.9</td>
<td>55.4±5.5</td>
<td>61.2±2.8</td>
<td>63.6±2.3</td>
<td>66.5±3.0</td>
</tr>
<tr>
<td>Colon weight-to-length ratio</td>
<td>2.11±0.43</td>
<td>3.31±0.17</td>
<td>3.36±0.12</td>
<td>3.07±0.19</td>
<td>3.66±0.27</td>
<td>3.33±0.17</td>
<td>3.35±0.16</td>
</tr>
</tbody>
</table>

Values are means ± SE. DSS, dextran sulfate sodium. *P < 0.05 relative to corresponding vehicle treated colitic group and not significantly different relative to control group. The rest of values are significantly different from the control noncolitic group, except colon weight in GI270384X pretreatment group.
colon were significantly attenuated by treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X (Table 1). This was accompanied by amelioration of histological inflammatory changes (Fig. 3) with a significant reduction in histological damage scores in the active treatment group (34.5 ± 3.5 vs. 22.7 ± 2.7; P < 0.01).

The beneficial effects of GI270384X in the DSS model of colitis were not reproduced in IL-10⁻/⁻ colitic mice. Compared with wild-type mice, IL-10⁻/⁻ mice had overt clinical signs of ileocolitis at the time of the study between 16 and 18 wk of age. Body weight tended to be lower in IL-10⁻/⁻ mice (23.8 ± 0.7) than in wild-type mice (25.7 ± 0.1), although this difference at week 16 did not reach statistical significance. Only minor changes in body weight were observed during the 2-wk study period, and these were similar in vehicle (-0.21 ± 1.15 g) and GI270384X-treated mice (-1.04 ± 1.54 g).

Blood in stool was detected daily in the majority of IL-10⁻/⁻ mice during the 2-wk study. When the intensity of diarrhea was scored as 0 (normal stools), 1 (soft stools), and 2 (liquid stools), the diarrhea score at the end of the study period was similar in vehicle (1.23 ± 0.16) and GI270384X (1.37 ± 0.18)-treated animals. At the beginning of the study period, both groups of IL-10⁻/⁻ mice had a similar DAI score: vehicle was 3.01 ± 0.91 and GI270384X was 3.25 ± 1.11. At the end of treatment, DAI score was also similar in vehicle-treated (3.50 ± 0.33) and GI270384X-treated (4.25 ± 0.49) groups of IL-10⁻/⁻ mice. DAI score was 0 in wild-type mice. Histological damage score analysis demonstrated a marked increase in histological inflammatory lesions in vehicle-treated IL-10⁻/⁻ mice (2.78 ± 0.25) compared with wild-type mice (1.36 ± 0.08), which were not modified by treatment with GI270384X (2.84 ± 0.27). Estimation of neutrophil infiltration of the colon measured as MPO activity was significantly increased in colon tissue samples of vehicle-treated IL-10⁻/⁻ mice (1.63 ± 0.04 U) compared with wild-type mice (0.07 ± 0.02 U). Treatment of IL-10⁻/⁻ mice with 25 mg·kg⁻¹·day⁻¹ GI270384X did not result in changes of MPO activity (1.79 ± 0.06).

Study 2: Dose-Response Study

After we observed a beneficial effect of treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X in 1% DSS-induced colitis, the efficacy of lower doses of the drug (3 and 10 mg·kg⁻¹·day⁻¹) was assessed in additional groups of mice.

As shown in Table 2, a clear-cut dose-response effect on clinical, pathological, and serological markers of colitis severity was observed across the range of doses tested. Although treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X was associated with a significant protection on all parameters tested, treatment with 10 mg·kg⁻¹·day⁻¹ afforded significant protection in body weight loss, DAI increase, and rectal bleeding scores at the end of the study. Colon shortening was also attenuated by treatment with 10 mg·kg⁻¹·day⁻¹ GI270384X but not colon weight or weight-to-length ratio. Treatment with 3 mg·kg⁻¹·day⁻¹ GI270384X did not afford significant amelioration of any of the parameters of colitis severity.

The elevated histological damage score associated with development of colitis was significantly reduced by treatment with 10 or 25 mg·kg⁻¹·day⁻¹ GI270384X but not by the dose of 3 mg·kg⁻¹·day⁻¹ (Fig. 4A). Levels of MPO activity, as a reflection of neutrophil infiltration, were significantly elevated in colitic animals treated with vehicle relative to the noncolitic group. Administration of 10 or 25 mg·kg⁻¹·day⁻¹ GI270384X but not of 3 mg·kg⁻¹·day⁻¹ completely prevented the increase in MPO activity in colonic tissue (Fig. 4B).

Levels of SAA and IL-6 were significantly increased in colitic animals compared with controls. As shown in Table 2,
only treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X significantly reduced circulating IL-6 levels, whereas treatment with either 10 or 25 mg·kg⁻¹·day⁻¹ GI270384X resulted in significant reductions in SAA levels.

**Study 3: Adhesion Molecule Expression**

Development of colitis by administration of 1% DSS in drinking water was associated with a significant increase in ICAM-1 expression in jejunum, ileum, cecum, and colon. Treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X from day 3 up to study completion at day 8 entirely abrogated ICAM-1 upregulation in all segments of small and large intestine (Fig. 5A).

Expression of VCAM-1 was also significantly increased in most splanchnic organs of animals with colitis induced by 1% DSS compared with controls. In contrast to the effects of
GI270384X on ICAM-1 upregulation, expression of VCAM-1 remained unmodified in animals treated with this drug (Fig. 5B).

In control animals, no expression of E-selectin was detected in any of the splanchnic organs. Induction of colitis by 1% DSS was associated to a relatively weak expression of E-selectin in cecum and colon, but expression levels of this adhesion molecule were not significantly modified by treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X (data not shown).

In more severe forms of colitis induced by 2% or 3% DSS, ICAM-1 upregulation was not affected by treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X either in the colon (with 2% DSS: vehicle 522 ± 27 ng Ab/g, GI270384X 419 ± 52 ng Ab/g, not significant; with 3% DSS: vehicle 767 ± 35 ng Ab/g, GI270384X 714 ± 39 ng Ab/g, not significant) or in the ileum (with 2% DSS: vehicle 614 ± 70 ng Ab/g, GI270384X 696 ± 94 ng Ab/g, not significant; with 3% DSS: vehicle 824 ± 107 ng Ab/g, GI270384X 783 ± 92 ng Ab/g, not significant). In other splanchnic organs including jejunum, stomach, pancreas, and mesentery, DSS-induced ICAM-1 upregulation was also not affected by GI270384X (data not shown).

Development of colitis in IL-10⁻/⁻ mice was associated with a significant increase in ICAM-1 expression in jejunum, cecum, colon, and mesentery (Table 3). A higher expression of ICAM-1 was also observed in the ileum of IL-10⁻/⁻ animals than in controls, although this difference did not reach statistical significance. Treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X during 2 wk marginally reduced ICAM-1 expression in these organs. Although significant differences between treated and untreated animals were not detected, the expression of ICAM-1 was reduced so that colitic animals had no longer a statistically significant increased expression of ICAM-1 relative to noncolitic littermate controls, expect for the cecum in which the group treated with GI270384X still had a significantly higher ICAM-1 expression than that shown in noncolitic controls (Table 3).

**Study 4: Leukocyte Recruitment**

Appearance of colonic inflammation was associated with a marked increase in leukocyte-endothelial cell interactions in colonic venules. As detailed in Table 4, mice with 1% DSS-induced colitis had a threefold increase in the flux of rolling leukocytes compared with control noncolitic animals. Rolling velocity was decreased (indicating a more stable tethering of rolling cells) in colitic animals, although this reduction did not reach statistical significance. The number of rolling leukocytes (cells rolling in a 100-μm segment of venule) was significantly increased in colitic mice. Treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X resulted in a significant reduction in the flux of rolling leukocytes and the number of rolling leukocytes in colonic venules of colitic mice. The most important determinant of leukocyte infiltration into an inflamed organ, i.e., the number of leukocytes firmly adhered to the venular wall, was markedly increased (20-fold) in colitic mice. Treatment with GI270384X significantly reduced the number of adherent cells relative to vehicle-treated colitic mice, although this number still remained significantly higher than that observed in control noncolitic animals (Fig. 6).

**DISCUSSION**

The results of the overall series of experiments performed in the model of DSS-induced colitis in mice indicate that treatment with GI270384X at the dose of 25 mg·kg⁻¹·day⁻¹ po does not have an effect on the clinical course or pathological changes in the most severe forms of colitis induced by 2% or 3% DSS, which are associated with a considerable loss in body weight (19.9 ± 2.6% and 27.7 ± 1.8%, respectively). By contrast, in a less severe form of colitis induced by 1% DSS that was associated with a 10.1 ± 1.4% loss in body weight, treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X had a beneficial effect on the course of colitis. This beneficial effect was corroborated in all four studies reported herein, which involved repeated series of experiments on a large number of animals. The loss in body weight and the increases in DAI, rectal bleeding score, and MPO activity, as well as the macroscopic and microscopic pathological changes in the colon, were all significantly attenuated by treatment with GI270384X. Furthermore, systemic biomarkers of inflammatory activity such as IL-6 and SAA concentration were also significantly reduced in response to treatment with GI270384X. This observation does not necessarily mean that GI270384X might only be effective in colitis of mild severity in humans. The colitis induced in mice by 2% or 3% DSS was extremely severe, and some mortality was observed. Human IBD is an exceptional cause of mortality, and a patient with active colitis with a 10% reduction in body weight over a period of 8 days, diarrhea, and macroscopic daily rectal bleeding, such as those seen in 1% DSS colitic mice, would be considered to have a moderate to severe flare of IBD. Another important consideration of the experimental design of the study is that GI270384X showed therapeutic efficacy in established DSS colitis. Demonstration of efficacy in established colitis, as opposed to a preventive effect, has a superior clinical relevance, since in the clinical setting we treat patients with active disease, and a significant proportion of treatments that are useful when used prophylactically has proven to be ineffective in established colitis.

The dose-response study showing a progressive increase in the number of parameters of colitis severity that were significantly reduced by increasing doses of the drug confirms the

**Table 3. ICAM-1 expression on mesenteric organs in wild-type and IL-10-deficient mice**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Wild-Type + Vehicle</th>
<th>IL-10-Deficient + Vehicle</th>
<th>IL-10-Deficient + GI270384X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>493 ± 40</td>
<td>704 ± 60*</td>
<td>639 ± 60</td>
</tr>
<tr>
<td>Cecum</td>
<td>310 ± 25</td>
<td>698 ± 76*</td>
<td>600 ± 63*</td>
</tr>
<tr>
<td>Ileum</td>
<td>418 ± 29</td>
<td>535 ± 73</td>
<td>528 ± 57</td>
</tr>
<tr>
<td>Jejunum</td>
<td>433 ± 54</td>
<td>630 ± 75*</td>
<td>529 ± 40</td>
</tr>
<tr>
<td>Stomach</td>
<td>308 ± 19</td>
<td>338 ± 39</td>
<td>349 ± 32</td>
</tr>
<tr>
<td>Mesentery</td>
<td>417 ± 27</td>
<td>665 ± 58*</td>
<td>547 ± 52</td>
</tr>
<tr>
<td>Pancreas</td>
<td>482 ± 33</td>
<td>569 ± 58</td>
<td>485 ± 32</td>
</tr>
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</table>

Values (expressed as ng antibody/g tissue) are means ± SE; n = no. of mice. Results show effects of treatment with GI270384X. *P < 0.05 vs. wild type.
The results of the overall series of experiments performed in IL-10−/− mice indicate that treatment with GI270384X is not effective in reducing intestinal inflammation in this model. No differences were observed in clinical parameters of colitis severity, macroscopic or microscopic inflammatory changes in the colon, and biochemical markers of colitis severity (MPO).

Treatment with GI270384X did not have a profound effect on ICAM-1 upregulation in colitic IL-10 knockout mice. In none of the organs studied was a significant reduction of ICAM-1 expression relative to vehicle-treated IL-10 KO mice observed, although a constant trend toward a reduction of ICAM-1 expression was noticeable, so that GI270384X-treated animals had no longer a statistically significant increased expression of ICAM-1 relative to noncolitic littermate controls in most of the segments of the intestine studied.

The results may appear to be at odds with those obtained in the initial part of this study in which a significant amelioration of DSS-induced colitis was observed in response to treatment with GI270384X, with a well-defined dose-response effect. The dose of the drug used in the experiments in IL-10−/− mice was 25 mg·kg⁻¹·day⁻¹, which uniformly ameliorated all parameters of colitis severity in the DSS-induced colitis model. The discrepancy of these observations may have several reasons. One of the factors that should be taken into account is that a beneficial effect of GI270384X in DSS-induced colitis was only observed in a model of moderate severity, i.e., that induced by 1% DSS, whereas in conditions of more severe colitis (induced by 2% or 3% DSS) the results of GI270384X treatment were negative. Another possible reason for the discrepancy between the DSS model and the IL-10−/− mice model, may be related to the ability of GI270384X to revert ICAM-1 upregulation in the colon of colitic animals. Whereas in 1% DSS-induced colitis treatment with GI270384X significantly reduced ICAM-1 expression in all segments of the gut studied, in IL-10−/− mice a nonsignificant reduction was observed. This differential response in modulation of ICAM-1 expression may be related to different pathophysiological mechanisms involved in the two models of colitis or may be because GI270384X can only effectively reduce ICAM-1 expression in the context of mild or moderate (not severe) disease. The latter possibility is supported by the results obtained in the DSS model of colitis of different severities.

The reduction in ICAM-1 expression in response to treatment with 25 mg·kg⁻¹·day⁻¹ GI270384X was associated with a significant reduction in leukocyte-endothelial cell interactions, as manifested by reduced rolling and firm adhesion of leukocytes in colonic venules.

The reduction in leukocyte rolling is probably not related to changes in ICAM-1 expression. Although recent evidence...
indicates that ICAM-1 may mediate rolling interactions in vitro (29), numerous experiments using anti-ICAM-1 antibodies in vivo have not observed an impact of ICAM-1 immunoblockade on rolling interactions (30, 34). Reduced rolling interactions do not seem to result from inhibition of E-selectin expression because expression was very low and not affected by GI270384X treatment. In that regard, it is interesting to note the effects of GI270384X reducing plasma SAA levels. Levels of SAA increase and correlate with disease activity in both animal models (33) and human disease (5) and has been used as a biomarker of disease severity. However, SAA may have a modulatory effect on the immune response. In that regard, it has been shown that SAA promotes leukocyte chemotaxis and cellular adhesion (1, 23, 39), activates proinflammatory gene expression in human and murine intestinal epithelial cells (13), and increases metalloprotease secretion in various cell types (20). Therefore, reduction of circulating SAA in response to treatment with GI270384X may have an additive effect to downregulation of ICAM-1 expression in reducing leukocyte recruitment into the inflamed intestine.

The reduction in leukocyte adhesion is most probably directly linked to the reduced ICAM-1 expression in the endothelium of the inflamed colon. This notion is in keeping with the observations of a study using ICAM-1-deficient mice showing an amelioration of intestinal inflammatory changes and reduced mortality in DSS-induced colitis (2) and with a study from our group demonstrating a significant reduction in leukocyte recruitment in response to ICAM-1 immunoneutralization in the trinitrobenzenesulfonic acid model of colitis in the rat (30). By contrast, another study from our group found no protection using anti-ICAM-1 blocking antibodies in the DSS model of colitis (34). However, in the latter study, a very severe form of colitis was induced by administration of 5% DSS during 5 days; according to the present results, ICAM-1 blockade may not be an effective form of treatment for extremely severe colitis. The clear relationship between efficacy of GI270384X treatment and the severity of colonic inflammation is an important piece of information that should be considered if treatment strategies based on modulation of ICAM-1 function are to be applied in the treatment of human IBD. So far, treatment of Crohn disease with an anti-ICAM-1 antisense oligonucleotide has produced inconsistent results. An initial study in patients with active Crohn disease (40) demonstrated an increase in the number of patients achieving remission and a reduction in steroid requirements in patients treated with active drug relative to those receiving placebo, but these results were not corroborated in a larger controlled study (32). More recently, a randomized, controlled, escalating dose study showed promising acute and long-term benefits of this drug in patients with mild to moderate descending ulcerative colitis (37).

In summary, the result of the present study show that GI270384X significantly reduces ICAM-1 expression in the inflamed colon to a level that results in diminished recruitment of inflammatory cells and amelioration of the inflammatory damage, although this beneficial effect is highly influenced by the severity of the underlying colitis. Therefore, small molecules such as GI270384X may represent an alternative to biologicals targeting adhesion molecules, with the potential advantage of low or absent immunogenicity.

GRANTS
This work was supported by Grant SAF 2005-03755 and SAF 2006-03074 from Ministerio de Educación y Ciencia, and a Grant from GlaxoSmithKline R&D Ltd. (Harlow, UK).

REFERENCES


