Peroxisome Proliferator-Activated Receptor \( \gamma \) Activation Alleviates Postoperative Ileus in Mice by Inhibition of Egr-1 Expression and Its Downstream Target Genes\(^S\)

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Received April 15, 2009; accepted August 4, 2009

ABSTRACT

Postoperative ileus, a major cause of morbidity after abdominal surgery, is characterized by intestinal dysmotility and a complex inflammatory cascade within the intestinal muscularis. Treatment with carbon monoxide (CO)—inhaled or intraperitoneal—has been shown to ameliorate bowel dysmotility caused by surgical manipulation of the gut in experimental animals. Recent evidence indicates that CO exerts its anti-inflammatory effects through the induction of peroxisome proliferator-activated receptor (PPAR)-\( \gamma \), a nuclear receptor whose activation has been linked to several physiological pathways, including those related to the regulation of intestinal inflammation. The purpose of this study was to evaluate pharmacological activation of PPAR\( \gamma \) in a murine model of postoperative ileus by use of the PPAR\( \gamma \) agonist rosiglitazone. Postoperative bowel dysmotility was induced by surgical manipulation of the colon. The functional severity of postoperative ileus was significantly ameliorated in mice pretreated with rosiglitazone (0.3 to 10 mg/kg i.p.); this was associated with a down-regulation of pro-inflammatory cytokines/chemokines, inducible nitric oxide synthase activity, cyclooxygenase-2 activity, as well as a decrease in leukocyte recruitment into the muscularis of both colon and jejunum. These anti-inflammatory effects were preceded by a PPAR\( \gamma \)-dependent down-regulation of early growth response (Egr)-1, a key regulator of inflammatory gene expression. In conclusion, these results indicate that rosiglitazone significantly attenuates postoperative ileus in mice by suppression of the muscularis inflammatory cascade through a PPAR\( \gamma \)-dependent down-regulation of Egr-1 and encourage the further clinical evaluation of synthetic PPAR\( \gamma \) agonists as pharmacological tool to prevent this postoperative event.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, belonging to the nuclear hormone receptor superfamily. The PPAR\( \gamma \) subtype is predominantly expressed in adipose tissue and colon and, to a lesser extent, in macrophages, kidney, liver, small intestine, and pancreas (Clark, 2002; Dubuquoy et al., 2006). Besides its well-known role in lipid and glucose metabolism, PPAR\( \gamma \) has also been demonstrated to play a pivotal role in the regulation of inflammatory/immune responses (Clark, 2002). In this context, it has been shown that synthetic PPAR\( \gamma \) agonists attenuate colonic damage in several models of colitis (Dubuquoy et al., 2006; Linard et al., 2008), whereas targeted deletion of PPAR\( \gamma \) in either intestinal epithelial cells (Adachi et al., 2006) or inflammatory cells (Shah et al., 2007) leads to further aggravation of dextran sulfate sodium-induced colitis. In an experimental model of intestinal ischemia/reperfusion (I/R) injury, a more severe injury was observed in PPAR\( \gamma \) \(-/-\) mice, whereas local and remote tissue injury was markedly attenuated in mice treated with a PPAR\( \gamma \) agonist. Thereby, it was demonstrated that PPAR\( \gamma \) ligands can inhibit the inflammatory response by decreasing interleukin (IL)-8, tumor necrosis factor-\( \alpha \), and inducible NO synthase (iNOS) expression (Nakajima et al., 2001).

Recent studies have demonstrated that a complex cascade of inflammatory responses occupies a key position in the functional severity of postoperative ileus.

ABBREVIATIONS: PPAR, peroxisome proliferator-activated receptor; BRL-49653, rosiglitazone; 5-[(4-(2-methyl-2-pyridinylamino)ethoxy)phenyl]-methyl-2,4-thiazolidinedione; CO, carbon monoxide; CM, colon manipulation; COX-2, cyclooxygenase-2; DMSO, dimethyl sulfoxide; Egr-1, early growth response-1; ELISA, enzyme-linked immunosorbent assay; FD70, fluorescein-labeled dextran, 70 kDa; GC, geometric center; GI, gastrointestinal; GDW662, 2-chloro-6-nitro-N-phenylbenzamide; HO, heme oxygenase; ICAM, intercellular adhesion molecule; IL, interleukin; NO, nitric oxide; iNOS, inducible NO synthase; I/R, ischemia/reperfusion; MCP, monocyte chemoattractant protein; MPO, myeloperoxidase; POI, postoperative ileus.
pathogenesis of postoperative ileus (POI; Bauer and Boeckxstaens, 2004). Activation of macrophages that reside in the intestinal muscularis has been implicated to play a critical role in the initiation of this postoperative muscle inflammation, resulting in the release of pro-inflammatory cytokines, chemokines, and adhesion molecules and the secretion of kinetically active substances such as NO (iNOS) and prostaglandins [cyclooxygenase (COX)-2; Kalff et al., 2000; Schwarz et al., 2001]. This local molecular inflammatory response is followed by a cellular inflammatory phase with the additional recruitment of circulating leukocytes and the subsequent further release of inflammatory mediators. In rodents, inactivation/depletion of the muscularis macrophage network (Wehner et al., 2007) as well as inhibition of leukocyte infiltration by intercellular adhesion molecule (ICAM)-1 blockade (The et al., 2005) has been reported to reduce surgically induced intestinal dysmotility, thereby underscoring the importance of intestinal inflammation in the pathogenesis of POI.

Previous studies have demonstrated that heme oxygenase (HO)-1 plays an important protective role in the pathogenesis of POI. Thereby, it was shown that treatment of mice with the HO-1 end product CO—either by inhalation or intraperitoneally—markedly reduces the development of POI (Moore et al., 2003; Nakao et al., 2006; De Backer et al., 2009). Recently, Bilban et al. (2006) reported that CO exerts its anti-inflammatory effects in macrophages through the induction of PPARγ. In accordance, Hoetzl et al. (2008) demonstrated that intraperitoneal administration of CO protect against ventilator-induced lung injury via activation of PPARγ and the subsequent inhibition of early growth response (Egr)-1. Taken together, we reasoned that PPARγ might be a therapeutic target for the prevention of POI.

In the current study, we demonstrate that intestinal motility is markedly impaired after surgical manipulation of the colon; however, treatment with rosiglitazone attenuates POI by suppression of the intestinal muscularis inflammatory cascade through a PPARγ-dependent down-regulation of Egr-1. These data suggest that PPARγ activation may be a new therapeutic strategy for the prevention of POI.

Materials and Methods

Animals. Male C57Bl6 mice (Janvier, Le Genest St-Ise, France), 6 to 12 weeks of age (20 to 25 g) were kept under environmentally controlled conditions (12h:12h light/darkness cycle; temperature 20 to 23°C; standard mouse chow and water ad libitum). All experiments were performed in accordance with European Union regulations for the handling and use of laboratory animals and approved by the Ethical Committee for Animal Experiments, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium.

Drug Injections. Rosiglitazone (BRL-49653) was obtained from Cayman Chemical (Ann Arbor, MI) and GW9662 was from Sigma-Aldrich (Steinheim, Germany). Rosiglitazone was dissolved in dimethyl sulfoxide (DMSO)/saline (1:10, 0.5 mg/ml) and injected intraperitoneally at concentrations of 0.3, 1, 3, or 10 mg/kg; DMSO/saline (1:10, total volume of 500 μl) was used as a control vehicle for rosiglitazone. GW9662 was dissolved in DMSO/saline (1:100, 0.5 mg/ml) and injected intraperitoneally (total volume, 100 μl) in a concentration of 1 mg/kg (see Study Protocol).

Surgical Procedure. Mice were anesthetized by inhalation of isoflurane (Porene; Abbott Labs, Louvain-la-Neuve, Belgium) at 5% induction and 2% maintenance dose. The surgical procedure was performed under aseptic conditions. Mice underwent midline laparotomy and colonic manipulation (CM), as described previously (Türler et al., 2002). In brief, the colon was exteriorized carefully and then compressed for 5 min by use of sterile moist cotton applicators. After repositioning of the bowel, the abdomen was closed using a two-layer continuous suture. Mice recovered from surgery in a temperature-controlled cage with free access to water, but not food.

Study Protocol. Mice were randomly assigned to eight experimental groups. Group I consisted of nontreated, nonoperated mice. Mice in group II underwent surgical manipulation of the colon (CM). Group III to VI consisted of manipulated mice pretreated with the PPARγ agonist rosiglitazone (0.3, 1, 3, or 10 mg/kg i.p.) at 1 h before surgery. Group VII consisted of manipulated mice pretreated with the PPARγ antagonist GW9662 (1 mg/kg i.p.) at 1 h 30 min before surgery. Group VIII consisted of manipulated mice treated with GW9662 (1 mg/kg i.p.) + rosiglitazone (3 mg/kg i.p.) at the same time points as mentioned above; the dose selection for rosiglitazone was based on results from our intestinal motility studies (see Fig. 1, A and B).

In an additional set of experiments, mice were divided into five experimental groups. Group IX consisted of nontreated, nonoperated mice. Group X consisted of nonoperated controls injected with rosiglitazone (3 mg/kg i.p.). Group XI consisted of manipulated mice without further intervention. Group XII consisted of manipulated mice injected with vehicle (500 μl of DMSO/saline, 1:10) at 1 h before surgery. Group XIII consisted of manipulated mice pretreated with rosiglitazone (3 mg/kg i.p.) at 1 h before surgery.

Animals were killed at 1, 6, or 24 h after the surgical procedure. The gastrointestinal (GI) tract was removed, opened along the mesenteric border, and washed with ice-cold Krebs-Henseleit buffer. The muscularis was isolated by scraping off the mucosa by use of a glass slide and stored at −80°C until further analysis.

Evaluation of Intestinal Motility. Intestinal transit and contractility were evaluated 24 h postoperatively by use of video fluorescence imaging (De Backer et al., 2008; see Supplemental Methods). Intestinal transit was evaluated by measuring the intestinal distribution of fluorescein-labeled dextran (70 kDa; FD70). Mice were gavaged with 200 μl of FD70 dissolved in H2O (25 mg/ml) at 22 h 30 min after surgery. Ninety minutes later, animals were killed by cervical dislocation, the GI tract was excised, and the fluorescent signal along the GI tract was measured by a CCD camera. The geometric center (GC) was calculated by the following formula: $\Sigma(%FD70 \text{ per segment}) \times \text{segment number} / 100$, as described previously (Moore et al., 2003). Immediately after, small intestinal contractility was evaluated by measuring the contraction amplitude of the spontaneous oscillatory contractions in the midjejunum. Therefore, the change in intestinal diameter (θ) within a 30-s period was calculated by the following equation: $[\theta_{\text{max}} - \theta_{\text{min}}] \times \theta_{\text{max}}$ and expressed as percentage of contraction amplitude (De Backer et al., 2008).

Enzyme-Linked Immunosorbent Assay. Intestinal muscularis samples—obtained at 1 h (only for HO-1) and 6 h after surgery—were homogenized in PBS and centrifuged at 10,000 g for 15 min at 4°C. Protein expression levels of IL-1β, IL-6, monocyte chemoattractant protein (MCP)-1 (Invitrogen, Merelbeke, Belgium), ICAM-1 (R&D Europe Ltd., Abingdon, Oxfordshire, UK) and HO-1 (Takara, Kyoto, Japan) were determined in the resulting supernatants by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's protocols.

Immunoblotting. Standard Western blotting techniques were used, as described previously (Moore et al., 2003). Nuclear and cytoplasmic extracts were prepared from intestinal muscularis samples—obtained at 1 and 6 h after surgery—using the NE-PER Extraction kit (Pierce Biotech, Aalst, Belgium). Equal amounts of protein (10 μg) were loaded onto NuPAGE-Novel 4 to 12% Bis-Tris electrophoresis gels (Invitrogen) and blotted onto nitrocellulose membranes (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Membranes were blocked in Tris-buffered saline/0.1% Tween 20 containing 5% nonfat dry milk and incubated overnight with...
appropriate antibodies for the detection of Egr-1 (1:200) and β-actin (1:500; Santa Cruz Biotechnology, Santa Cruz, CA). Horseradish peroxidase-linked secondary antibodies (Cell Signaling Technology Inc., Danvers, MA) were visualized by use of SuperSignal West Femto Substrate (Pierce Biotech).

Leukocyte Infiltration. Polymorphonuclear leukocyte infiltration was assessed by measuring myeloperoxidase (MPO) activity in ileal segments at 24 h after surgery, according to a previously described protocol (De Backer et al., 2009; see Supplemental Methods). In addition, MPO-positive cells were detected in whole mounts of ileal muscularis tissue (2 to 4 cm distal from the cecum) by use of a mixture of Hanker-Yates reagent (Polysciences, Warrington, PA), Krebs-Ringer buffer, and 3% hydrogen peroxide (Sigma-Aldrich) for 10 min (Moore et al., 2003).

Measurement of iNOS/COX-2 Enzyme Activity. Inducible NO synthase enzyme activity in intestinal muscularis samples—obtained at 6 h after surgery—was assayed by measuring myeloperoxidase (MPO) activity in ileal segments at 24 h after surgery, according to a previously described protocol (De Backer et al., 2009; see Supplemental Methods). In addition, MPO-positive cells were detected in whole mounts of ileal muscularis tissue (2 to 4 cm distal from the cecum) by use of a mixture of Hanker-Yates reagent (Polysciences, Warrington, PA), Krebs-Ringer buffer, and 3% hydrogen peroxide (Sigma-Aldrich) for 10 min (Moore et al., 2003).

Measurement of Egr-1 DNA-Binding Activity. Binding of Egr-1 to the Egr-1 consensus binding-sequence 5’-GATC-CAGCGGGGGCCAGCGGGGGCG-3’ was measured by ELISA-based assay (Panomics, Milan, Italy) with nuclear extracts prepared from intestinal muscularis samples, obtained at 1 and 6 h after surgery. These assays are performed in 96-well plates coated with an oligonucleotide containing the consensus binding sequence. The active form in nuclear extracts is detected by use of antibodies specific for an epitope that is accessible only when the appropriate subunit is activated and bound to its target DNA.

Statistics. Data are expressed as mean ± S.E.M., unless stated otherwise. Statistical analysis was performed by use of analysis of variance followed by Bonferroni’s multiple comparison test. P values of <0.05 were considered statistically significant.

Results

Rosiglitazone Attenuates Surgically Induced Intestinal Dysmotility. The primary efficacy endpoint of this study was restoration of intestinal transit after colonic manipulation; improvement of surgically induced intestinal contractile dysfunction was the secondary endpoint, as measured at 24 h postoperatively.

Intestinal transit was evaluated by measuring the intestinal distribution of fluorescein-labeled dextran (FD70) at 90 min after oral gavage. In control mice, the nonabsorbable FD70 was primarily localized in the distal segments of the small intestine (GC = 9.1 ± 0.3). In nontreated manipulated mice, we visualized the highest fluorescence intensity in the more proximal segments of the intestinal tract (GC = 5.8 ± 0.2), a finding that is consistent with the presence of ileus. Pretreatment of mice with rosiglitazone attenuated this sur-
gically induced delay in intestinal transit in a dose-dependent manner (GC = 7.9 ± 0.3 at a dosage of 3 mg/kg); however, rosiglitazone treatment did not result in a complete restoration of intestinal transit compared with nonoperated mice (p < 0.05 for CM versus control; Fig. 1A).

In parallel with the study above, we also evaluated intestinal contractile activity in the midjejunum by use of spatio-temporal motility mapping. As indicated in Fig. 1B, the small intestine showed vigorous contractile activity in control mice resulting in a mean contraction amplitude (Amp-P50mean) of 23.1 ± 3%. As reported previously, surgical manipulation of the colon caused a marked suppression of small intestinal contractility (Amp-P50mean of 9.3 ± 1.8%); however, this impairment of contractile activity was dose-dependently ameliorated by pretreatment with rosiglitazone (Amp-P50mean of 18.4 ± 2.5% at a dosage of 3 mg/kg; Fig. 1B). Based on the results above, we selected a dosage of 3 mg/kg rosiglitazone for further investigation of the molecular mechanisms implicated in its protective effect against POI.

Administration of vehicle (DMSO/saline) had no effect on the surgically induced delay in intestinal transit (GC = 5.5 ± 0.4; Fig. 1C) nor did it alter intestinal contractility after colonic manipulation (Amp-P50mean of 8.9 ± 2.6%; Fig. 1D).

Rosiglitazone Reduces CM-Induced Inflammatory Responses. Surgical manipulation of the colon initiates a complex inflammatory cascade within the colonic muscularis, which is characterized by the release of inflammatory mediators and the recruitment of leukocytes (Türler et al., 2002). Recently, it was shown that selective colonic manipulation also initiates a distant inflammatory response in the small intestinal muscularis that significantly contributes to the pan-enteric development of POI (Türler et al., 2007). Therefore, we sought to determine whether the postoperative inflammatory response within both the colonic and small intestinal muscularis, which has been shown to settle within 6 to 24 h after surgical manipulation, would be altered by rosiglitazone treatment.

Previous studies have demonstrated the functional importance of the kinetically active substances NO and prostaglandins in the pathogenesis of POI (Kalff et al., 2000; Schwarz et al., 2001). Therefore, we wanted to determine whether iNOS and COX-2 enzyme activity levels would be altered by rosiglitazone treatment. As expected, surgical manipulation of the colon caused a significant increase in iNOS and COX-2 enzyme activity in the muscularis of both colon and jejunum; however, enzyme activity levels were markedly higher in colonic tissue samples compared with jejunal tissue samples (Fig. 2, A and B). When manipulated mice were pretreated with rosiglitazone, we observed an overall reduction in iNOS and COX-2 enzyme activity levels compared with nontreated manipulated mice (p < 0.05; n = 5–7); vehicle administration of vehicle (DMSO/saline) had no effect on the surgically induced delay in intestinal transit (GC = 5.5 ± 0.4; Fig. 1C) nor did it alter intestinal contractility after colonic manipulation (Amp-P50mean of 8.9 ± 2.6%; Fig. 1D).

Treatment with rosiglitazone did not have any effect on intestinal motility per se in control mice compared with naive controls (Fig. 1, C and D).

Fig. 2. Effect of rosiglitazone treatment on intestinal muscularis inflammatory responses after colonic manipulation, as evaluated for iNOS enzyme activity (A), COX-2 enzyme activity (B), and IL-1β, IL-6, MCP-1, and ICAM-1 protein expression (C). Intestinal tissue samples were obtained at 6 h after surgery. Nonmanipulated mice were used as controls. Data are expressed as median, interquartile range (box), and 95% confidence interval (whiskers; A and B) or as mean ± S.E.M. (C; n = 5–7). *, p < 0.05 for CM versus control mice; §, p < 0.05 for rosiglitazone (Rosi)-treated versus nontreated CM mice. Rosi, rosiglitazone.

Fig. 3. Effect of rosiglitazone treatment on leukocyte infiltration after colonic manipulation. A, MPO-positive leukocytes within Hanker-Yates stained muscularis whole mounts (magnification, 200×) prepared from the jejunum and colon. B, the histogram shows the quantification of leukocytes in muscularis tissue samples, as determined by MPO assay. Intestinal tissue samples were obtained at 24 h after surgery. Nonmanipulated mice were used as controls. Data are expressed as mean ± S.E.M. (n = 5–7). *, p < 0.05 for CM versus control mice; §, p < 0.05 for rosiglitazone (Rosi)-treated versus nontreated CM mice.
was without any effect on iNOS and COX-2 activity levels at 6 h after surgery (data not shown).

The regulation of other inflammatory mediators involved in the pathogenesis of POI was studied by ELISA. As shown in Fig. 2C, colonic manipulation resulted in a significant increase in IL-1β, IL-6, MCP-1, and ICAM-1 expression in the isolated muscularis of both colon and jejunum. In accordance with the potent reduction of iNOS/COX-2 enzyme activity by rosiglitazone shown above, this surgically induced increase in inflammatory parameters was significantly reduced in mice pretreated with rosiglitazone (p < 0.05; n = 5–7); only the reduction of IL-1β in jejunal tissue samples did not reach significance.

As a result of the increased chemokine/adhesion molecule expression, this early molecular inflammatory response is followed by the subsequent infiltration of circulating leukocytes into the intestinal muscularis (The et al., 2005). Herein, we demonstrate that surgical manipulation of the colon results in a massive recruitment of leukocytes into the muscularis of both colon and jejunum; with the highest density of leukocytes in muscularis whole mounts of the manipulated colon (Fig. 3A). This cellular infiltration was markedly reduced in mice pretreated with rosiglitazone. Figure 3B shows the quantification of MPO-positive leukocytes in muscularis tissue samples, as determined by MPO assay. Statistical analysis shows a significant increase in colonic and jejunal MPO activity after surgical manipulation which was significantly reduced by rosiglitazone treatment (p < 0.05; n = 5–7).

**Rosiglitazone Reduces CM-Induced Egr-1 Expression.** The role of Egr-1 in the early phase of the postoperative muscularis inflammatory cascade was investigated by Western blot analysis. Egr-1 is a member of the immediate-early response transcription factor family and functions as a “master switch” that triggers the expression of numerous key inflammatory mediators (Hoetzel et al., 2008). Surgical manipulation of the colon caused a rapid, significant up-regulation of Egr-1 protein expression in nuclear extracts of colonic tissue samples, which remained elevated up to 6 h after surgery. In contrast, Egr-1 expression was only increased at the 6-h time point in nuclear extracts of jejunal tissue samples (Fig. 4A). In rosiglitazone-treated manipulated mice, nuclear Egr-1 expression was significantly reduced compared with nontreated manipulated mice (p < 0.05; n = 5–7). A similar result was observed in cytoplasmic extracts from the same tissue samples (Fig. 4B), demonstrating that PPARγ activation leads to a down-regulation of Egr-1 expression and...
not merely a reduced translocation of Egr-1 from the cytoplasm to the nucleus.

Influence of GW9662 on Rosiglitazone-Mediated Protective Effects. Additional confirmation that PPARγ activation provides protection against POI was obtained in mice pretreated with GW9662. Administration of GW9662 alone to manipulated mice did not result in a significant alteration in intestinal motility compared with nontreated manipulated mice (Fig. 5). However, coadministration of GW9662 nearly abolished the salutary effect of rosiglitazone treatment on intestinal dysmotility after surgical manipulation of the colon \( (p < 0.05; n = 5–7) \).

Next, we investigated the influence of GW9662 on the molecular inflammatory response after manipulation of the colon. As shown in Fig. 6A, GW9662 alone did not affect the CM-induced increase in Egr-1 DNA-binding activity; whereas coadministration of GW9662 and rosiglitazone abrogated the suppression of Egr-1 DNA-binding activity by rosiglitazone treatment \( (p < 0.05; n = 5–7) \). A similar result was observed when studying the influence of GW9662 on iNOS and COX-2 activity; GW9662 did not exacerbate intestinal inflammation when administered alone, but significantly reversed the inhibitory effect of rosiglitazone treatment on iNOS and COX-2 activity in our POI model \( (p < 0.05; n = 5–7) \).

Discussion

POI is characterized by dysmotility of the GI tract that occurs after essentially every abdominal procedure. It is a major contributor to postoperative discomfort and results in prolonged hospitalization and increased patient morbidity. Recent evidence indicates that a complex cascade of inflammatory responses can be attributed as the root cause of POI after abdominal surgery (Bauer, 2008). Despite this novel insight, there is no pharmacological treatment yet available to prevent this postoperative event. In the present study, we...
demonstrate that rosiglitazone alleviates POI through a PPARγ-dependent down-regulation of Egr-1, a key regulator of inflammatory gene expression.

Previous studies have demonstrated that surgical manipulation of the colon initiates an inflammatory cascade within both the colonic and small intestinal muscle layer that consists of 1) the induction of proinflammatory cytokines, chemokines, and adhesion molecules; 2) the additional recruitment of circulating leukocytes into the muscularis; and 3) the release of kinetically active substances, such as NO and prostaglandins, that directly inhibit smooth muscle contractility (Türler et al., 2002, 2007; Bauer and Boeckxstaens, 2004). Herein, we show that pretreatment of mice with rosiglitazone significantly attenuates these intestinal muscularis inflammatory responses, an effect that was abrogated by the PPARγ antagonist GW9662. In contrast to earlier studies reporting aggravation of intestinal injury in PPARγ−/− mice after intestinal I/R injury (Nakajima et al., 2001) or dextran sulfate sodium-induced colitis (Adachi et al., 2006; Shah et al., 2007), we did not observe worsening of intestinal injury in mice pretreated with GW9662 alone compared with nontreated manipulated mice. A search of the literature, however, revealed that GW9662 modifies a conserved cysteine residue in the PPARγ molecule, interfering with ligand-binding but not with DNA-binding (Leesnitzer et al., 2002; Todorov et al., 2007). Therefore, GW9662 does not affect basal PPARγ activity but only its ligand-induced activation.

Although our knowledge of the inflammatory cascade and pathophysiological effects underlying POI has grown widely in recent years, the seminal molecular triggering event(s) that initiate POI are still not known. Recent studies have shown that surgical manipulation of the intestine activates the dense network of normally quiescent macrophages by phosphorylation of mitogen-activated protein kinases [extracellular signal-regulated kinases (ERK1/2), c-Jun N-terminal kinases, and p38; De Backer et al., 2008] and the activation of specific transcription factors such as nuclear factor-κB and signal transducer and activator of transcription (STAT)-3 (Wehner et al., 2005). Very recently, Bauer’s group demonstrated that Egr-1 occupies a key position in the intestinal muscularis inflammatory cascade after surgical manipulation (Schmidt et al., 2008). The functional severity of POI was significantly ameliorated in Egr-1−/− mice and chimera wild-type mice transplanted with Egr-1−/− bone marrow. Based on these results, the authors concluded that “the Egr-1 transcription factor could be a promising checkpoint for the therapeutic targeting of POI” (Schmidt et al., 2008). Our current results demonstrate that Egr-1 expression/activity is highly up-regulated in the manipulated colon as early as after 1 h after surgery. In contrast, Egr-1 expression/activity was only increased at the 6-h time point in the jejunum, indicating that this distant inflammatory response only occurs after a certain time interval. Pretreatment of mice with rosiglitazone significantly prevented this surgically induced Egr-1 induction, an effect that was abrogated by GW9662. This finding is consistent with a previous study reporting that activation of PPARγ suppresses ischemic induction of Egr-1, thereby providing protection against I/R-induced lung injury (Okada et al., 2002). Very recently, Wu et al. (2009) also reported that rosiglitazone attenuates bleomycin-induced skin inflammation-fibrosis by inhibition of Egr-1 induction. Our findings are further supported by recent studies reporting that Egr-1 expression/activity is suppressed by CO through a PPARγ-dependent mechanism. In murine macrophage cultures, the inhibitory effect of CO on Egr-1 expression was abrogated by PPARγ inhibition, either pharmacologically or genetically. In the same study, CO also protected mice against endotoxin-induced lung injury by inhibition of Egr-1, an effect that was blocked by GW9662 (Bilban et al., 2006). Very recently, Hoetzl et al. (2008) also demonstrated that inhaled CO protects against ventilator-induced lung injury via PPARγ activation and the subsequent inhibition of Egr-1.

These studies suggest a role for PPARγ as a downstream mediator of CO-mediated anti-inflammatory effects; in contrast, other studies recently identified HO-1 as a target gene of PPARγ (Lin et al., 2006; Krönke et al., 2007; Ptasinska et al., 2007). This finding opens up the possibility that the protective effects of rosiglitazone as seen in this study should be ascribed to the HO-1/CO signaling pathway. In our POI model, HO-1 protein expression was significantly up-regulated at 6 h after colonic manipulation; however, rosiglitazone treatment partially prevented this surgically induced HO-1 induction (Fig. 7). This finding is consistent with the role of HO-1 as an inducible stress protein that is up-regulated as a consequence of inflammatory tissue injury; however, it does not support the existence of a positive feedback
loop between PPARγ and HO-1/CO. Nevertheless, this result does not exclude the possibility that CO-mediated protection against POI is mediated by PPARγ.

In conclusion, our study demonstrates that rosiglitazone significantly attenuates POI by suppression of the muscularis inflammatory cascade through a PPARγ-dependent down-regulation of Egr-1. Future studies will be needed to further unravel the interplay between PPARγ/Egr-1 and other transcription factors, such as nuclear factor κB and STAT proteins, as well as the mitogen-activated protein kinases. Taken together, our data suggest that PPARγ activation may represent a new pharmacological approach to prevent POI and encourage further clinical evaluation of PPARγ agonists as pharmacological tool to prevent this postoperative event.

Acknowledgments
We thank Valère Geers and Elke De Vuyst for technical assistance in the laboratory.

References


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