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Protection against 2,4,6-trinitrobenzenesulphonic acid-induced colonic inflammation in mice by the marine products bolinaquinone and petrosaspongiolide M

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Abstract

Proinflammatory mediators, namely eicosanoids, reactive oxygen and nitrogen species and cytokines, are clearly involved in the pathogenesis of intestinal bowel disease. bolinaquinone (BQ) and petrosaspongiolide M (PT), two marine products with potent anti-inflammatory action, have been shown to control the production of mediators in acute and chronic inflammatory processes. Hence, we have tested here the hypothesis that BQ and PT could ameliorate inflammation and oxidative stress parameters in 2,4,6-trinitrobenzenesulphonic acid (TNBS)-induced colitis in Balb/c mice. BQ and PT were given orally in doses of 10 or 20 mg/kg/day. Treatment of the animals with BQ or PT at the highest dose significantly protected against TNBS-induced inflammation, as assessed by a reduced colonic weight/length ratio and histological scoring. Neutrophilic infiltration, interleukin (IL)-1β and prostaglandin E_2 (PGE_2) levels, as well as cyclooxygenase-2 (COX-2) protein expression were inhibited by both compounds. Colonic nitrite and nitrate levels and protein expression of inducible nitric oxide synthase (iNOS) were also lower in the treated groups in comparison to the TNBS control. BQ and PT reduced nitrotyrosine immunodetection and colonic superoxide anion production. Neither compound inhibited the expression of the protective protein heme oxygenase-1 (HO-1), although they reduced the extension of apoptosis. Our study also indicated that PT could interfere with the translocation of p65 into the nucleus, a key step in nuclear factor-κB (NF-κB) activation. Altogether, the results suggest that BQ and PT can have potential protective actions in intestinal inflammatory diseases.

Keywords: Bolinaquinone; Petrosaspongiolide M; Intestinal inflammation; 2,4,6-Trinitrobenzenesulphonic acid; Balb/c mice; Nuclear factor-κB

1. Introduction

Inflammatory bowel disease (IBD) is a chronic condition of the intestine of unknown etiology involving multiple immune, genetic and environmental factors [1]. Increased levels of inflammatory cytokines are secreted in the colonic mucosa of IBD patients by lymphocytes, macrophages and neutrophils, leading to the production of other inflammatory mediators such as nitric oxide (NO), reactive oxygen species (ROS), eicosanoids, etc. [2].

Gut inflammation is linked with the activation of nuclear factor κB (NF-κB), which is involved in the regulation of inflammatory genes including those encoding cytokines, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), adhesion molecules, etc. [3]. High levels of NO are generated by iNOS, which is induced by endotoxins, cytokines and other stimuli during inflammatory responses [4]. In IBD and animal models of colitis, NO overproduction can result in mucosal injury. The cytotoxic effects of this free radical are mediated in part by peroxynitrite produced by the reaction of NO and superoxide anion [5]. The massive infiltration of leukocytes in IBD are

Abbreviations: BQ, bolinaquinone; COX-2, cyclooxygenase-2; Dex, dexamethasone; HO-1, heme oxygenase-1; IBD, intestinal bowel disease; IL, interleukin; MPO, myeloperoxidase; NF-κB, nuclear factor-κB; NO, nitric oxide; NO_x^-, $NO_2^- + NO_3^-$; iNOS, inducible nitric oxide synthase; PT, petrosaspongiolide M; PGE_2, prostaglandin E2; PLA_2, phospholipase A2; ROS, reactive oxygen species; Sh, Sham; TNBS, 2,4,6-trinitrobenzenesulphonic acid; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling

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thought to produce large amounts of ROS that would participate in intestinal damage. Oxidative stress can thus be a pathogenic factor in this condition where the antioxidant mechanisms within the intestinal mucosa are seriously impaired [6].

An increased production of eicosanoids has been demonstrated in human disease and experimental models of intestinal inflammation. Thus, prostaglandin E_2 (PGE_2), a major local inflammatory mediator that might come from activated neutrophils and monocytes, is considered as a marker of colitis. Nevertheless, these mediators may play a complex role, and selective inhibition of a specific eicosanoid pathway such as COX-2 has given controversial results [7]. Another enzyme relevant in inflammatory processes, phospholipase A_2 (PLA_2), initiates the synthesis of different lipid mediators including eicosanoids, lysophospholipids and platelet activating factor. Activation of the secretory form of this enzyme has also been implicated in IBD [8]. Therefore, a number of new potential targets are being pursued for the development of IBD therapeutics. New therapies for this condition are still needed due to the failure of standard treatments to induce remission in about 30% of patients (reviewed in [9]).

Bolinaquinone (BQ) and petrosaspongiolide M (PT) are two marine metabolites isolated from *Dysidea* sp. and *Petrosaspongia nigra*, respectively. We have previously shown their anti-inflammatory activity in acute and chronic models such as carrageenan edema and adjuvant arthritis [10,11]. These agents inhibited human synovial PLA_2 activity, the infiltration of leukocytes and the generation of eicosanoids, cytokines and NO in vitro and in vivo. If BQ and PT affect these mechanisms within the gut, they might modify the inflammatory response associated with IBD. We therefore sought to determine if these marine compounds could exert protective effects in intestinal inflammatory disorders.

Hapten-induced colonic inflammation is a widely used animal model of human IBD. Intrarectal delivery of TNBS induces weight loss and transmural inflammation with histopathologic features bearing similarities to Crohn's disease [12]. In this study we have assessed the ability of BQ and PT to reduce the severity of colitis using the TNBS model in mice. In order to gain a better insight into the mechanisms of action of BQ and PT, we have also examined the effects of both compounds on the expression of several proteins relevant to the inflammatory process, the occurrence of apoptosis and the production of inflammatory mediators.

2. Materials and methods

2.1. Reagents

BQ (MW 358) and PT (MW 460) were isolated from the sponges *Dysidea* sp. and *Petrosaspongia nigra* following published procedures [13,14]. The [5,6,8,11,12,14,15(*n*)-^{3}H]PGE_2 was from Amersham Biosciences. Polyclonal antibodies against COX-2, iNOS and nitrotyrosine were purchased from Cayman Chemical Co. Polyclonal antibody against heme oxygenase-1 (HO-1) came from StressGen Biotech. The peroxidase-conjugated goat anti-rabbit IgG was from Dako and the polyclonal antibody against p65 were purchased from Santa Cruz Biotechnology. All the other reagents were from Sigma–Aldrich.

2.2. Study protocol

All experiments were approved by the institutional Animal Care and Use Committee and were carried out in accordance with European Union guidelines for the handling and use of laboratory animals. Male Balb/c mice (10-week-old) were purchased from Harlan Iberica. After a period of adaptation, weight matched animals (24.7 ± 0.5 g) were randomized into seven groups according to colitis and drug administration. Group sham (Sh): sham colitis-induced mice; group TNBS: non-treated colitis-induced mice; group dexamethasone (Dx): dexamethasone-treated colitis-induced mice; group BQ: BQ-treated colitis-induced mice; group PT: PT-treated colitis-induced mice. Dexamethasone (2 mg/kg/day), BQ and PT (10 or 20 mg/kg/day) were suspended in olive oil and administered p.o. once a day for 4 days, beginning 24 h prior to colitis induction. Olive oil alone was administrated to both groups Sh and TNBS. Acute colitis was induced according to published methods [15,16]. Briefly, mice (fasted for 24 h) were anesthetized with ketamine and xylazine, and colitis was induced by intrarectal administration of 1.5 mg of TNBS in 35% ethanol. The Sh group received 35% ethanol alone through the same technique. The total injection volume was of 100 μL. The mice were killed 72 h after TNBS administration. The colon was removed by cutting at the pubis symphysis and at the caecum and gently washed with 0.9% saline solution. The colon was blotted dry, measured and weighed. Immediately after weighing, a 15 mm full thickness segment was cut from the distal region to the macroscopically most intensively affected region for the superoxide anion production assay, and a 6 mm segment for histological analyses was taken from the adjacent region. From this distal macroscopically most intensively affected region a 3 cm full thickness segment was cut longitudinally into three strips for measurement of myeloperoxidase (MPO) activity, PGE_2, interleukin(IL)-1β, NO_x^- (NO_2^-+ NO_3^-) levels, and NF-κB p65 protein expression.

2.3. Colitis severity

Colonic length and weight were measured as gross indicators of colitis. For histological morphometry, colon tissues were fixed in 4% formalin in 0.1 M phosphate buffer, dehydrated with increasing concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5 μm thick)

were mounted on slides, cleared, hydrated and stained with hematoxylin and eosin. Histological scoring was performed in a blinded manner [17]: (0) histological findings identical to normal mice; (1) mild mucosal and/or submucosal inflammatory infiltrate and edema, punctuate mucosal erosions often associated with capillary proliferation, muscularis mucosae intact; (2) grade 1 changes involving 50% of the specimen; (3) prominent inflammatory infiltrate and edema frequently with deeper areas of ulceration extending through the muscularis mucosae into the submucosa, rare inflammatory cells invading the muscularis propria but without muscle necrosis; (4) grade 3 changes involving 50% of the specimen; (5) extensive ulceration with coagulative necrosis bordered underneath by numerous neutrophils and lesser numbers of mononuclear cells, necrosis extends deeply into the muscularis mucosae; (6) grade 5 changes involving 50% of the specimen.

2.4. MPO measurement

Colonic tissue MPO activity was determined as previously reported [18]. Briefly, tissue strips were suspended in potassium phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (pH 6.0; 50 mg of tissue per mL) and then homogenized for 30 s using a Polytron homogenizer. After homogenates were centrifuged at 10,000 × *g* for 15 min, the supernatants were collected to determine the tissue levels of MPO activity. One unit of MPO was defined as the amount needed to degrade 1 μmol of hydrogen peroxide in 1 min at 22 °C.

2.5. Detection of superoxide

Detection of superoxide in colonic tissues was performed as previously reported [18]. Briefly, tissue strips were incubated in Hank's balanced salt solution (HBSS), pH 7.4 gassed in 95% O_2, 5% CO_2 at 37 °C for 5 min. Tissue strips were placed in plastic scintillation vials containing 0.25 mM lucigenin in a final volume of 1 mL of HBSS buffer. Blanks of reaction were included and substracted from the results obtained with the samples. The chemiluminescence elicited by superoxide in the presence of lucigenin was measured in a Microbeta counter (Wallac) for 2 min. Results were expressed as c.p.m. per mg of wet tissue.

2.6. Colonic NO_x^- (NO_2^-+NO_3^-), IL-1β and PGE_2 levels

Tissue strips were homogenized for 15 s in HEPES buffer (40 mM, pH 7.4) containing sucrose (320 mM). NO_x^- (NO_2^- + NO_3^-), interleukin (IL)-1β and PGE_2 levels were determined in the supernatants (10,000 × *g* for 20 min at 4 °C). NO_x^- was calculated by first reducing NO_3^- into NO_2^- by nitrate reductase, and NO_2^- was determined fluorometrically in microtiter plates using a standard curve of sodium nitrite [19]. IL-1β levels were measured by time-resolved fluoroimmunoassay, while PGE_2 levels were determined by radioimmunoassay [20].

2.7. Immunohistochemical study

Colonic tissues were fixed in 4% formalin in 0.1 M phosphate buffer, dehydrated with increasing concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5 μm thick) were mounted on slides, cleared and hydrated. After inhibition of endogenous peroxidases, tissues were treated with a buffered blocking solution (10% BSA in PBS) for 15 min. The sections were then incubated with primary antibody (anti-COX-2, anti-iNOS, anti-HO-1 or anti-nitrotyrosine, 1/50 in PBS-Tween 20, v/v) or control solutions at 37 °C for 45 min. Controls included buffer alone or non-specific purified rabbit IgG. Samples were washed with PBS-Tween 20 and incubated with secondary antibody (goat anti-rabbit IgG, peroxidase-conjugated, 1/250 in PBS-Tween 20, v/v) at 37 °C for 30 min. Thereafter the sections were washed as before and incubated with 3,3′-diaminobenzidine solution in the dark at room temperature for 10 min. After washing with water, samples were mounted with Dako® faramount aqueous mounting medium (Dako Corporation) and observed with a Nikon Eclipse E600FN microscope (Nikon Intruments Inc.).

2.8. Nuclear extraction and Western blotting

Colonic strips were homogenized with a Polytron homogenizer in a buffer containing 10 mM HEPES/KOH, pH 7.9, 1.5 mM KCl, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulphonylfluoride and 0.6% Nonidet P40 for 1 min. The homogenates were centrifuged (8,000 × *g*, 5 min, 4 °C) and the supernatant (cytosolic extract) was collected. The pellets were solubilized in a buffer containing 20 mM HEPES/KOH, pH 7.9, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulphonylfluoride, 2 mM benzamidine, 5 mg/mL leupeptin and 25% glycerol. After centrifugation at 15,000 × *g* for 2 min at 4 °C, the supernatant (nuclear extract) was collected and used for Western blotting. Protein was measured by the Bradford method using BSA as standard. Equal amounts of protein were loaded on 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Membranes were blocked in PBS-Tween 20 containing 3% (w/v) unfatted milk and incubated with polyclonal antibody against p65 (1/750). Finally, membranes were incubated with peroxydase-conjugated goat anti-rabbit IgG (1/10,000). The immunoreactive bands were visualized using an enhanced chemiluminescence system (Amersham Biosciences).

2.9. Detection of TUNEL-positive cells

Colonic tissue fixed in 4% formalin in 0.1 M phosphate buffer, was dehydrated and embedded in paraffin. The in

situ terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay was performed on 5 μm thickness sections using the 'In situ cell death detection kit, POD' (Roche Applied Science), following the manufacturer's instructions. The results were expressed as the number of apoptotic nuclei/150 μm^2 of mucosa.

2.10. Statistical analysis

The results are presented as mean ± S.E.M.; *n* represents the number of animals or experiments. The level of statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons of all groups in relation with the TNBS control group. Bonferroni post hoc test was used to evaluate the statistical significance among the BQ and PT groups.

3. Results

3.1. Effects on colitis severity

Seventy-two hours after intrarectal administration of TNBS, mice showed prostration, piloerection and hypomotility. A significantly increased weight/length ratio of the mice colon, an indicator of inflammation, was observed in TNBS-treated mice in comparison to sham animals. Oral administration of dexamethasone (2 mg/kg/day), BQ at 20 mg/kg/day or PT at 10 and 20 mg/kg/day significantly reduced the weight/length ratio (Fig. 1A). TNBS induced mucosal thickening, crypt damage, increase of lymphoid follicle size and loss of goblet cells. The mucosal injury produced by TNBS was characterized by necrosis of the epithelium, focal ulceration of the mucosa and diffuse infiltration of neutrophils and lymphocytes in the mucosa and submucosa. As shown by histologic examination of mice colon, dexamethasone as well as BQ and PT at the highest dose significantly ameliorated the signs of colitis in comparison to the TNBS control mice (Fig. 1B). As expected, dexamethasone significantly reduced colonic MPO activity, an index of neutrophilic infiltration in the mucosa. PT administration also resulted in decreased MPO activity, whereas BQ was effective at the highest dose (Fig. 1C).

3.2. Effects on inflammatory mediators

TNBS administration resulted in increased production of inflammatory mediators in colonic tissues. The PGE_2 content of the colon was significantly reduced by both marine metabolites (Fig. 2A), whereas dexamethasone greatly lowered the levels of this eicosanoid to an extent similar to sham animals. Increased NO_x^- levels were detected in the colon of the TNBS group in comparison with sham animals, but were significantly reduced by dexamethasone, BQ and PT (Fig. 2B). As measured by chemiluminescence, ex vivo colonic superoxide generation in the TNBS control group was increased with respect to sham animals, and the oral administration of BQ and PT at the highest dose significantly inhibited this parameter (Fig. 2C). IL-1β was significantly elevated in the colon 72 h after TNBS instillation. Reductions in the levels of this cytokine were seen in the groups treated with dexamethasone, and both BQ and PT at 20 mg/kg/day (Fig. 2D).

3.3. Effects on protein expression

Immunohistochemical analyses revealed increased immunoreactivity for COX-2, iNOS, nitrotyrosine and HO-1 proteins in the colon of control mice 72 h after TNBS administration, as compared with sham animals (Fig. 3). We observed that COX-2 and iNOS protein expression was inhibited in colonic samples of animals treated with dexamethasone, BQ or PT. In addition these treatments also reduced nitrotyrosine immunoreactivity. In the present experiments, we observed HO-1 immunostaining located at or near the inflammatory cells infiltrating the mucosa in colon from TNBS control mice. HO-1 immunostaining was also detected in colon from BQ and PT groups with a different localization, since HO-1 was present near or at the top of the crypts. The increased HO-1 expression induced by TNBS was suppressed by dexamethasone.

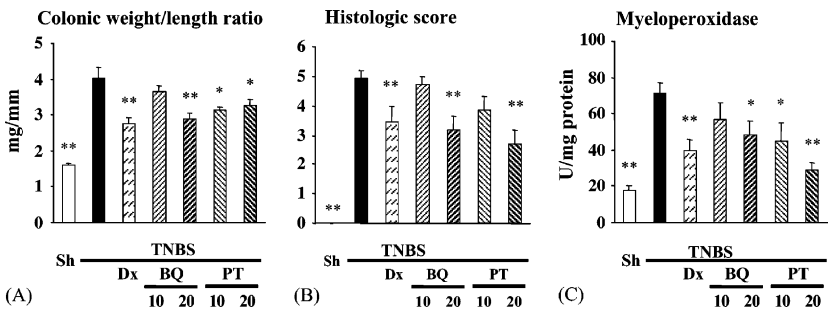

Fig. 1. Effects of BQ (10 and 20 mg/kg/day), PT (10 and 20 mg/kg/day) and Dx (2 mg/kg/day) on TNBS-induced colitis: (A) colonic weight/length ratio; (B) histologic score; (C) MPO activity in colonic tissues. Data represent the mean ± S.E.M. ($n = 6$–12); $^{*}P < 0.05$; $^{**}P < 0.01$ with respect to the TNBS control group (Dunnett). MPO activity of PT (20 mg/kg/day) was different from that of BQ (20 mg/kg/day) (Bonferroni, $P < 0.05$).

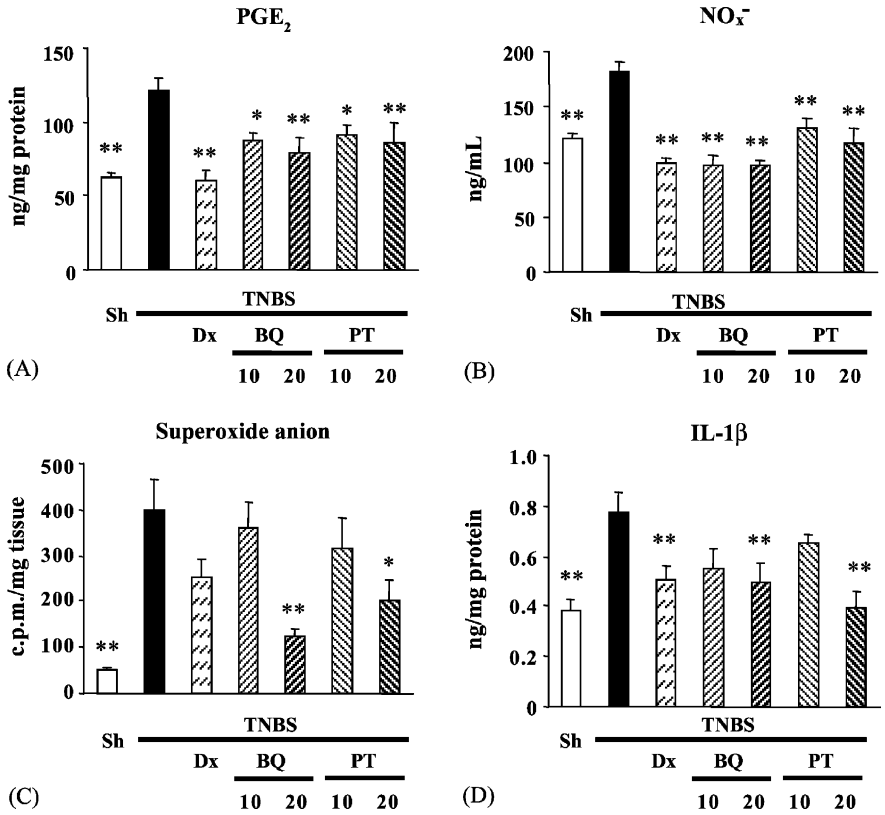

Fig. 2. Effects of BQ (10 and 20 mg/kg/day), PT (10 and 20 mg/kg/day) and Dx (2 mg/kg/day) on TNBS-induced colitis: (A) PGE_2; (B) NO_x^-; (C) superoxide generation; (D) IL-1β levels in colonic tissues. Data represent the mean ± S.E.M. ($n = 6$–12); $^{*}P < 0.05$; $^{**}P < 0.01$ with respect to the TNBS control group (Dunnett). Superoxide generation of BQ (20 mg/kg/day) was different from that of PT (20 mg/kg/day) (Bonferroni, $P < 0.01$).

3.4. Effects on apoptosis

The extent of cell apoptosis was assessed by the TUNEL assay in sections of colonic tissue. Some TUNEL-positive epithelial cells were present in sham mice (Fig. 4). Increased TUNEL staining was observed in TNBS-instilled animals, whereas dexamethasone, BQ or PT treatments partially reduced the number of TUNEL-positive cells.

3.5. Effects on p65 expression

To assess the in vivo inhibition of NF-κB activation by marine metabolites, we analyzed by Western blotting the expression of p65 in nuclear extracts of mouse colon. Inflamed colonic tissues in the TNBS control group showed a higher expression of p65 in the nuclear fraction (Fig. 5), indicating an increased translocation of this protein into the nucleus. The nuclear translocation of p65 induced by TNBS was inhibited by dexamethasone and PT.

4. Discussion

The results of this study have demonstrated that prophylactic administration of BQ or PT by oral route attenuated TNBS-induced colitis in Balb/c mice, as shown by clinical and histological examinations. This confirms the potent anti-inflammatory effects of these marine products in other models of inflammatory diseases [10,11]. These effects were accompanied by a modulation of both inflammatory and oxidative stress parameters.

Although the etiology of IBD remains unknown, a dysregulated immune response has been widely accepted as a possible mechanism in the pathogenesis of this condition. Increased NF-κB activation and high production of cytokines such as tumor necrosis factor-α, IL-6 and IL-1β have been detected in mononuclear cells recruited by the IBD mucosa [21]. In addition, there is good evidence that IL-1β helps to propagate a local systemic inflammatory process by activating a cascade of immune cells with induction of PLA_2, COX-2 and iNOS, which would account for the production of high levels of platelet activating factor, PGE_2 and NO [22]. Our results indicate that the level of IL-1β, a marker of intestinal inflammation, was enhanced in the colon of TNBS control mice, whereas a significant inhibition of this cytokine was observed in the BQ and PT groups. Our data also demonstrate that the COX-2 immunoreactivity and colonic PGE_2 overproduction induced by TNBS administration were reduced by both marine compounds.

It has been suggested that the infiltration of leukocytes into the mucosa contributes significantly to tissue necrosis

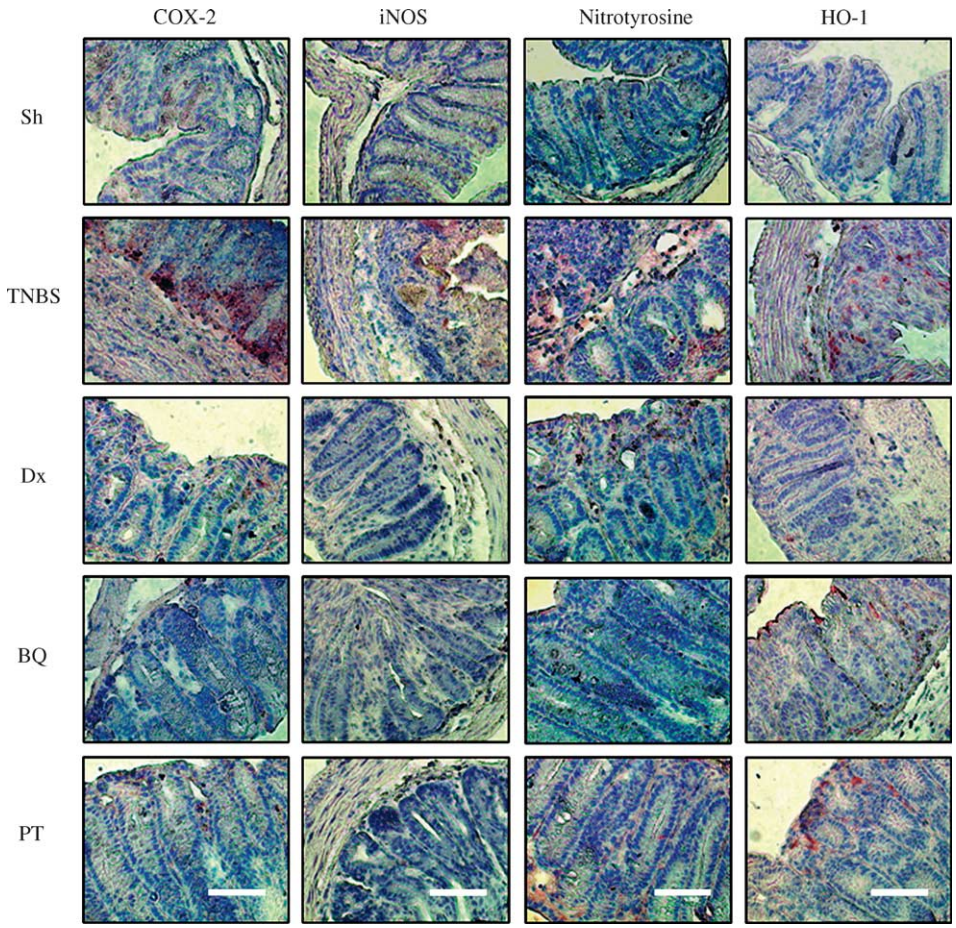

Fig. 3. Effects of BQ (20 mg/kg/day), PT (20 mg/kg/day) and Dx (2 mg/kg/day) on COX-2, iNOS, nitrotyrosine, and HO-1 immunohistochemistry. The photomicrographs were obtained as video images (magnification 200×). Scale bars: 100 μm.

and mucosal dysfunction as they represent the major source of ROS in the inflamed colonic mucosa [23,24]. Quantitatively, the principal free radical is superoxide anion, which is transformed into the secondary oxidant H_2O_2 by superoxide dismutase. In addition, neutrophils can also release proteases, lactoferrin and lipid mediators that contribute to colon injury. Reduction of leukocyte migration or activation in inflamed intestinal tissue participates in the anti-inflammatory effects of IBD therapies such as corticosteroids or 5-aminosalicylic acid [24,25]. Our data indicate that this mechanism is involved in the intestinal anti-inflammatory activity of both marine metabolites.

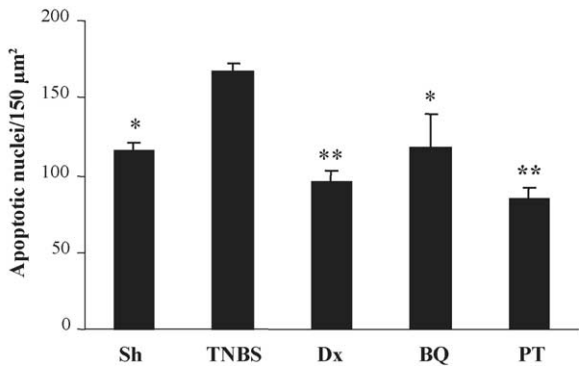

Fig. 4. Effects of BQ (20 mg/kg/day), PT (20 mg/kg/day) and Dx (2 mg/kg/day) on TUNEL-positive cells. Data represent the mean ± S.E.M. (n = 6–12); $^{*}P < 0.05$; $^{**}P < 0.01$ with respect to the TNBS control group (Dunnett).

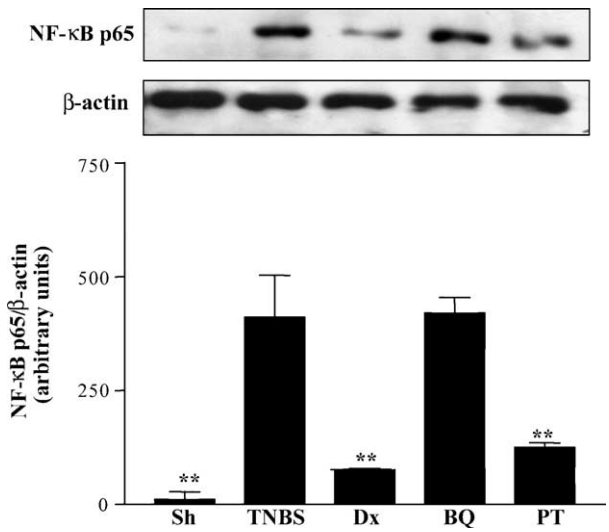

Fig. 5. Effects of BQ (20 mg/kg/day), PT (20 mg/kg/day) and Dx (2 mg/kg/day) on NF-κBp65 protein expression in nuclear extracts from colonic tissues. Densitometric analysis is expressed as the NF-κBp65/β-actin ratio. Data represent the mean ± S.E.M. (n = 3); $^{**}P < 0.01$ with respect to the TNBS control group (Dunnett).

The production of high levels of reactive nitrogen species by iNOS could contribute to tissue injury in IBD [26]. In addition, TNBS administration to iNOS-deficient mice has demonstrated the role of this enzyme in the production of nitrosative and oxidative damage in this experimental model [27]. Accordingly, induction of iNOS in the inflamed human colonic epithelium is associated with the formation of peroxynitrite and the nitration of cellular proteins [26]. In our experiments, administration of PT or BQ considerably reduced iNOS expression, NO_x^- levels and nitrotyrosine staining. Although 3-nitrotyrosine has been proposed as an index of peroxynitrite formation [28], other studies have shown that nitration of tyrosine residues can be the result of oxidation pathways dependent on hypochlorous acid or MPO activity and nitrite-derived species [29]. Inflamed mucosa from patients with IBD contains macrophages able to undergo a respiratory burst upon stimulation [30], resulting in high mucosal levels of ROS and nitrogen species [6]. Interestingly, oxidative injury correlates with disease severity and tissue injury may depend on cytoskeletal disruption by nitrosative species and ROS [31]. The nitration of tyrosine residues on key cellular proteins seriously compromises cell functions. Protein nitration has been detected in human IBD and animal models of intestinal inflammation and can reflect oxidative stress. In the TNBS-induced colitis, superoxide production and nitrotyrosine immunoreactivity were reduced by both marine metabolites, although our results suggest a higher effect of BQ to control oxidative stress. This is in line with the observation that BQ inhibits the activation of human neutrophils and the generation of superoxide anion in vitro [13].

Resistance of lamina propria T cells to apoptosis may be a factor in the perpetuation of chronic inflammation in IBD [32]. Nevertheless, during colonic inflammation there is a significant increase of apoptosis in lamina propria leukocytes and epithelium that may lead to a breakdown of the epithelial barrier function in the intestine [33]. We have observed that colonic cell death was associated with apoptosis in the colon 72 h after administration of TNBS, which is in accordance with previous studies showing significantly increased apoptosis in epithelial cells in TNBS-induced colitis, where peroxynitrite is a major contributor to colon epithelial cell apoptosis [34]. Our results suggest that BQ and PT protect from apoptosis in this experimental model, which may be due to inhibitory effects on iNOS expression and production of NO and related reactive species.

HO-1 is rapidly induced in response to cytokines, NO and peroxynitrite, ROS, etc. This protein can be a marker of oxidative stress or play a protective role against oxidative injury (reviewed in [35]). Previous reports support the concept that HO-1 exerts protective effects on TNBS colitis, likely due to decreased free radical production and inhibition of iNOS expression in colonic tissues [36]. We observed HO-1 immunostaining in colon from BQ and PT groups with a different localization with respect to the TNBS control, since HO-1 was present near or at the top of the crypts. The lack of HO-1 detection in the lower portions of the crypts is consistent with the fact that both free radical production and MPO activity were significantly lower in BQ and PT groups, as compared with TNBS control group.

Increased synthesis of proinflammatory cytokines activates NF-κB in intestinal inflammation. The activated form of this transcription factor has been detected in mononuclear cells and epithelial cells of inflamed colon [37] and is inhibited by corticosteroids by stabilizing the NF-κB inhibitory protein-α [38]. In addition, selective downregulation of p65 by specific antisense oligonucleotides inhibits experimental colitis and may be a beneficial approach in the treatment of IBD [39]. We have previously demonstrated that PT inhibits NF-κB activation and p65 translocation in mouse peritoneal macrophages [20]. Our results in the TNBS model have confirmed that PT inhibits the translocation of p65 into the nucleus, thus suggesting that inhibition of NF-κB activation is a likely mechanism through which this marine metabolite modulates intestinal inflammation.

Available therapies in IBD are frequently associated with issues of toxicity or efficacy. We have previously reported that BQ and PT exert therapeutic effects in chronic inflammatory conditions without producing the gastrointestinal complications often associated with anti-inflammatory drugs [40,41]. Together with the present study, these data suggest that both marine metabolites may be candidates for protective strategies in intestinal inflammation.

In conclusion, we found that oral administration of BQ and PT significantly reduced a wide range of inflammatory mediators relevant in intestinal inflammation and prevented the development of TNBS-induced colitis, suggesting a chemopreventive potential in inflammatory intestinal diseases.

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