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Dietary supplementation of resveratrol attenuates chronic colonic inflammation in mice

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ABSTRACT

Ulcerative colitis is a nonspecific inflammatory disorder characterized by oxidative and nitrosative stress, leucocyte infiltration and upregulation of inflammatory mediators. Resveratrol is a polyphenolic compound found in grapes and wine, with multiple pharmacological actions, mainly anti-inflammatory, antioxidant, antitumour and immunomodulatory activities. The aim of this study was to investigate the effect of dietary resveratrol on chronic dextran sulphate sodium (DSS)-induced colitis. Six-week-old mice were randomized into two dietary groups: one standard diet and the other enriched with resveratrol at 20 mg/kg of diet. After 30 days, mice were exposed to 3% DSS for 5 days developing acute colitis that progressed to severe chronic inflammation after 21 days of water. Our results demonstrated that resveratrol group significantly attenuated the clinical signs such as loss of body weight, diarrhea and rectal bleeding improving results from disease activity index and inflammatory score. Moreover, the totality of resveratrol-fed animals survived and finished the treatment while animals fed with standard diet showed a mortality of 40%. Three weeks after DSS removal, the polyphenol caused substantial reductions of the rise of pro-inflammatory cytokines, TNF-α and IL-1β and an increase of the anti-inflammatory cytokine IL-10. Also resveratrol reduced prostaglandin E synthase-1 (PGES-1), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) proteins expression, via downregulation of p38, a mitogen-activated protein kinases (MAPK) signal pathway. We conclude that resveratrol diet represents a novel approach to the treatment of chronic intestinal inflammation.

1. Introduction

Inflammatory bowel disease is a chronic pathology by uncontrolled inflammation of the intestinal mucosa which can affect part of the gastrointestinal tract. Pathophysiological bases of this disease involve genetic factors, immune dysregulation, barrier dysfunction, and a loss of immune tolerance toward the enteric flora (Kucharzik et al., 2006; Sánchez-Muñoz et al., 2008). Increase of inflammatory mediators, including reactive oxygen species such as nitric oxide, prostaglandins and inflammatory cytokines play an important role in immune dysregulation (Kolios et al., 2004; Rojas-Cartagena et al., 2005; Atreya and Neurath, 2005; Ito et al., 2006). Furthermore, reactive oxygen species can activate diverse downstream signalling pathways, for instance mitogen-activated protein kinases (MAPKs) which lead to the activation of transcription factors modulating a number of different steps in the inflammatory cascade. These include production of pro-inflammatory cytokines (tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, interferon (IFN)-γ, IL-6, IL-12 and IL-17 among others) in different cell-types, degranulation of neutrophils, as well as the expression of important determining parameters namely prostaglandin E_2-synthesizing enzymes, including prostaglandin E synthase (PGES)-1 and cyclooxygenase (COX)-2, and inducible nitric oxide synthase (iNOS) (Dubuquoy et al., 2002; Collino et al., 2006; Pecchi et al., 2009).

Resveratrol (trans-3,4,5-trihydroxystilbene), a natural polyphenol, is found in a large number of plant species including some components of the human diet, such as various fruits and vegetables and is abundant in grapes and in red wines. The last years, it has been the focus of numerous *in vitro* and *in vivo* studies where its biological attributes have been investigated, which include essentially antioxidant and anti-inflammatory activities, anti-platelet aggregation effect, anti-atherogenic property, oestrogen-like growth promoting effect, growth-inhibiting activity, immunomodulation and chemoprevention (Jang et al., 1997; Bradamante et al., 2004; de la Lastra and Villegas, 2007; Shakibaei et al., 2009). Among the possible mechanisms responsible for its biological activities are downregulation of the inflammatory response through inhibition of synthesis and release of pro-inflammatory mediators, modification of eicosanoid synthesis, inhibition of Kupffer cells and adhesion molecules, inhibition of activated immune cells, or iNOS and COX-2 downregulation via its

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inhibitory effects on NF-κβ or AP-1 (Jang et al., 1997; Bertelli, 1998; de la Lastra and Villegas, 2005). Moreover, resveratrol has been shown to produce no adverse effects, even when consumed at high concentrations.

Previous studies by our research group have showed the beneficial effect of intragastric resveratrol in an acute and chronic-induced trinitrobenzenesulphonic acid colitis (Martín et al., 2004, 2006); these effects were observed despite extremely low bioavailability and rapid clearance from the circulation (Walle et al., 2004). However, there is no report related to dietary resveratrol on chronic experimental colitis. Thus, the present study was designed to examine the protective/preventive effects of dietary resveratrol intake in a chronic colitis model induced by dextran sulphate sodium (DSS) in C57BL/6 mice, which is useful to identify and validate new therapies for treatment of inflammatory bowel disease (Melgar et al., 2008) by macroscopic and histology parameters and to analyze the mechanisms involved in its effects as inflammatory response such as cytokines production, PGES-1, COX-2, iNOS expression and finally, we studied the role of p38 MAPK signalling pathway in the beneficial effects of resveratrol on chronic colonic inflammation.

2. Material and methods

2.1. Animals and treatment

A total of 50 6-week-old female C57BL/6 mice (Charles River, Tokyo, Japan) were used in this study. They were acclimatized in our Animal Laboratory Center under standard conditions (temperature 24–25.8 °C, humidity 70–75%, lighting regimen of 12L/12D) and were fed pellet diets and water *ad libitum*. Mice were randomized into two dietary groups: 25 mice were fed with standard diet and the rest were fed with resveratrol-enriched diet at 20 mg/kg of diet (Sigma-Aldrich Company Ltd. Spain) (Table 1) during all experimental period. Resveratrol group consumed an average of 3 g/day of diet resulting in a dose of 3 mg/kg body weight of resveratrol ingested. The administered dose of resveratrol was chosen based on analyses described in the literature (Kimura and Okuda, 2001) and was equivalent to 0.429 mg/kg/day in humans (30 mg resveratrol in a 70 kg person) according to the human equivalent formula (Reagan-shaw et al., 2008). All diets were prepared by mixing the respective compounds under yellow light and stored at −80 °C. Fresh diet was provided daily. Experiments followed a protocol observed by the Animal Ethics Committee of the University of Seville and all experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Counsel 86/609/EC).

2.2. Induction of colitis

During 30 days after weaning, 15 mice into each diet received 3% DSS (DSS group; MW: 40,000, ICN Pharmaceuticals, Costa Mesa, CA) in the drinking water for 5 days followed by a regime of 21 days of water, reflecting chronic inflammation. Control healthy mice (10 mice) were allowed to drink only water (Melgar et al., 2005). Then animals were sacrificed by an overdose of i.p. chloral hydrate. Disease activity index was evaluated as the average of score of clinical parameters described by Melgar et al. with slight modifications (Melgar et al., 2005) which included daily monitoring body weight changes, rectal bleeding and stool consistency or diarrhea from when DSS was administered as shown in Table 2. At the end of the experimental period, the colon was removed, slightly cleaned in physiological saline to remove fecal residues, weighed and measured in order to evaluate variations in the weight/length as an inflammation index. The macroscopic appearance of the colon (inflammatory score), based on the degree of inflammation and the presence of oedema and/or ulcerations was also evaluated (Table 2).

2.3. Histology

Samples of three regions (proximal, middle and rectum) were excised out of every segment, fixed in 4% buffered formaldehyde, then submerged into 20–30% sucrose and after embedded in tissue freezing medium (Leica Instruments GmbH, Nussloch, Germany), and finally were frozen in liquid nitrogen. 7 μm thick slices were obtained by utilizing a cryostat (HM 525, Microm, Walldorf, Germany) and stored at −70 °C until use. The samples were stained with hematoxylin–eosin in accordance with the standard procedures for histological evaluation of colonic damage. Histological study was representative of 3 animals per group.

2.4. Assessment of TNF-α, IL-1β and IL-10

Colon samples were weighed and homogenized, after thawing, in 0.3 ml phosphate buffer saline solution (PBS, pH = 7.2) 1% bovine serum albumin (BSA) at 4 °C. They were centrifuged at 12,000 *g* for 10 min. Mucosal cytokine levels were assayed twice with quantitative TNF-α, IL-1β and IL-10 ELISA kits (eBioscience Inc, San Diego, USA). TNF-α, IL-1β and IL-10 values were expressed as pg/mg tissue.

2.5. Western blot analysis

Frozen colonic tissues were weighed and homogenized in ice cold buffer (50 mM Tris–HCl, pH 7.5, 8 mM MgCl2, 5 mM EGTA, 0.5 mM EDTA, 0.01 mg/ml leupeptin, 0.01 mg/ml pepstatin, 0.01 mg/ml aprotinin, 1 mM PMSF and 250 mM NaCl). Homogenates were centrifuged (12,000 *g*, 15 min, 4 °C) and the supernatants were collected and stored at 80 °C. Protein concentration of the homogenate was determined following Bradford colorimetric method (Bradford, 1976). Aliquots of supernatants containing equal amounts of protein (50 μg) were separated on 10% acrylamide gel by sodium dodecyl sulfatepolyacryamide gel electrophoresis. In the next step,

Table 1
Composition of experimental diets.

Ingredients	Standard diet (g/kg of diet)	Resveratrol-enriched diet (g/kg of diet)
Casein	200	200
DL-Methionine	3	3
Cornstarch	150	150
Sucrose	449.91	449.89
Cellulose	50	50
Sun flower	100	100
Mineral mix	35	35
Vitamin mix	10	10
Choline bitartrate	2	2
Fe (sulphate)	$90*10^{-3}$	$90*10^{-3}$
Resveratrol	–	$20*10^{-3}$

Table 2
Scoring of inflammation by means of clinical parameters during treatment and macroscopic inflammatory score at the end of treatment.

Score	Bleeding	Weight loss (% of initial wt)	Stool consistency	Inflammatory Score
0	Normal	<1	Normal pellets	Normal
1	Slightly bloody	1–4.99	Slightly loose feces	Slight inflammation
2	Bloody	5–10	Loose feces	Moderate inflammation and/or edema
3	Blood in whole colon	>10	Watery diarrhea	Heavy inflammation and/or ulcerations and/or edema

the proteins were electrophoretically transferred onto a nitrocellulose membrane and incubated with specific primary antibodies: anti-COX-2 and iNOS, both at a dilution of 1:3000 (Cayman Chemical, Michigan, USA), anti-PGES-1 at a dilution of 1:500 (Cayman Chemical, Michigan, USA) and anti-phospho p38 MAPK and p38 MAPK at a dilution of 1:500 and 1:1000 respectively (Cell Signalling Technology Inc, Danvers, USA). Each filter was washed three times for 15 min and incubated with the secondary horseradish peroxidase linked anti-rabbit (for COX-2, iNOS and PGES-1) (Pierce Chemical Company, Rockford, IL, USA) and anti-mouse (for MAPKs) (Sigma-Aldrich, MO, USA). To prove equal loading, the blots were analyzed for β-actin expression using an anti-β-actin antibody (Sigma-Aldrich, MO, USA). Immunodetection was performed using enhanced chemiluminescence's light-detecting kit (SuperSignal1 West Pico Chemiluminescent Substrate, Pierce, IL, USA). Densitometry data were studied following normalization to the control (housekeeping gene). The signals were analyzed and quantified by a Scientific Imaging Systems (KODAK 1D, Image Analysis Software). The figures shown are representative of colonic mucosa from 4 animals per group.

2.6. Statistical analysis

All values in the figures and text are expressed as arithmetic means ± standard error (S.E.M.). Data were evaluated with Graph Pad Prism® Version 2.01 software. The statistical significance of any difference in each parameter among the groups was evaluated by one-way analysis of variance (ANOVA), using Tukey–Kramer multiple comparisons test as post hoc test. Inflammatory score was evaluated by non-parametric Kruskal–Wallis Test. P values of <0.05 were considered statistically significant. In the experiment involving histology, the figures shown are representative of at least 3 experiments performed on different days.

3. Results

3.1. Therapeutic efficacy of dietary resveratrol for chronic experimental DSS model

Clinical signs on exposure to 3% DSS in the standard diet group for 5 days were loss of body weight, not formed stool or diarrhea and rectal bleeding. The signs were aggravated when disease progressed to the chronic phase. Acute disease did not resolve after DSS removal, instead it progressed to a severe chronic colitis, although paradoxically, diarrhea and weight loss did not correlate with the severity of the inflammation. Significant loss of body weight was observed 1 week after DSS removal in standard diet group vs. sham animals ($P<0.05$) (Fig. 1A). Signs of diarrhea were clear from day 3 ($P<0.05$), but peak levels were found at day 5 ($P<0.001$), and 1, 2 and 3 weeks after DSS ($P<0.001$, $P<0.05$ and $P<0.05$ respectively) (Fig. 1B). Rectal bleeding was significant at day 5 with respect to sham group ($P<0.001$) however, was gradually decreased as the chronic inflammation progressed (Fig. 1C). In the same line disease activity index, it showed a significant increase at 5 days of DSS exposure, and 1 and 2 weeks after DSS removal ($P<0.001$, $P<0.01$ and $P<0.05$ respectively) (Fig. 2). By contrast, dietary resveratrol counteracted all these clinical signs. Although the animals also showed weight loss, it was significantly lower in relation to that observed in the standard diet at 1 week ($P<0.05$). Stool consistency showed better results at day 5 of DSS administration ($P<0.001$), and at 1, 2 and 3 weeks after DSS removal ($P<0.01$, $P<0.05$ and $P<0.05$, respectively). Finally, rectal bleeding also improved at day 5 ($P<0.001$) vs. standard diet group. Therefore, results from disease activity index improved significantly at 5 days of DSS treatment, and 1 and 2 weeks ($P<0.001$, $P<0.05$ and $P<0.05$ respectively) vs. standard diet-DSS animals. Moreover, the totality of resveratrol-fed animals survived and finished the treatment while animals fed with standard diet showed a mortality of 40% (6/15).

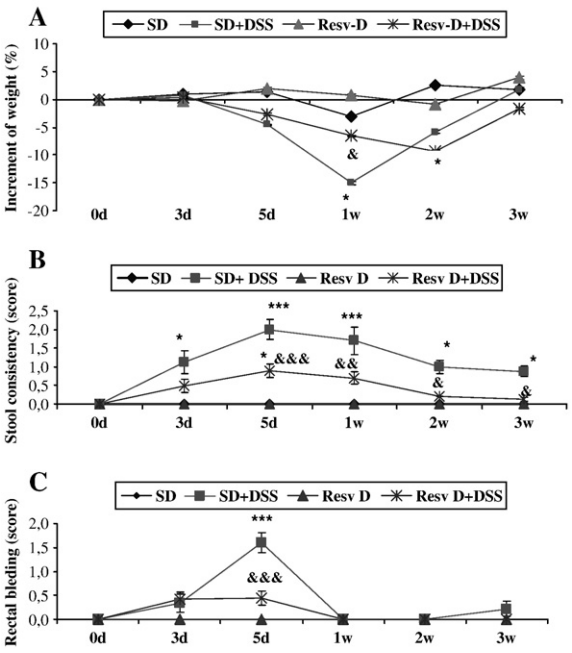

Fig. 1. Effect of resveratrol supplemented diet (Resv D) on clinical markers analyzed during acute and chronic colitis by dextran sodium sulphate (DSS) in C57BL/6 mice. Body weight was calculated by dividing body weight on the specified day by body weight at day 0 and expressed in percentage (A). Stool consistency (B) and rectal bleeding (C) were scored separately on a scale of 0–3 every 2 days for DSS treatment and for 1, 2 or 3 weeks after. Data are expressed as the means ± S.E.M. (*) $P<0.05$ and (***) $P<0.001$ vs. sham group; (&) $P<0.05$, (&&) $P<0.01$ and (&&&) $P<0.001$ vs. standard diet (SD) + DSS group.

3.2. Effect of resveratrol diet on colon weight/length and macroscopic inflammation

Control DSS-treated group showed a relation weight/length of 118.3 ± 15.1 mg/cm that was decreased to 84.4 ± 3.6 mg/cm, $P<0.01$ in the DSS-resveratrol group, demonstrating a first anti-inflammatory effect of resveratrol administration. Moreover, macroscopic inflammatory score in group fed resveratrol was significantly lower (0.75 ± 0.11, $P<0.05$) vs. DSS-control group (1.75 ± 0.21) at the end of treatment (Fig. 3).

3.3. Histological improvement of DSS-induced chronic colitis after dietary resveratrol

Histopathological analysis was carried out by hematoxylin/eosin method of the proximal, middle and rectum of the colon (Fig. 4). Slides from sham animals in both diets showed a normal structure of

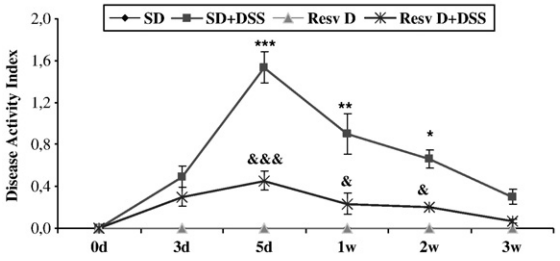

Fig. 2. Disease activity index after resveratrol supplemented diet (Resv D) during acute and chronic colitis by dextran sodium sulphate (DSS) in C57BL/6 mice. Disease activity index was evaluated as average of score of clinical parameters as body weight changes, rectal bleeding and stool consistency or diarrhea. Data are expressed as the means ± S.E.M. (*) $P<0.05$, (**) $P<0.01$ and (***) $P<0.001$ vs. sham group; (&) $P<0.05$ and (&&&) $P<0.001$ vs. standard diet (SD) + DSS group.

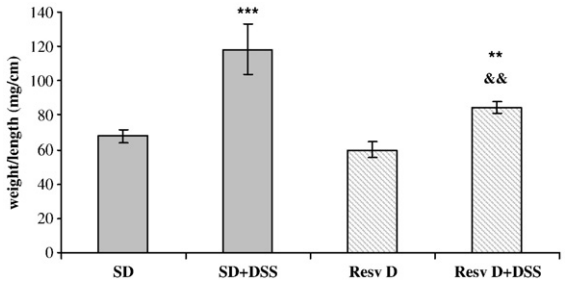

Fig. 3. Effect of resveratrol supplemented diet (Resv D) on weight/length of the colon in the experimental animal model of colitis by dextran sodium sulphate (DSS). Data are expressed as the means ± S.E.M. (**) $P<0.01$ and (***) $P<0.001$ vs. sham group; (&&) $P<0.01$ vs. standard diet (SD) + DSS group.

the colon. On the contrary, samples of mice on the standard diet after treatment with DSS presented loss of crypts, reduction of goblet cells, focal ulcerations and infiltration of inflammatory cells to the mucosa, principally in middle and rectum (Fig. 4D, E, and F). However, treatment with resveratrol dramatically reduced histological signs of cell damage. It could be observed exfoliation of epithelial cells, slightly infiltration of inflammatory cells and glandular hypertrophy, also regeneration of crypts and reepithelialization were observed in some areas (Fig. 4J, K, and L).

3.4. Effect of dietary resveratrol on colonic cytokines levels in DSS-induced chronic colitis

In animals fed with standard diet, chronic colonic injury induced by DSS administration was also characterized by an increase of the

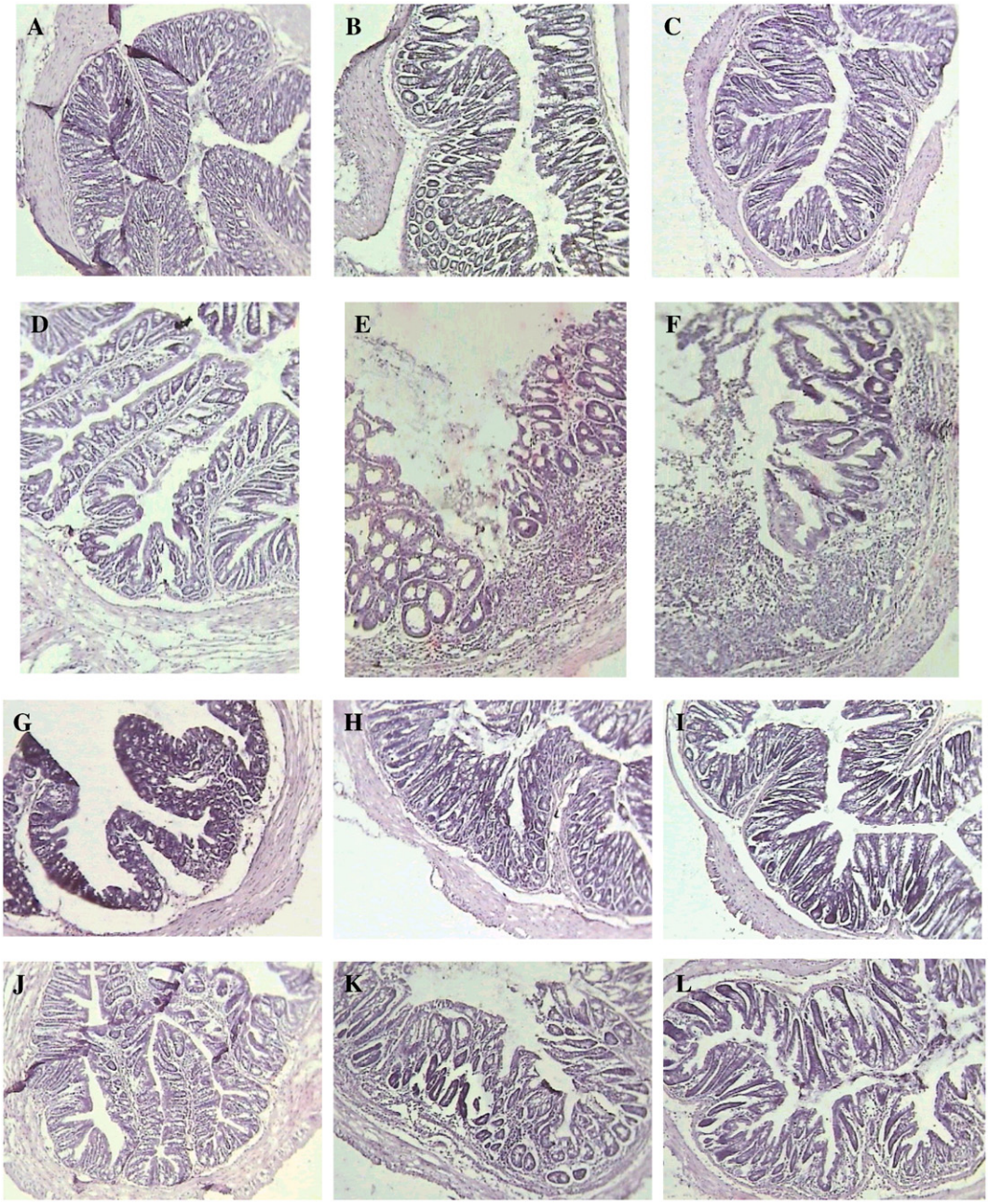

Fig. 4. Histopathology sections of proximal, middle and rectum of colonic lesions of mice fed with functional diet (D, E and F respectively) or supplemented with resveratrol diet (J, K, and L) and treated with 3% dextran sodium sulphate (DSS) for 5 days followed by 3 weeks of water. Histology sections of sham animals fed with functional diet (A, B, C) or supplemented with resveratrol diet (G, H, and I). Hematoxylin and eosin stain. Original magnification 20×.

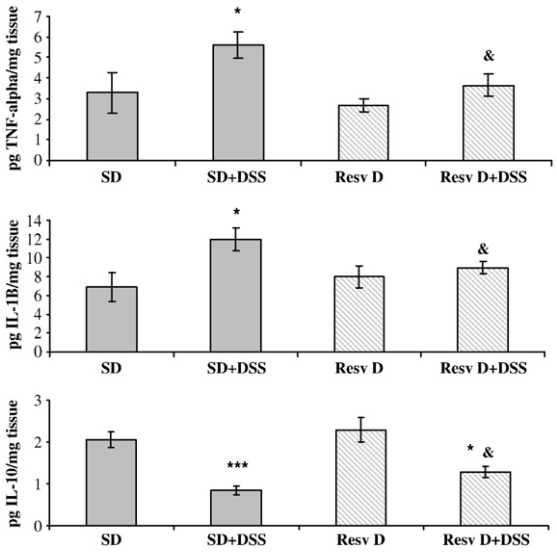

Fig. 5. Effect of resveratrol supplemented diet (Resv D) on factor of necrosis tumoral alpha (TNF-α) (A), interleukins (IL)-1β (B) and IL-10 (C) in the colon tissue after 3% of dextran sodium sulphate (DSS) for 5 days followed by 3 weeks of water. Data are expressed as the means ± S.E.M. (*) $P<0.05$ and (***) $P<0.001$ vs. sham group; (&) $P<0.05$ vs. standard diet (SD) + DSS group.

pro-inflammatory cytokines TNF-α and IL-1β ($P<0.05$), and a reduction of the anti-inflammatory cytokine IL-10 ($P<0.001$) compared with sham control group. In contrast, the levels of these cytokines were modified after resveratrol-enriched diet, TNF-α and IL-1β were significantly reduced ($P<0.05$) and IL-10 significantly augmented ($P<0.05$) compared with the effects observed for DSS-standard diet group (Fig. 5).

3.5. Effect of dietary resveratrol on colonic expression of inflammatory proteins in DSS-induced chronic colitis

The levels of protein expression were measured by western blotting of cytosolic extracts from colonic mucosa. As shown in Fig. 6, DSS exposure caused significant expression of PGES-1 and COX-2 in animals fed with standard diet ($P<0.05$ and $P<0.001$ respectively), on the contrary resveratrol diet induced downregulation of both proteins in DSS group ($P<0.01$ and $P<0.05$ respectively vs. standard diet-DSS group).

In the same way iNOS was significantly expressed in DSS group fed with standard diet ($P<0.001$) and resveratrol diet significantly reduced its expression ($P<0.05$ vs. standard diet) (Fig. 7).

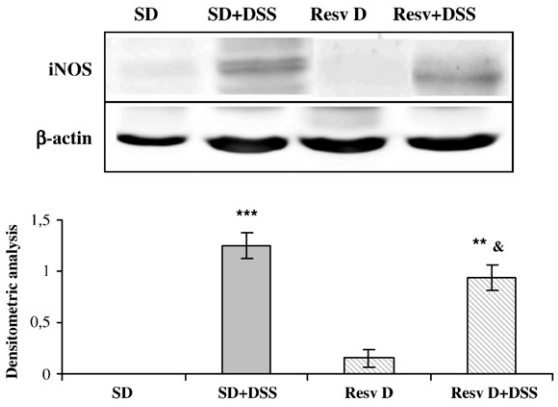

Fig. 7. Effect of resveratrol supplemented diet (Resv D) on expression of inducible nitric oxide synthase (iNOS) enzyme in the colon tissue after 3% of dextran sodium sulphate (DSS) for 5 days followed by 3 weeks of water. Western blotting with antibodies against iNOS has been described in Material and methods section. Data are expressed as the means ± S.E.M. (**) $P<0.01$ and (***) $P<0.001$ vs. sham group; (&) $P<0.05$ vs. standard diet (SD) + DSS group.

We also examined the expression and activation of the p38 MAPK by western blot analysis using phosphospecific MAPK antibodies. To standardize protein loading in each line, blots were stripped and reproved with the corresponding antibodies against p38 MAPK. Administration of DSS resulted in a significant increase in the phosphorylation of p38 MAPK protein ($P<0.05$), indicating that the p38 MAPK protein activation could be induced at the chronic stage of colonic lesion caused by DSS. Interestingly, administration of resveratrol was able to diminish p38 MAPK protein activation ($P<0.05$) (Fig. 8).

4. Discussion

In the present study, results indicate that dietary administration of 3 mg/kg/day of resveratrol reduced the severity and extension of progressive chronic colonic damage induced by a short 5-day exposure of DSS followed by a 3 week rest period in C57BL/6 mice. In control animals, the acute phase (day 5) was characterized by clinical signs, i.e. diarrhea, bloody feces, and body weight loss and the chronic phase (day 21) was characterized by recovered body weight, no/few clinical signs, high inflammatory markers and histopathological changes (Melgar et al., 2005). Resveratrol counteracted significantly the clinical signs reducing the inflammatory process. Moreover, there was an attenuation of morphological signs of cell damage, the colonic mucosa showed areas of exfoliation of epithelial cells, reduction of inflammatory cells infiltration and glandular hypertrophy, as well as some areas of intact epithelium. These results are

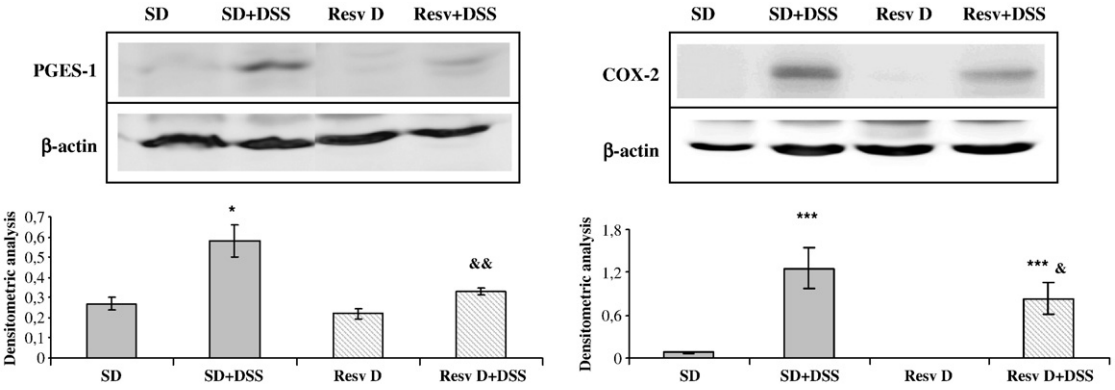

Fig. 6. Effect of resveratrol supplemented diet (Resv D) on prostaglandin E_2-synthesizing enzymes in the colon tissue after 3% of dextran sodium sulphate (DSS) for 5 days followed by 3 weeks of water. Western blotting with antibodies against prostaglandin E synthase (PGES)-1 (A) and cyclooxygenase-2 (COX-2) (B) has been described in Material and methods section. Data are expressed as the means ± S.E.M. (*) $P<0.05$ and (***) $P<0.001$ vs. sham group; (&) $P<0.05$ and (&&) $P<0.01$ vs. standard diet (SD) + DSS group.

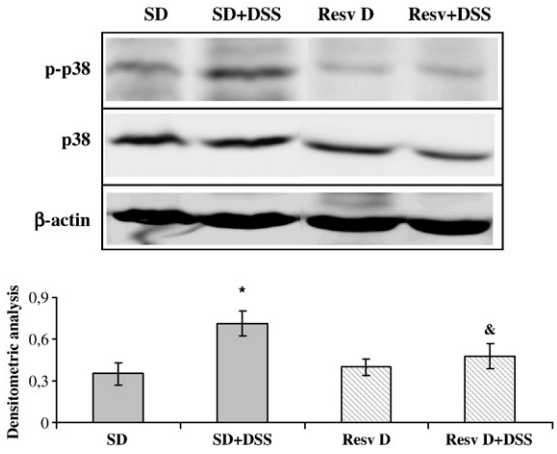

Fig. 8. Effect of resveratrol supplemented diet (Resv D) on expression and activation of the p38 MAPK using phosphospecific MAPK antibodies in the colon tissue after 3% of dextran sodium sulphate (DSS) for 5 days followed by 3 weeks of water. Data are expressed as the means ± S.E.M. (*) $P<0.05$ vs. sham group.

consistent with previous data using different models of colonic damage, where it was documented that resveratrol, as well as other stilbenes such as 2,3,5,4′-tetrahydroxystilbene-2-O-beta-D-glucoside (Wang et al., 2008), was capable of decreasing the degree of inflammation associated with experimental colitis (Martín et al., 2004, 2006; Larrosa et al., 2009). Although bioavailability of resveratrol is low after oral ingestion (Walle et al., 2004), it has been suggested that luminal resveratrol may have topical activity on colonic epithelial cells independent of systemic absorption. All these observations may have significance on the beneficial effect of resveratrol in ulcerative colitis.

An important finding was the high reduction of mortality in DSS-resveratrol animals. The mechanism by which resveratrol reduces DSS-induced mortality are not clear, although its antioxidant, anti-inflammatory and anti-infectious properties may play a role (Kawada et al., 1998; Mahady et al., 2003; Docherty et al., 2001; Bujanda et al., 2006; Kwon et al., 2008). This result is in agreement with other studies where resveratrol administration did not increase mortality in alcohol-induced liver damage in mice (Kwon et al., 2008; Bujanda et al., 2008).

Infiltration of inflammatory cells into the mucosa has been suggested to contribute significantly to mucosal dysfunction associated with colitis as they represent a major source of reactive oxygen and nitrogen species in the inflamed colonic mucosa (Martín et al., 2006; Deniz et al., 2004). Moreover, human and animal studies support the idea that cytokines, such as TNF-α and IL-1β, are correlated with the development of colonic inflammation (Podolsky, 1991a,b; Rojas-Cartagena et al., 2005) helping to propagate the extension of a local or systemic inflammatory process. Blocking of the action of endogenous TNF-α and IL-1β attenuates acute and chronic experimental colitis and its systemic complications (Rogler and Andus, 1998; Arai et al., 1998). Our data showed that resveratrol diet was able to diminish both parameters, which is in line with the above mentioned previous reports, thus inhibiting the pro-inflammatory response. In addition, the degree of inflammation and damage induced by DSS was paralleled to low levels of the anti-inflammatory cytokine IL-10 in colonic specimens. A previous study by Camacho-Barquero et al. (2007) documented a downregulation of this cytokine in an experimental model of chronic ulcerative colitis, interestingly our study reports for the first time the increase in IL-10 levels induced by the stilbene. Accordingly, the ability of resveratrol to partially reduce the inflammatory cell infiltrate in the colon could in part explain the observed modulation in the levels of these cytokines (Martín et al., 2004, 2006).

Prostaglandin E_2-synthesizing enzymes and iNOS are enzymes that play a pivotal role in mediating inflammation and contribute to DSS-induced inflammation (Hennebert et al., 2008). In this regard, COX-2 activation produces excessive PGE_2 and TXB_2, which are important inflammatory mediators that plays important roles in cell adhesion, angiogenesis and contribute to the intestinal hyperaemia, oedema and even dysfunction (Subbaramaiah et al., 2004). Likewise, iNOS activation leads to excessive production of NO which may be detrimental to the integrity of the colonic based on the generation of reactive nitrogen species causing cellular degeneration in various tissues and contributing to the development of intestinal damage (Dignass et al., 1995). Additionally, iNOS acts in synergy with COX-2 to promote the inflammatory reaction (Itzkowitz, 2006). Furthermore, both COX-2 and iNOS expression are upregulated by MAPK in intestinal epithelial cells (Kim et al., 2005a,b).

In the present study, we have also demonstrated that (1) macroscopic damage was associated with PGES-1, COX-2 and iNOS overexpression and (2) resveratrol treatment reduced COX-2 and iNOS immunosignals and returned PGES-1 to basal levels. These results are in line with previous *in vivo* studies by Martín et al. (2004, 2006), who documented a decrease of both PGE_2 levels and COX-2 protein expression on colonic mucosa in acute and chronic inflammatory conditions. Indeed, it has further been reported that this polyphenol decreased the levels of the enzyme PGES-1 during early inflammation in colonic mucosa (Larrosa et al., 2009). Our data are also consistent with prior *in vitro* studies in human mammary, oral epithelial cells, mouse macrophages and beta-amyloid-treated C6 glioma cells, in which resveratrol inhibited COX-2 expression and production of prostaglandin E_2 and reduced inflammation by down-regulation of iNOS mRNA and protein (Subbaramaiah et al., 1998; Martinez and Moreno, 2000; Leiro et al., 2004; Kim et al., 2006). In contrast, Jang et al. (1997) detected no influence on induction of COX-2 on phorbol ester-mediated induction of COX-2 in mouse skin and Candelario-Jalil et al. (2007) showed that resveratrol is the first known inhibitor which specifically prevents PGES-1 expression without affecting COX-2 levels in LPS-activated microglia, indicating that the effect of resveratrol on these enzymes depends on tissue and/or species studied.

p38 MAPK is a key modulator of several target genes that ultimately control infiltration of monocytic cells, acute intestinal inflammation and intestinal electrolyte and water secretion. The importance of p38 MAPK in ulcerative colitis is supported by recent experiments where the use of p38 MAPK inhibitors abrogated colitis (Waetzig et al., 2002). Moreover, a recent study has demonstrated that it can be effective for human inflammatory bowel disease (Hommes et al., 2002). They also regulate cytokine production in response to a variety of stimuli (Kyriakis and Avruch, 2001) and up-regulate COX-2 and iNOS expression in intestinal epithelial cells (Kim et al., 2005a,b). Interestingly our study reports, for the first time, a reduction of the expression of p-p38 MAPK by the stilbene. Several *in vitro* studies have determined that the modulation of p38 and p42/44 cascades is a key event in the resveratrol action (El Mowafy and White, 1999). In line with these findings, studies with resveratrol tetramer isolated from *Vitis vinifera* roots suppressed LPS-induced NO production by inhibiting p38 phosphorylation activation (Sun et al., 2009). Likewise, a methylated derivative of resveratrol has shown to inhibit invasion of human lung adenocarcinoma by suppressing the MAPK pathway (Yang et al., 2009).

In conclusion, we have demonstrated that dietary supplementation of resveratrol exerted a significant beneficial effect in chronic DSS-induced colitis. The anti-inflammatory effects seem to be related to a cytokines modulation and a reduction of PGES-1, COX-2 and iNOS expression in colonic mucosa, in addition to other possible mechanisms, via downregulation of p-38 MAPK pathway. We conclude that resveratrol diet represents a novel approach to the treatment of chronic intestinal inflammation.

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