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Monochloramine induces acute and protracted colitis in the rat: Response to pharmacological treatment

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Abstract

Monochloramine is a powerful oxidative molecule that is produced in inflammatory sites. We investigated the effect of intrarectally administered monochloramine (3.2 mg) in the rat. A single enema induced after 24 h an intense inflammatory reaction characterized by mucosal necrosis, submucosal edema, hemorrhage and colonic thickening, as well as induction of nitric oxide synthase and tumor necrosis factor and an increase in the interferon γ/interleukin 4 ratio. The inflammatory response peaked 3–5 days after monochloramine administration and then followed a extended recovery phase. At 1 week there was substantial but incomplete mucosal repair, submucosal edema, neutrophil/macrophage infiltration and increased myeloperoxydase and alkaline phosphatase activities. Oxidative stress, as determined by malonyldialdehyde levels, was prominent only in the acute phase (3–5 days). Monochloramine colitis was amenable to pharmacological treatment with sulphasalazine or prednisolone, suggesting that it may be used as an experimental model of inflammatory bowel disease. In conclusion, monochloramine induces acute and protracted colonic inflammation in the rat. Locally produced monochloramine might contribute to the perpetuation of inflammatory bowel disease.

Keywords: Monochloramine; Oxidative stress; Inflammatory bowel disease; Alkaline phosphatase; Myeloperoxidase

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Introduction

Inflammatory bowel disease (IBD) is a group of two generally related but distinct diseases, namely ulcerative colitis and Crohn's disease. Both are multifactorial disorders typically presenting with diarrhea, rectal bleeding and abdominal pain (Sands, 2000). While the exact causative factors of IBD have not been identified, current understanding of this condition has implicated an exacerbated immune response to otherwise innocuous stimuli, probably belonging to luminal bacteria which are a normal part of the intestinal flora (Guarner and Malagelada, 2003). In order to explore the etiopathogenic mechanisms underlying the initiation, progression and chronicity of IBD, a number of experimental animal models have been used, but the complexity of these disorders is not easily reproducible, and there is no ideal model of IBD (Hoffmann et al., 2002–2003). Instead, there are multiple valuable experimental models available to study the different components involved in IBD and their use depends on the questions being addressed.

Chemically induced models consist normally in the luminal instillation of agents that are toxic to the colonic mucosa or that are capable of eliciting an immune response. The most widely used is probably trinitrobenzenesulfonic acid (TNBS) induced colitis (Morris et al., 1989; Sanchez de Medina et al., 1996). This model shares many of the histopatological and clinical features of human Crohn's disease and it is a simple and reasonably reproducible model. The mechanism of TNBS apparently involves its capacity to act as a hapten as well as direct toxic effects on the mucosa which are mediated by reactive oxygen species (Grisham et al., 1991). On the other hand, continuous administration of dextran sulfate sodium or carragenan in drinking water induces a more chronic type of colitis, and the lengthy period of induction allows investigation of early preclinical phases of the inflammatory response (Stucchi et al., 2000; Onderdonk, 1985). Manipulation of doses in these models can vary the intensity of the response. Many other models produce only acute, self limited colitis, including acetic acid, phorbol esters, etc. (Kim and Berstad, 1992; Hoffmann et al., 2002–2003).

The HLA-B27/β_2m transgenic rat represents a chronic intestinal inflammation model induced by a human class I major histocompatibility complex molecule which is linked to human autoimmune disease (Hammer et al., 1990). This model allows the study of the complex interactions between the flora, the immune system and susceptibility genes in disease induction. Over-expression (transgenic) or deletion (knockout) of specific genes have led to the development of a number of rodent models to determine the functions of cytokines in vivo (interleukin 2, interleukin 10, T cell receptor, etc.) and to evaluate the results of the application of some of the existing therapeutic alternatives. In other cases colonic inflammation is elicited by the transfer of immune cells from donor animals. Despite the claimed 'spontaneous' nature of these models, it must be noted that the technical manouvers used to induce colitis in these animals are strictly unrelated to IBD and, in fact, the resultant inflammation does not match the pathophysiology of the human condition (Neurath et al., 2000).

Intestinal inflammation is accompanied by excesive production of reactive oxygen and nitrogen metabolites (Kruidenier and Verspaget, 2002). It is also known that the DNA damage caused by oxidative stress is a major contributor to colorectal cancer development in IBD patients. It has previously been shown that the balance between the most important antioxidants in the intestinal mucosa is seriously impaired in IBD patients compared with normal mucosa (Kruidenier et al., 2003). Chronic inflammation is associated with the genesis of reactive oxygen species, which in turn may activate certain transcription factors that further stimulate the inflammatory response, such as NF-kB (Magnani et al., 2000). In this context, chloramine derivatives, including monochloramine, are relatively long lived

physiological oxidants relevant to the pathogenesis of inflammation in the gastrointestinal tract (Witko-Sarsat et al., 1995). We have tested the hypothesis that oxidants may contribute significantly to the inflammatory response observed in IBD by examining the effects of a number of oxidative molecules on the rat colon. We found that monochloramine was a powerful inducer of colitis compared with agents such as hydrogen peroxide or tert-butyl hydroperoxide and therefore we proceeded to characterize in detail the intestinal proinflammatory effects of the former. Finally, we determined the response to colonic antiinflammatory drugs to examine the feasibility of monochloramine colitis as an IBD model.

Materials and methods

Animals and colitis induction

Female Wistar rats (200–220 g) provided by the Laboratory Animal Service of the University of Granada and Harlan Interfauna Ibérica (Barcelona, Spain) were used for the experiments. This study was carried out in accordance with the Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of the European Union (86/609/EEC) and was approved by the Ethics Committee of the University of Granada. Colitis was induced in animals fasted overnight by the administration of an enema containing 0.25 ml of monochloramine (NH_2Cl) 0.25 M, equivalent to 3.2 mg/rat. Monochloramine was obtained fresh every time by reacting equal amounts of sodium hypochloride (Aldrich, Madrid, Spain) and ammonium chloride. The reaction was followed at 242 nm (Musch et al., 1999). Animals were sacrificed after either 24 hours or 1 week (7 rats per group). In a second experiment animals were sacrificed 1, 3, 5, 7 or 14 days after monochloramine administration (5 rats per group). Control animals received a saline enema.

Pharmacological treatment

In order to examine the susceptibility to pharmacological manipulation of monochloramine colitis, some animals (7 per group) were treated with prednisolone (20 mg/kg) or sulphasalazine (500 mg/kg) daily, starting 3 days before colitis induction and continuing until the animals were sacrificed. Drugs were delivered by means of an oesophageal catheter.

Assesment of intestinal damage

Animals were killed by cervical dislocation. The colon was removed and placed on an ice-cold plate, cleaned of fat and mesentery, and blotted on filter paper. Each specimen was weighed and its length measured under a constant load (2 g). The intestinal segments were subsequently divided longitudinally in 3–4 pieces and immediately frozen in liquid nitrogen for biochemical determinations. Colon damage was scored for visible damage on a 0 to 25 scale according to the following criterion: adhesions (0–3), obstruction (0–2), thickening (0–2), hyperemia (0–3), fibrosis (0–3), necrosis (0–5), scarring and deformation (0–7). One of the segments was used to measure myeloperoxidase (MPO) activity, an index of neutrophil accumulation. MPO was determined spectrophotometrically, according to the technique described by Krawisz et al. (1984). The results are expressed as myeloperoxidase units (μmol/min) per milligram of protein (Smith et al., 1985). Another piece of tissue was destined

for determination of alkaline phosphatase (AP) activity, which was performed spectrophotometrically, using disodium nitrophenylphosphate (5.5 mM) as substrate in 50 mM glycine buffer with 0.5 mM $MgCl_2$ (pH = 10.5) (Bessey et al., 1946). The sensitivity of alkaline phosphatase to chemical inhibitors (levamisole, homoarginine) was also measured. The enzymatic activity is expressed as mU/mg protein (Smith et al., 1985). Malondialdehyde (MDA), an index of oxidative stress, was quantitated in a separate sample as thiobarbituric acid reactive substances (TBARS) by the method of Zingarelli et al. (1999).

Histological study

Tissue samples from the distal colon were obtained from the same areas (4 cm from the anus) in all animals and fixed in 4% phosphate-buffered formaldehyde. Paraffin sections were prepared and stained with haematoxylin and eosin.

Western blot

The samples were homogenized in ice-cold lysis buffer (0.1% SDS, 0.1% sodium deoxycholate, 1% Triton X-100 in phosphate buffered saline) with freshly added protease inhibitors (phenylmethylsulfonil fluoride, aprotinin, leupeptin, iodoacetamide, 1,10-phenantroline). Protein concentrations were determined by the bicinchoninic acid assay (Smith et al., 1985), using bovine serum albumin as standard. Samples were boiled for 4 minutes in Laemmli buffer, separated by SDS-PAGE, transferred to a nitrocellulose membrane and blocked with 5% (w/v) nonfat dry milk in Tris buffered saline. Equal loading was routinely checked by reversible red Ponceau staining. After incubation with the pertinent antibodies the bands were detected by enhanced chemiluminiscence (NEN, Zaventem, Belgium). The antibody for inducible nitric oxide synthase (iNOS) was purchased from BD Biosciences. The B4-78 antibody against human tissue nonspecific AP developed by Dr Katzmann (Lawson et al., 1985) was obtained from the Development Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA 52242. The antiserum against rat neutrophils was obtained from Accurate Chemical and Scientific Corporation (Westbury, NY). All secondary antibodies were from Sigma (Madrid, Spain).

Cytokine determination

For the determination of interleukin-1β (IL-1β) and tumor necrosis factor (TNF) one segment of colonic tissue was processed for RNA obtention using Trizol (Life Technologies, Gaitherburg, MD) following the manufacturer's instructions. RNA integrity was checked by gel electrophoresis and retrotranscribed (First-strand cDNA synthesis kit, Amersham Pharmacia Biotech, Barcelona, Spain), and the resulting cDNA was amplified by PCR using specific primers for IL-1β (sense 5′-AAT GAC CTG TTC TTT GAG GCT GAC-3′; antisense 5′-CGA GAT GCT GCT GTG AGA TTT GAA G-3′), TNF (sense 5′-TGT GCC TCA GCC TCT TCT CAT TC-3′; antisense 5′-CAT TTG GGA ACT TCT CCT CCT TG-3′) and β-actin (sense 5′-GGC CAA CCG TGA AAA GAT G-3′; antisense 5′-GGA TCT TCA TGA GGT AGT CTG TC-3′). For the determination of interleukin 4 (IL-4) and interferon γ (IFN-γ) colonic samples were homogenized in phosphate buffered saline containing 1% Triton X-100, 0.1% sodium

deoxycholate, 0.1% sodium dodecylsulphate and protease inhibitors, cleared by centrifugation and analyzed by enzyme linked immunoassay (Biosource International, Camarillo, CA).

Reagents

Except where indicated, all chemicals were obtained from Sigma (Madrid, Spain).

Statistics

Results are expressed as mean ± standard error of the mean (S.E.M.). Differences among means were tested for statistical significance by one way analysis of variance and a posteriori least significance tests on preselected pairs. Colonic damage score were tested for normality and equal variance and subsequently analyzed by one way analysis of variance. Differences between group pairs were established with Student's unpaired *t* test. Differences in the incidence of diarrhea were analyzed by the χ^2-square test. All statistical analyses were carried out with the SigmaStat program (Jandel Corporation, San Rafael, CA). Statistical significance was set at $P < 0.05$.

Results

Colonic inflammation

Colonic instillation of monochloramine elicited an intense inflammatory response after 1 day characterized by mucosal necrosis, edema, hemorrhage and loss of crypts (Fig. 1, see also Fig. 7). The mucosa appeared as an eosinophilic layer, representing the necrosed epithelial cells, which also contained a significant number of erythrocytes. The submucosa was dilated as a consequence of edema but the inflammatory infiltrate was relatively mild in some cases. Macroscopically, this was associated to a 60% increase in the colonic weight/length ratio, which in turn resulted from a combination of reduced colonic length (Table 1) and higher colonic weight (not shown). The colonic damage score was also significantly increased by monochloramine administration (Table 1). The mean linear extension of the necrosed area was 3.4 ± 0.5 cm. Adhesions with adjacent organs were present in some cases. Monochloramine acute colitis was also characterized by biochemical changes. Thus myeloperoxidase activity, a marker of neutrophils and other leukocytes, was significantly increased 24 h after the enema (Fig. 2), as was MDA, a marker of oxidative stress (Fig. 3). However, the levels of alkaline phosphatase activity were not altered by monochloramine at this time point (Fig. 4). Moreover, no changes were detected in the AP sensitivity to levamisole. Colonic function was also altered by monochloramine challenge, since all animals suffered from diarrhea.

Colonic status was also examined 1 week after monochloramine instillation. Histological analysis of colonic samples showed a general restoration of normal mucosal architecture but still with some rests of ulcerations and sometimes necrosis (Fig. 1). The presence of inflammatory cells (neutrophils and macrophages) was more prominent and consistent among experimental animals. Colonic shortening and thickening persisted or were actually aggravated at this time point (Table 1). Fibrosis was not apparent. At the biochemical level, MPO activity was similar to that observed at 24 h, although the variability was clearly smaller (Fig. 2). However, MDA colonic levels were not significantly elevated (Fig. 3). On the other

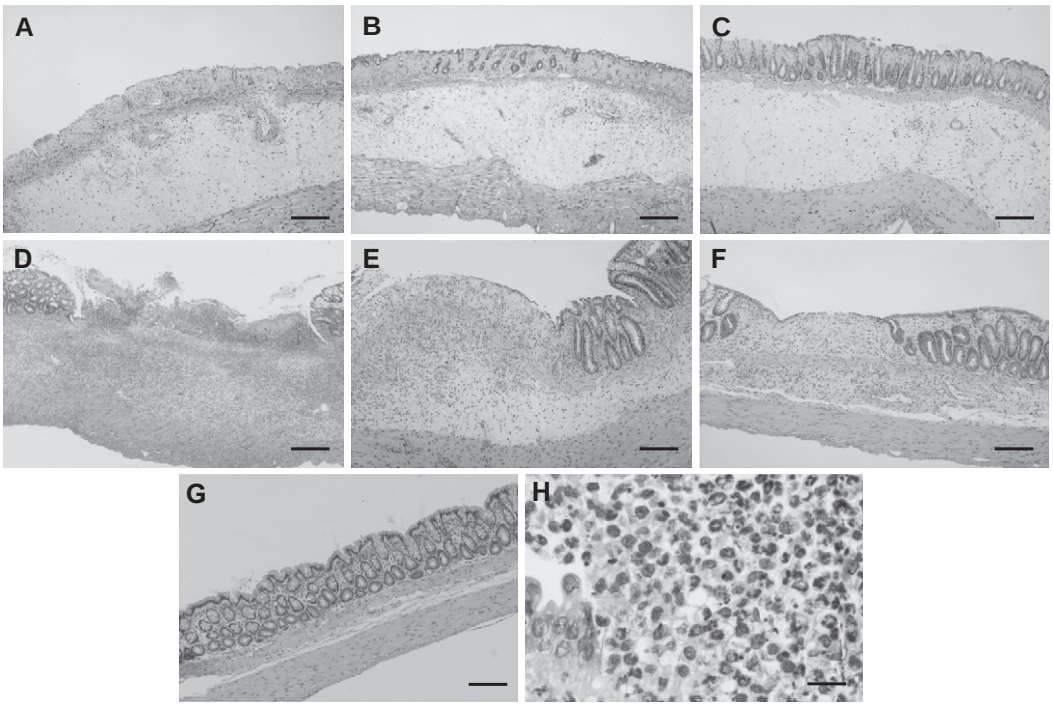

Fig. 1. Histological sections of monochloramine treated rats. Scale bar: 50 μm. A, D: Control group, 24 h and 1 week; B, E: Prednisolone group, 24 h and 1 week; C, F: Sulphasalazine group, 24 h and 1 week; G: Uninflamed group; H: Detail of control group at 1 week, showing neutrophil and macrophage infiltration (scale bar: 5 μm).

hand, AP activity was dramatically increased at 1 week compared with the normal, uninflamed animals (Fig. 4). This effect was associated with a significantly higher sensitivity to the AP inhibitor levamisole (Fig. 4) and also homoarginine (not shown). Finally, diarrhea was not widespread at this stage.

Western blot analysis of tissue lysates using a polyclonal antibody against rat neutrophil antigens showed a clear increase in the presence of these cells in the inflamed colon, both at 1 day and at 1 week (Fig. 5). The expression of iNOS was enhanced also in the colitic animals, specially in the acute stage (Fig. 5). Finally, the involvement of tissue nonspecific AP was confirmed by immunoblotting (Fig. 5).

Table 1
Macroscopic parameters in monochloramine acute and chronic rat colitis and effects of prednisolone and sulphasalazine

		Weight/length ratio (mg/cm)	Colonic length (cm)	Colonic damage score
Uninflamed		68.2 ± 3.3	17.3 ± 0.5	0
1 d colitis	Control	109.3 ± 9.7^{+}	14.1 ± 0.4^{+}	6.1 ± 0.9^{+}
	Prednisolone	97.3 ± 8.7^{+}	14.0 ± 0.7^{+}	5.3 ± 1.0^{+}
	Sulphasalazine	82.4 ± 6.1*	14.9 ± 0.4^{+}	3.9 ± 0.3^{+}*
7 d colitis	Control	123.3 ± 11.9^{+}	13.7 ± 0.4^{+}	10.4 ± 1.2^{+}
	Prednisolone	84.1 ± 5.9*	14.4 ± 0.5^{+}	5.7 ± 0.9^{+}*
	Sulphasalazine	73.9 ± 4.7*	15.9 ± 0.5^{+}*	5.4 ± 1.6^{+}*

* $P < 0.05$ vs. control.
$^{+}$ $P < 0.05$ vs. uninflamed.

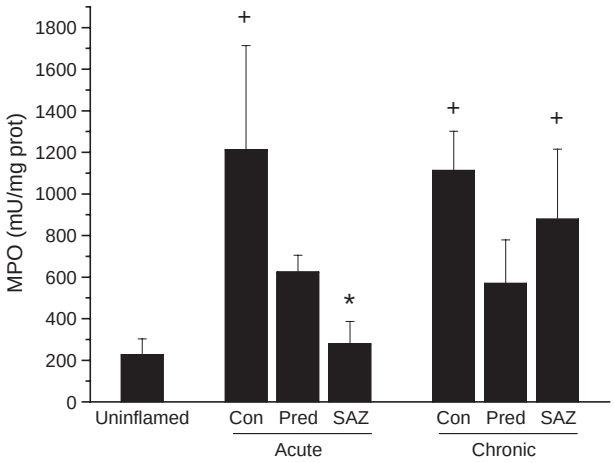

Fig. 2. Myeloperoxidase colonic activity in rat monochloramine colitis. $^{+}P < 0.05$ vs. uninflamed group; $*P < 0.05$ vs. control group. Con: control; Pred: prednisolone; SAZ: sulphasalazine.

Cytokine profile

Next we aimed to characterize the nature of the inflammatory response to monochloramine by measuring various relevant cytokines. First we determined the levels of IFN-γ and IL-4 as representative Th-1 and Th-2 cytokines, respectively, and calculated the IFN-γ/IL-4 ratio. The results obtained show a marked increase in this parameter at 24 h (4.1 ± 0.8 vs. 1.0 ± 0.3, $P < 0.05$ by one-way analysis of variance) but not 1 week (0.8 ± 0.2, not significantly different from the control group). On the other hand, the colonic expression of TNF, as assessed by RT-PCR, was significantly increased at 1 but not 7 days. The expression of IL-1β, on the other hand, was also significantly augmented, both at the mRNA (Fig. 6) and at the protein level (Fig. 5).

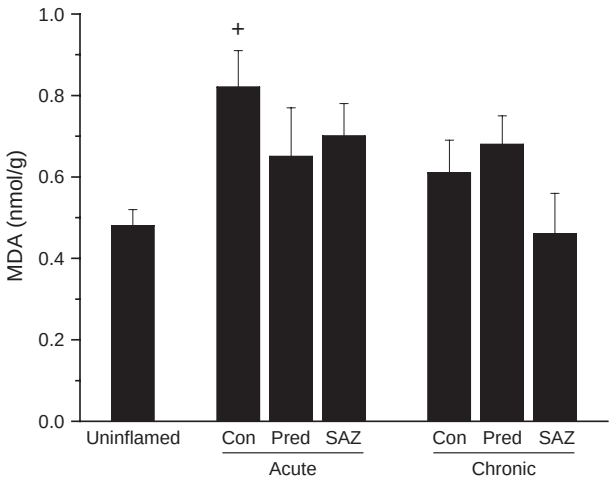

Fig. 3. Malondialdehyde colonic levels in rat monochloramine colitis. $^{+}P < 0.05$ vs. uninflamed group. Con: control; Pred: prednisolone; SAZ: sulphasalazine.

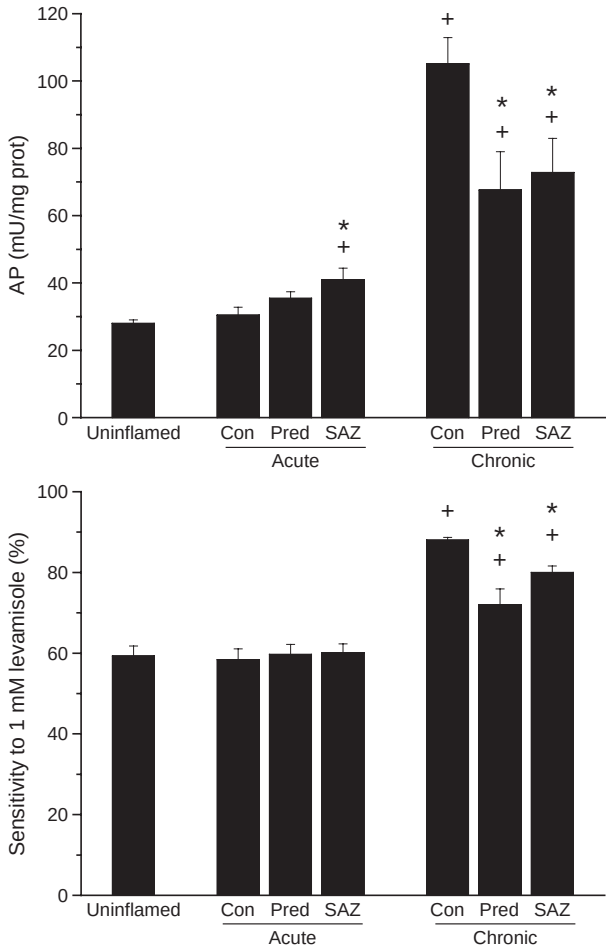

Fig. 4. Alkaline phosphatase colonic activity and sensitivity to levamisole in rat monochloramine colitis. $^{+}P < 0.05$ vs. uninflamed group; $*P < 0.05$ vs. control group. Con: control; Pred: prednisolone; SAZ: sulphasalazine.

Response to pharmacological treatment

Next we set out to verify whether monochloramine colitis is amenable to pharmacological manipulation. To this end prednisolone (20 mg/kg·day) or sulphasalazine (500 mg/kg·day) were administered, starting 3 days before monochloramine challenge and continuing until the animals were sacrificed. Treatment with prednisolone had little if any effect on the acute phase of monochloramine colitis. Thus MPO, MDA, colonic weight/length ratio and damage score were lower than in the control group, but this difference did not reach statistical significance (Figs. 2 and 3, Table 1). At the histological level, prednisolone treated animals did show some preservation of the crypts (Fig. 1). There was no change in the incidence of diarrhea. The effect was however marked at 1 week, with significant reduction in colonic weight/length ratio, damage score and AP activity, including sensitivity to levamisole (Table 1, Fig. 4). Amelioration of inflammatory status was observed as well at the microscopic level (Fig. 1), with some evidence of a smaller degree of infiltration. In this regard,

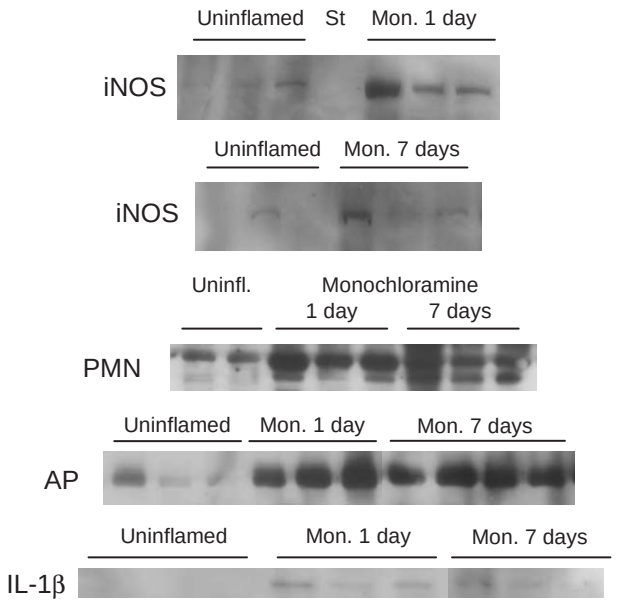

Fig. 5. Western blot analysis of colonic levels of iNOS, polymorphonuclear neutrophils (PMN), tissue nonspecific alkaline phosphatase and IL-1β in rat monochloramine colitis. Several samples per group are shown. St: standard.

mean MPO levels were the lowest of all animals at 1 week, even though the differences were not significant.

On the other hand, sulphasalazine treatment resulted in a more pronounced therapeutic effect. Thus mucosal crypts were better preserved in animals treated with this drug at 24 h of colitis, specially at the base (Fig. 1). Epithelial cells were also partially protected, so that an epithelial layer was visible in many cases. Diarrhea was present in only 40% of animals ($P < 0.05$). Colonic weight/length ratio, damage score and MPO were significantly lower in the sulphasalazine group than in the control (Fig. 2, Table 1).

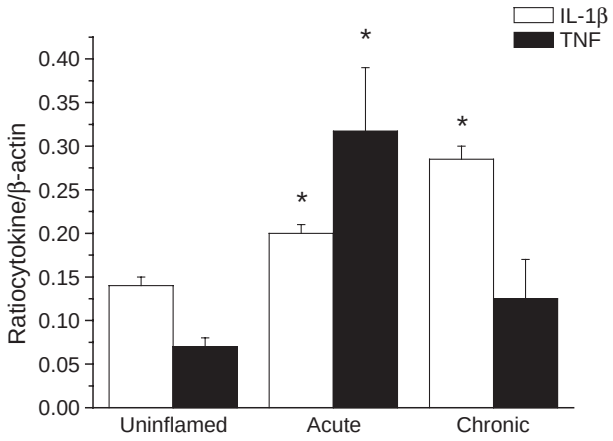

Fig. 6. Colonic cytokine expression in the rat monochloramine colitis. TNF and IL-1β were determined by RT-PCR and normalized to the level of β-actin. *$P < 0.05$ vs. uninflamed group.

Table 2
Macroscopic parameters 1, 3, 5, 7 and 14 days after monochloramine administration

	Weight/length ratio (mg/cm)	Colonic length (cm)
Uninflamed	54.8 ± 2.1	18.9 ± 0.5
1 day	95.9 ± 5.0*	14.5 ± 0.4*
3 days	95.0 ± 6.7*	14.2 ± 0.5*
5 days	95.9 ± 11.5*	16.6 ± 0.6*
7 days	78.4 ± 2.5	17.1 ± 0.7*
14 days	63.8 ± 3.4	17.8 ± 0.3

* $P < 0.05$ vs. uninflamed.

In addition, MDA levels were not significantly different from those of the uninflamed group (Fig. 3). AP levels were slightly but significantly increased (Fig. 4). After 1 week, sulphasalazine maintained all these effects except the lowering of MPO. Colonic shortening was significantly counteracted by the treatment at this stage (Table 1). Histologically, these animals showed only low grade infiltration together with epithelial restoration which apparently resulted from epithelial cell hyperplasia (Fig. 1). New microvessels were also generated.

Time course

In an attempt to further characterize the time course of monochloramine induced colitis an additional experiment was carried out in which colitic status was examined at 1, 3, 5, 7 and 14 days after monochloramine administration. The results are shown in Table 2 and Figs. 7 and 8. The groups that

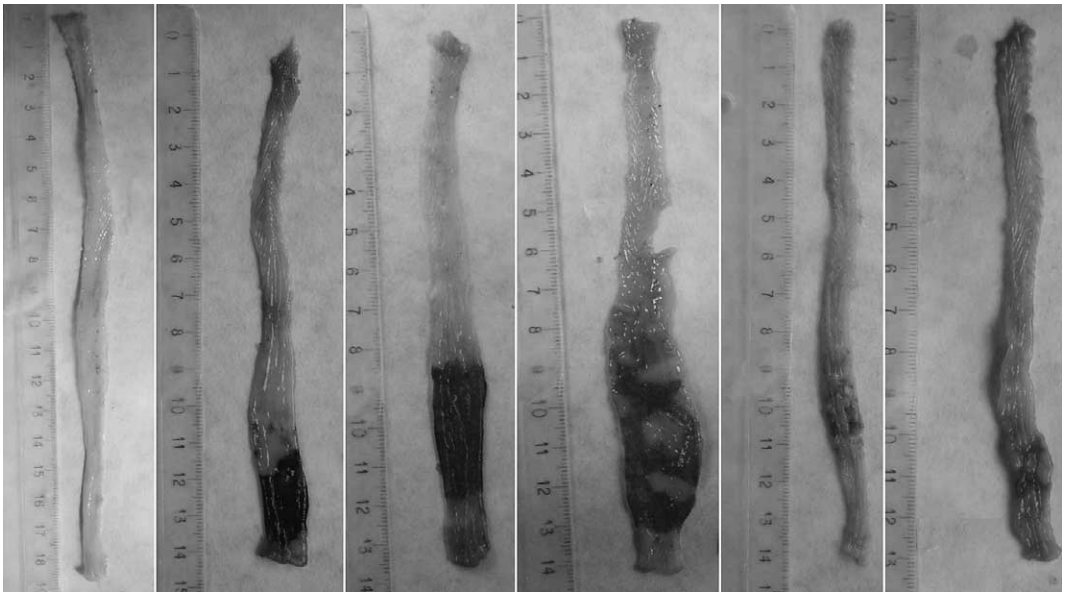

Fig. 7. Macroscopic appearance of the uninflamed rat colon (left) and of monochloramine induced colitis after 1, 3, 5, 7 and 14 days (left to right). Darkened areas correspond to epithelial necrosis and later to scarring. Please note that the scales are slightly different.

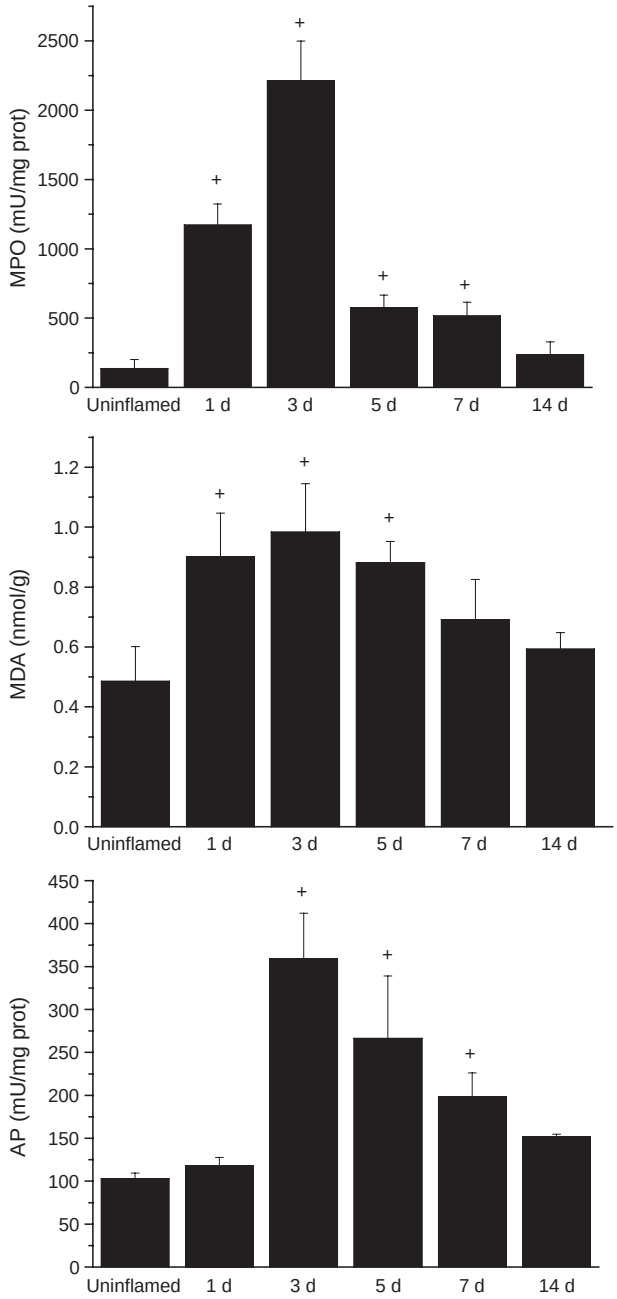

Fig. 8. Myeloperoxidase, malondialdehyde and alkaline phosphatase colonic levels in normal (uninflamed) rats and in rats 1, 3, 5, 7 and 14 days after monochloramine administration. $^{+}P < 0.05$ vs. uninflamed group (n=5).

received monochloramine lost a maximum of 5.8 ± 0.1% of body weight after 2 days, while the controls had practically regained their initial weight (−0.9 ± 0.2%, $P < 0.05$ vs. combined monochloramine groups). This difference disappeared at the fourth day after colitis induction. During this 4 day period

monochloramine treated rats also suffered anorexia (food intake 8.2 ± 0.6 vs. 16.6 ± 0.8 g rat^{-1} day^{-1}, $P < 0.05$). The morphological and biochemical data indicate that inflammatory injury was maximal between the third and fifth day and then faded slowly. However, even after 14 days there were still signs of the inflammatory reaction (Fig. 7). Both MPO and AP colonic activities peaked at 3 d after induction but the increase in MPO clearly preceded that of AP. The sensitivity to levamisole was augmented in parallel to the increase in AP activity (data not shown). Oxidative stress, as determined by MDA levels, was relatively constant during the first 5 days. After 2 weeks the 3 parameters were fully normalized. Thus the results of this experiment are similar to those mentioned above, except that recovery appeared to be slightly faster. This difference may be due to the fact that the animals used in both experiments were supplied by different vendors.

Discussion

Inflammatory bowel disease is characterized by intense oxidative stress, i.e. an imbalance between the attack of oxidative molecules and the defense provided by antioxidative mechanisms like glutathione peroxidase, vitamin E, ascorbic acid, etc. (Kruidenier et al., 2003). While the causative factors contributing to the etiopathogenesis of IBD are not known, it is commonly accepted that oxidative stress is an important determinant of its pathophysiology (Kruidenier and Verspaget, 2002; Yamada and Grisham, 1991). In fact, both Crohn's disease and ulcerative colitis are characterized by significant infiltration in the intestinal wall of activated inflammatory cells, which in turn produce and release a number of inflammatory mediators, including cytokines, chemokines and various oxidative molecules including superoxide, hydrogen peroxide, hydroxyl radical, etc. In particular, infiltrating neutrophils are notorious sources of such reactive oxygen species, including hypochlorous acid (HClO), a powerful oxidant generated by the enzyme myeloperoxidase which they use to kill target microorganisms. However, in the context of IBD, the major target of these ROS are probably normal cells of the mucosa, which would act as 'innocent bystanders' (Yamada and Grisham, 1991). Furthermore, oxidative stress may be capable to contribute or even initiate an inflammatory response, since it has been shown to activate signalling pathways such as NF-kB driven gene transcription (Magnani et al., 2000). Among the genes controlled by NF-kB are TNF, iNOS, ICAM-1 and interleukin 8 (Magnani et al., 2000). Thus antioxidants may be relevant for the treatment of IBD (Aghdassi et al., 2003; Natarajan et al., 2001; Gonzalez et al., 2001; Sanchez de Medina et al., 1996; Galvez et al., 1997).

Monochloramine is one of the most powerful oxidative mediators produced in the inflamed intestine. It is produced locally by the reaction of leukocyte derived hypochlorous acid with primary amines present in the inflammatory site (Grisham et al., 1990; Yamada and Grisham, 1991; Ogino et al., 1997; Tamai et al., 1991; Suzuki et al., 1991). The present study was undertaken to study the effects of intrarectally instilled monochloramine in the colon, using the rat as experimental model, in order to determine its relevance in the perpetuation of colonic inflammation.

Administration of a monochloramine enema elicited an intense inflammatory response in the rat colon which extended up to 1 week after induction, with some signs still visible after 14 days. This prolongation of colonic inflammation is significant if we consider that monochloramine is a relatively unstable molecule. In fact, it has to be obtained fresh before use rather than purchased. Therefore, it is unlikely that the inflammation observed at 1 week is caused directly by

monochloramine. Our results indicate that inflammation peaks 3–5 days after monochloramine administration and then fades slowly. This is similar to the situation in TNBS colitis, in which an intense acute colonic inflammatory response is followed by a prolongued recovery phase that leaves some sequelae such as fibrosis and scars (Morris et al., 1989). We can only speculate about the mechanism of action of monochloramine, but the fact that the severity of inflammation continued to increase after the first day suggests the existence of an amplification mechanism that perpetuates the response to this rather short lived chemical. Thus the two-fold increase in MPO activity between days 1 and 3 postchallenge reflects the occurrence of active recruitment of neutrophils at a time point where administered monochloramine can be assumed to have cleared completely. It is likely that epithelial disruption (Grisham et al., 1990; Ropeleski et al., 2003) may be the triggering event for extended inflammation, by allowing the unrestricted access to the mucosa of luminal antigens, which in turn may elicit an immunological reaction. This would in turn further stimulate the production of oxidative molecules, resulting in perpetuation of oxidative stress, as reflected in the increased colonic MDA levels. Interestingly, the best correlation among macroscopic damage and biochemical parameters was not found with MPO or MDA but with alkaline phosphatase (0.74, $P < 0.001$). Alkaline phosphatase is a sensitive biochemical marker of intestinal inflammation (Sanchez de Medina et al., 1996; Gonzalez et al., 2001; Sanchez de Medina et al., 2002) which is increased as a consequence of induction of the tissue nonspecific isoform, also known as bone/liver/kidney isoform (Sanchez de Medina et al., 2004). In both experiments carried out in this study the increase in MPO preceded the change in AP activity, which was apparent after 3 days, although upregulated expression of tissue nonspecific AP, as assessed by Western blot, was detectable already after 24 h. This discrepancy may be attributed to a difference in sensitivity between both techniques. Because induction of this AP isoform takes place in the intestinal epithelium (Sanchez de Medina et al., 2004) as a consequence of oxidative stress (unpublished data) and epithelial necrosis was maximal between 3 and 5 days postchallenge, it is logical that AP activity also peaked during this period. Furthermore, the time sequence indicated in Fig. 8 suggests that infiltrating neutrophils are the main culprits for epithelial injury at this time point. Histological analysis is consistent with this interpretation, since the characteristics of the cell infiltrate were different at 24 h and at 1 week; neutrophils, which are characteristic of acute/active lesions, were predominant at 24 h, whereas a mixed neutrophil/macrophage infiltrate was observed in the later phase. This may be interpreted as a sign of a more chronic type of inflammatory response.

The determination of the cytokine profile associated with the inflammatory response suggests that monochloramine colitis proceeds as a Th-1 type of immunological reaction (D'Haens, 2003), since the IFN-γ/IL-4 ratio was augmented, at least in initial phase. This type of response is characteristic of Crohn's disease but not ulcerative colitis (Neurath et al., 2002; Mariani et al., 2000). On the other hand, TNF seemed to play an important role as well in the acute phase of colitis, while IL-1β was increased at both time points. Both cytokines play an important role in IBD (D'Haens, 2003) as well as in well known experimental models of colitis such as that elicited by trinitrobenzenesulfonic acid or dextran sulfate sodium (Ohkawara et al., 2002; Armstrong et al., 2001; Gonzalez et al., 2001).

A second objective of our study was to characterize monochloramine induced colonic inflammation as an experimental model. This involved testing of drugs with known therapeutic effects on IBD, such as prednisolone and sulphasalazine, the rationale being that a good model of IBD should show a good correlation with the human correlate in terms of response to drugs. Our results clearly demonstrate that

both treatments are beneficial in monochloramine colitis, although sulphasalazine was generally superior. This fact may be related to the antioxidative effects of sulphasalazine (Miles and Grisham, 1994). While it could be argued that no drug treatment suppressed completely the inflammatory response, this is usually the case with experimental models of colitis.

In order to consider the effects of monochloramine on the rat colon, it may be useful to compare them with those of other oxidative molecules. Long lasting colitis can be induced in experimental animals with either trinitrobenzenesulfonic acid or iodoacetamide (Morris et al., 1989; Sanchez de Medina et al., 1996; Rachmilewitz et al., 1995). Iodoacetamide is believed to act by blocking free sulfhydryl groups in the intestinal mucosa, while TNBS has a mixed action as free radical generator and hapten (Morris et al., 1989; Grisham et al., 1991). We have examined the effects of other oxidative molecules, including hydrogen peroxide (1.2 mg/rat), tert-butyl hydroperoxide (3.1 mg/rat) and ferrous sulfate/ascorbic acid (7.0/4.4 μg/rat). One week after administration, these agents produce mild hyperemia and bowel wall thickening at most, without significant changes in the biochemical parameters (unpublished results). Thus monochloramine is specially powerful as an inducer of colonic inflammation.

It is also worth considering monochloramine colitis in comparison with the most widely used model of intestinal inflammation, i.e. TNBS colitis. The main difference between both models is that TNBS evoked injury is maximal at 24 h and then slowly fades, whereas monochloramine colitis appears to actually increase with time for 3–5 days before a recovery phase is observed. Thus monochloramine colitis may be considered more similar to the human condition. Other advantages of monochloramine colitis include higher affordability as well as avoidance of the confounding effects of ethanol, which is used as a barrier breaker in the TNBS model, thus causing toxic acute colitis itself.

In conclusion, monochloramine induces acute and protracted inflammation in the rat colon which seems to be driven by Th-1 lymphocytes and is amenable to pharmacological modulation. This finding supports the hypothesis that monochloramine may play an important role in the perpetuation of colonic inflammation. Monochloramine colitis may be used as a valid model of IBD for the testing of novel drug treatments.

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