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European Journal of Pharmacology 374 (1999) 277–283

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Effect of MEN 11467, a new tachykinin NK_1 receptor antagonist, in acute rectocolitis induced by acetic acid in guinea-pigs

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Received 4 February 1999; received in revised form 21 April 1999; accepted 26 April 1999

Abstract

The aim of this study was to evaluate the effect of MEN 11467 (1*R*,2*S*)-2-*N*[1(*H*)indol-3-yl-carbonyl]-1-*N*{*N*-(*p*-tolylacetyl)-*N*-(methyl)-D-3(2-Naphthyl)alanyl}diaminocyclohexane), a new potent tachykinin NK_1 receptor antagonist, in an experimental model of acute rectocolitis induced by an enema with 7.5% acetic acid in guinea-pigs. This effect was compared to that of mesalazine (5-amino-2-hydroxybenzoic acid). The injury was quantified visually by using a macroscopic injury score and histologically by using a necrosis score. In addition, changes in myeloperoxidase activity, a marker for neutrophil infiltration, and plasma protein extravasation were evaluated. The injury caused by 7.5% acetic acid was mild, affecting the superficial layers and producing a strong edema of the submucosa. A single administration of MEN 11467 (0.3–10 mg/kg s.c., 1 h before acetic acid) reduced the macroscopic damage and necrosis score and the increase in plasma protein extravasation induced by 7.5% acetic acid in the early acute phase of the injury (death at 2.5 h). Mesalazine (100 mg/kg p.o., 1 h before) reduced the macroscopic score but not the plasma protein extravasation. Repeated administration of MEN 11467 (1–3 mg/kg s.c., −1, +6 and +23 h after 7.5% acetic acid) reduced the macroscopic score and myeloperoxidase activity but not the plasma protein extravasation induced in the late phase of acute injury (death at 24 h). At this time mesalazine markedly reduced the macroscopic score, myeloperoxidase activity and plasma protein extravasation induced by 7.5% acetic acid. These results suggest a greater involvement of tachykinin NK_1 receptors in the early phase than in the late phase of colonic inflammation in response to chemical injury.

Keywords: Experimental colitis; Inflammatory bowel disease; MEN 11467; Tachykinin NK_1 receptor; Substance P; Tachykinin

1. Introduction

Inflammatory bowel diseases (Crohn's disease and ulcerative colitis) are chronic inflammatory pathologies of unknown etiology. Many inflammatory mediators, such as eicosanoids, cytokines, tumor necrosis factor-α or tachykinins, have been proposed to play a role in the pathogenesis of inflammatory bowel disease (Elson et al., 1995; Holzer, 1998; Quartara and Maggi, 1998). Among the tachykinins, substance P is known to have proinflammatory properties under different pathological conditions, causing plasma protein extravasation and edema, and to interact with the neuroimmune system (Holzer, 1998; Quartara and Maggi, 1998). However, our understanding of the role of substance P in intestinal inflammation is still limited. With regard to the involvement of tachykinins in inflammatory bowel disease, Mantyh et al. (1988) demonstrated that patients with overt ulcerative colitis and Crohn's disease have an extremely high expression of tachykinin NK_1 receptors (1000–2000 times higher than normal subjects) in surgical specimens of colon. Moreover several studies have reported changes in tissue concentrations of substance P in inflammatory bowel diseases (Goldin et al., 1989; Bernstein et al., 1993; Renzi et al., 1998). These data support the hypothesis that tachykinins and their receptors could be involved in inflammatory bowel disease pathogenesis (Quartara and Maggi, 1998). Several chemical agents have been used to induce experimental colitis in animals (Elson et al., 1995); among them, an enema of acetic acid has been shown to induce transient inflammation and necrosis of the mucosa of the rectocolon in a

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00313-1

dose-dependent fashion (MacPherson and Pfeiffer, 1976). The pseudopeptide MEN 11467 is a new tachykinin NK_1 receptor antagonist (Cirillo et al., 1998a) which potently antagonizes both tachykinin and antigen-mediated inflammatory responses of the respiratory tract 'in vivo' and which possesses a nanomolar affinity for guinea-pig and human tachykinin NK_1 receptors (Cirillo et al., 1998a). Therefore, the aim of this study was to evaluate the ability of MEN 11467 to inhibit the inflammatory responses induced by an enema of diluted acetic acid in the rectocolon of guinea-pigs.

2. Materials and methods

2.1. Animals

Male Dunkin–Hartley guinea-pigs, 300–350 g body weight, were purchased from Charles River (Calco, Italy). Animals were fasted for at least 24 h before experiments.

2.2. Induction of rectocolitis and evaluation of macroscopic and necrosis scores

Guinea-pigs were anaesthetised with pentothal sodium (50 mg/kg s.c.). Twenty minutes later, a rubber cannula (8 cm) was inserted into the colon through the anus. Acetic acid (7.5%) was instilled intrarectally, the cannula was left in the colon for 30 s and then the animal was placed in its cage to recover from anaesthesia. The control group was treated with saline intrarectally. The animals were killed at different times in order to establish the peak of injury, and in the experiments with drug treatment two times were chosen: 2.5 or 24 h after the enema of 7.5% acetic acid. In the first group (death at 2.5 h), animals received MEN 11467 p.o. or mesalazine s.c. 1 h before the administration of 7.5% acetic acid, and in the second group (death at 24 h) animals received supplementary treatments 6 and 23 h after the 7.5% acetic acid enema. For the evaluation of the tissue damage, each animal was killed with CO_2, and the distal colon was excised (5–7 cm) from a fixed site. The damage in all specimens was immediately evaluated macroscopically and scored. Half of the specimen was then placed in Bouin's solution (5% picric acid, 5% formalin and 1% acetic acid) for histological examination and the remaining half was rapidly frozen in liquid nitrogen and stored at -80°C for myeloperoxidase determination.

Assessment of macroscopic changes of the colonic mucosa was performed according to McCafferty et al. (1994), with minor modifications. Macroscopic scores were assigned as follows: 0 = normal appearance, 1 = hyperaemia of the mucosa in less than 50% of the specimen, absence of macroscopic ulceration, 2 = strong hyperaemia of the mucosa extending throughout the specimen, bowel wall thickening, absence of macroscopic ulcerations, 3 = severe hyperaemia, with numerous areas of ulceration (size < 0.5 cm), 4 = severe hyperaemia, with numerous areas of ulceration (size > 0.5 cm).

Histological examination of the tissue samples was performed as follows: after dehydration and embedding in paraffin, tissue sections (5 μm thickness) were stained with haematoxylin and eosin. The severity of mucosal necrosis was measured according to the following score: 1 = Minimum: focal points of damage (change in architecture of the layers) in the whole section, 2 = Slight: damaged areas in less than 20% of the whole section, 3 = Moderate: damaged areas in 20 to 50% of the whole section, 4 = Marked: damaged areas in 50 to 75% of the whole section, 5 = Severe: damaged areas involving the whole section.

2.3. Myeloperoxidase activity

Specimens of guinea-pig rectocolon (400–700 mg) were homogenized in 0.5% hexadecyltrimethylammonium bromide and 50 mM potassium phosphate buffer (pH = 6.0), using a Polytron tissue homogenizer. After homogenization, the homogenizer was rinsed twice with 1 ml of hexadecyltrimethylammonium bromide buffer. The pooled homogenate and washes were sonicated for 15 s and the released enzyme was separated from insoluble cellular debris by centrifugation at $3000 \times g$ for 10 min. Myeloperoxidase activity was measured spectrophotometrically: 0.1 ml of supernatant was added to 2.9 ml of 50 mM potassium phosphate buffer, pH = 6.0, containing 0.167 mg/ml *o*-dianosine hydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 1 and 5 min was measured with a spectrophotometer (Beckman DU-7, wavelength = 460 nm). One unit of myeloperoxidase activity is defined as the amount able to degrade 1 μmol of peroxide per minute at 25°C.

2.4. Evaluation of plasma protein extravasation of guinea-pig rectocolon

Animals were surgically prepared according to Cirillo et al. (1998b) and killed at 2.5, 24 or 72 h after 7.5% acetic acid enema. Evans Blue was administered intravenously (20 mg/kg in saline containing 210 I.U./2 ml heparin) and then, 10 min later, the animals were perfused via the left ventricle with saline 100 ml/10 min to wash the dye out of the vasculature. In some experiments the plasma protein extravasation was evaluated in the presence of the tachykinin NK_1 receptor agonist, $[Sar^9,Met(O_2)^{11}]$substance P (1 nmol/kg i.v. given 5 min before death). The rectocolon was excised, weighed and the dye was extracted in 2 ml of formamide (50°C for 24 h). The amount of Evans blue was determined by spectrophotometry (wave length = 620 nm) and expressed as ng/mg of wet tissue. The amount of non-specific dye leakage in guinea-pig

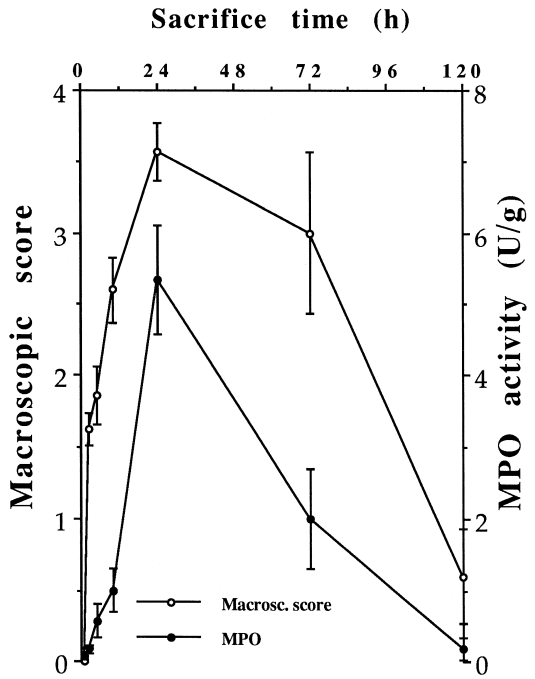

Fig. 1. Time course (0–120 h) of changes in the macroscopic damage score and myeloperoxidase activity after an enema of 7.5% acetic acid-induced acute colitis in guinea-pigs.

rectocolon was 10.6 ± 0.1 ng Evans blue/mg tissue (n = 4). This value was subtracted from the plasma protein extravasation data presented in results, tables or figures.

2.5. Data analysis

All data in the text are means ± S.E.M. Statistical significance was evaluated by means of one-way analysis of variance followed by Dunnett's test.

2.6. Drugs

MEN 11467 or (1*R*,2*S*)-2-*N*[1(*H*)indol-3-yl-carbonyl]-1-*N*{*N*-(*p*-tolylacetyl)-*N*-(methyl)-D-3(2-Naphthyl)alanyl}-diaminocyclohexane) was synthetized at the Chemistry Department, Menarini Ricerche (Pomezia, Italy), dissolved in saline containing 2% Tween 80 and administered s.c. at the doses of 0.3, 3 and 10 mg/kg in a volume of 4 ml/kg. [Sar^9,Met(O$_2$)^{11}]substance P was purchased from Peninsula Laboratories (Belmont, CA, USA) and dissolved in saline. Mesalazine tablets (Asacol® 400, Bracco, Italy) were powdered and suspended with 0.5% carboxymethylcellulose, and then given orally to the animals at the dose of 100 mg/kg in a volume of 5 ml/kg. Acetic acid (Sigma) was diluted in saline to 7.5% concentration and administered intrarectally in a volume of 0.3 ml/animal. Pentothal sodium (Abbott, Italy) was dissolved in saline and given in a volume of 1 ml/kg.

3. Results

3.1. Time course of acid acetic-induced injury

The effects of an enema of 7.5% acetic acid were studied at different times, ranging from 2.5 to 120 h after instillation. The macroscopic inspection revealed the presence of gross damage (recorded as macroscopic score) in the rectocolon between 2.5 and 24 h, whereas a significant increase of myeloperoxidase activity occurred only from 10 h onwards (Fig. 1). Both the macroscopic damage score and myeloperoxidase activity peaked at 24 h, and then slowly declined (Fig. 1). As shown in Fig. 2, 7.5% acetic acid induced a significant ($P < 0.01$) increase of plasma protein extravasation in groups killed at 2.5 and 24 h as compared to control (time 0). Lower values of plasma protein extravasation were recorded at 72 h after the 7.5% acetic acid enema. The administration of a selective tachykinin NK$_1$ receptor agonist, [Sar^9,Met(O$_2$)^{11}]substance P, produced a further significant increase in plasma protein extravasation at 2.5 and 24 h above that elicited by the 7.5% acetic acid enema (Fig. 2).

3.2. Effects of MEN 11467 on acid acetic-induced injury (death at 2.5 h after 7.5% acetic acid enema)

A typical microscopical picture of 7.5% acetic acid injury is shown in Fig. 3B. The mucosal architecture and the epithelial layer were completely damaged and in this zone there was limited infiltration of inflammatory cells (for comparison see Fig. 3A = controls). In the submucosa there was marked edema which caused detachment of the layers. In the group of MEN 11467 (3 mg/kg s.c.)-treated animals (Fig. 3C), the epithelium and the mucosa were protected from the inflammatory insult but the edema was

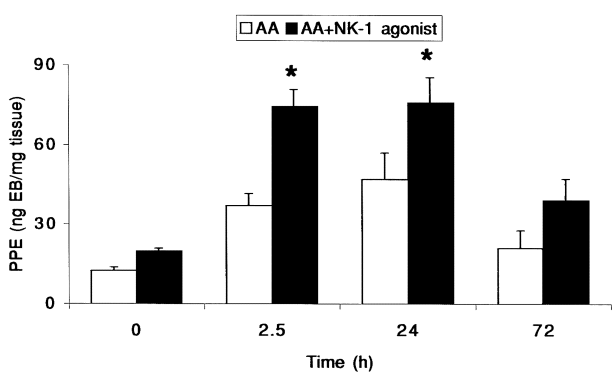

Fig. 2. Time course (2.5–72 h) of plasma protein extravasation (PPE), expressed as ng Evans blue/mg tissue, after an enema of 7.5% acetic acid (AA) in guinea-pig rectocolon in the absence (empty bars) or in the presence (filled bars) of the NK$_1$ receptor agonist, [Sar^9,Met(O$_2$)^{11}]substance P given 10 min before death.

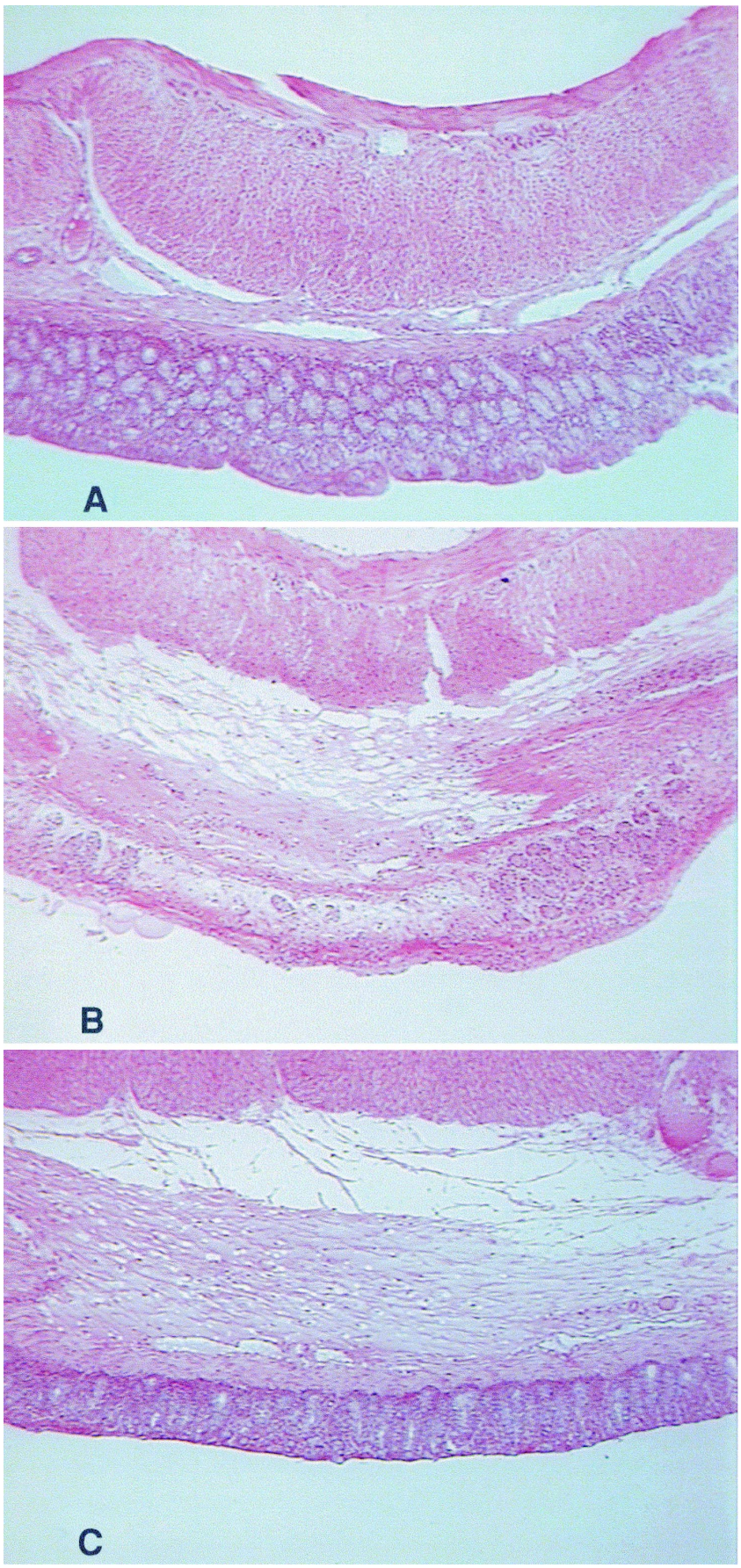
A
B
C

Table 1
Effect of MEN 11467 and mesalazine on macroscopic damage score, necrosis score and plasma protein extravasation (PPE) in 7.5% acetic acid-induced rectocolitis

Group	Dose (mg/kg)	*n*	Macroscopic score	Necrosis score	*n*	PPE (ng EB/mg tissue)
Saline	–	6	0 ± 0	0 ± 0	10	12 ± 2
Acetic acid	–	14	1.9 ± 0.06	3.1 ± 0.3	14	37 ± 5[a]
MEN 11467	0.3 s.c.	6	1.5 ± 0.2[b]	1.8 ± 0.3[b]	5	24 ± 3
MEN 11467	3 s.c.	9	0.4 ± 0.05[c]	2.1 ± 0.35[b]	9	21 ± 3[b]
MEN 11467	10 s.c.	8	0.6 ± 0.1[c]	2.0 ± 0.04[b]	–	n.t.
Mesalazine	100 p.o.	7	0.9 ± 0.2[c]	n.t.	4	30 ± 3

The animals were killed at 2.5 h after the 7.5% acetic acid enema.
[a] $P < 0.01$ vs. saline.
[b] $P < 0.05$ vs. acetic acid group.
[c] $P < 0.01$ vs. acetic acid group; n.t. = not tested.

Table 2
Effect of MEN 11467 and mesalazine on macroscopic damage score, myeloperoxidase activity (MPO) and plasma protein extravasation (PPE) in 7.5% acetic acid-induced rectocolitis

Group	Dose (mg/kg)	*n*	Macroscopic score	MPO (U/g)	*n*	PPE (ng EB/mg tissue)	*n*	PPE (+NK_1 agonist) (ng EB/mg tissue)
Saline	–	4	0 ± 0	0 ± 0	10	12 ± 2	4	20 ± 1
Acetic acid	–	28	3.8 ± 0.1	5.7 ± 0.5	7	47 ± 10[a]	9	76 ± 9[a]
MEN 11467	1 × 3 s.c.	7	3.0 ± 0.2[b]	6.3 ± 1.3	–	n.t.	–	n.t.
MEN 11467	3 × 3 s.c.	24	2.5 ± 0.2[c]	3.2 ± 0.5[b]	5	48 ± 11	9	47 ± 8[b]
Mesalazine	100 × 3 p.o.	7	0.7 ± 0.2[c]	0.2 ± 0.1[c]	5	11 ± 3[c]	–	n.t.

Plasma protein extravasation was evaluated also in presence of a tachykinin NK_1 receptor agonist, [Sar^9,$Met(O_2)^{11}$]substance P (last column). The animals were killed 24 h after 7.5% acetic acid enema.
[a] $P < 0.01$ vs. saline.
[b] $P < 0.05$ vs. acetic acid group.
[c] $P < 0.01$ vs. acetic acid group; n.t. = not tested.

still evident in the submucosa. As shown above, an enema of 7.5% acetic acid in guinea-pigs produced marked injury of the rectocolon, measured as macroscopic and necrosis scores or as an increase in plasma protein extravasation, when compared to the effects of saline (Table 1). Pretreatment with MEN 11467 (0.3, 3 and 10 mg/kg s.c., 1 h before the 7.5% acetic acid enema) produced a significant inhibition of macroscopic and necrosis scores and plasma protein extravasation (Table 1). Mesalazine (100 mg/kg p.o., 1 h before 7.5% acetic acid) significantly reduced the macroscopic score without affecting the increase in plasma protein extravasation.

3.3. Effects of MEN 11467 on acid acetic-induced injury (death at 24 h after 7.5% acetic acid enema)

As shown in Table 2, mesalazine (100 mg/kg p.o.) and MEN 11467 (1 and 3 mg/kg s.c.) administered 1 h before and 6 or 23 h after the 7.5% acetic acid enema significantly reduced both the macroscopic damage score and myeloperoxidase activity. Mesalazine (100 mg/kg × 3 p.o.) but not MEN 11467 (3 mg/kg × 3 s.c.) reduced the increase in plasma protein extravasation. The dose of 3 mg/kg of MEN 11467 induced a significant ($P < 0.05$) reduction of the plasma protein extravasation induced by the tachykinin NK_1 receptor agonist, [Sar^9,$Met(O_2)^{11}$]substance P, when compared to the effect of saline (Table 2).

4. Discussion

Acetic acid injury in the guinea-pig rectocolon was characterised by inflammation that affected the superficial layers, causing destruction of the mucosa, infiltration of inflammatory cells and submucosal edema. Unlike other chemical agents inducing colitis (e.g., trinitrobenzenesulphonic acid), acetic acid produces a transient inflammation which did not progress into chronic inflammation. The tissue recruitment of neutrophils and the increase in vascular permeability were concomitant to the development of

Fig. 3. Light microscopy images (100 ×) of rectocolon after 7.5% acetic acid enema (the animals were killed 2.5 h after 7.5% acetic acid). A = control treated with saline enema; B = acetic acid treated: severe alteration of mucosal architecture, limited infiltration of inflammatory cells and marked edema of the submucosa; C = acetic acid + MEN 11467 (3 mg/kg s.c., 1 h before) treated animals: protection of superficial layers but presence of edema in the submucosa.

macroscopic damage, peaked at 24 h and decreased 72–120 h after the challenge. As shown by light microscopy, MEN 11467 was able to protect the superficial layers of the colon from the chemical insult. In particular, MEN 11467 at doses that have a tachykinin NK_1 antagonist effect in other functional models (Cirillo et al., 1998a) markedly reduced the extent of tissue injury, possibly by inhibiting the acetic acid-induced increase in tissue permeability during the early phase of damage. These results are in keeping with those obtained with other tachykinin NK_1 receptor antagonists (RP 67580 and CP 96,345) which reduce the early but not the late phases of inflammation in trinitrobenzensulphonic acid-induced colitis in rats (Evangelista et al., 1996; Reinshagen et al., 1998; Wallace et al., 1998). In the latter model of colitis, time-course studies have shown an increased transcription of β-preprotachykinins mRNA along with unaltered or even decreased SP tissue levels, which points to an enhanced release of neuropeptide during the initial phases of colitis (Renzi et al., 1994).

MEN 11467 also reduced significantly the intensity of injury and the infiltration of neutrophils at 24 h after instillation of acetic acid. The extent of injury inhibition afforded by MEN 11467 at the peak of the inflammatory reaction was lower than that observed in the early phases, indicating that the injury is characterized by an early tachykinin-dependent and a late, probably non tachykinin-dependent, process. This was confirmed by the findings obtained with mesalazine, which strongly inhibited the injury at the late time, probably by interfering with other late-phase mediators.

In particular, the increase in tissue permeability at 24 h after the acetic acid enema was left unaltered by the tachykinin NK_1 receptor antagonist. However, in the presence of a selective tachykinin NK_1 receptor agonist, MEN 11467 reduced the increase in permeability, decreasing only the amount of plasma protein extravasation specifically sustained by the activation of NK_1 receptors. It is very interesting to note that, under basal conditions, the administration of a NK_1 receptor agonist did not produce any significant increase in plasma extravasation whereas permeability was enhanced during the inflammation induced by acetic acid, possibly as a result of a 'de novo' expression of NK_1 tachykinin receptors (Mantyh et al., 1988).

The involvement of substance P and tachykinin NK_1 receptors in models of colitis is a matter of debate and depends on the species, inducing agent, localisation of inflammation and potency and/or stability of the tachykinin NK_1 receptor antagonists used. Therefore, the results obtained with antagonists are different: LY-303870 inhibited acute inflammation of spontaneous colitis induced in cotton-top tamarin (Wood et al., 1996) and SR 140333 reduced chronic colitis induced by trinitrobenzensulphonic acid in rats (Mazelin et al., 1998), but RP 67580 did not affect trinitrobenzensulphonic acid-induced colitis or ileitis in guinea pigs and rats (Evangelista et al., 1996; Reinshagen et al., 1998; Wallace et al., 1998). In the latter species a down-regulation of tachykinin NK_1 receptors has been reported during trinitrobenzensulphonic acid colitis (Evangelista et al., 1996). Moreover, mice genetically deficient in the tachykinin NK_1 receptor have reduced mucosal ulceration induced by trinitrobenzensulphonic acid as compared to wild-type mice (Castagliuolo et al., 1998).

Taking into consideration that guinea-pigs are reported to be similar to human in terms of the sensitivity of tachykinin NK_1 receptors, as shown by the use of structurally different tachykinin NK_1 receptor antagonists (Barr and Watson, 1993; Quartara and Maggi, 1998), the results of the present study support the view that the acute colitis induced by diluted acetic acid in guinea-pigs is affected by MEN 11467 via a specific blockade of tachykinin NK_1 receptors. Early phases of inflammation were more sensitive to the tachykinin NK_1 receptor antagonist effects, suggesting the participation of tachykinin NK_1 receptors in the initiation and propagation of intestinal inflammation.

Acknowledgements

This work was supported in part by Istituto Mobiliare Italiano (contract no. 56665). We would like to thank Dr. A. Argentino-Storino for her histological work, Dr. A. Lecci for his helpful advice and suggestions and Miss C. Azzurrini for her secretarial assistance.

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