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European Journal of Pharmacology 522 (2005) 130–138
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# Inhibitory effects of histamine $H_4$ receptor antagonists on experimental colitis in the rat

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Received 28 April 2005; received in revised form 1 July 2005; accepted 18 August 2005
Available online 6 October 2005

**Abstract**

The histamine $H_4$ receptor is a G-protein coupled receptor with little homology to the pro-inflammatory histamine $H_1$ receptor, expressed on cells of the immune system with hematopoietic lineage such as eosinophils and mast cells. The effects of the recently described highly selective histamine $H_4$ receptor antagonists JNJ 10191584 and JNJ 7777120 have now been investigated on the acute colitis provoked by trinitrobenzene sulphonic acid over 3 days in the rat. Treatment with JNJ 10191584 (10–100 mg/kg p.o., b.i.d.) caused a dose-dependent reduction in macroscopic damage, inhibition of the TNBS-provoked elevation of both colonic myeloperoxidase and tumour necrosis factor-α (TNF-α), and a reduction in the histologically assessed increase in mucosal and submucosal thickness and neutrophil infiltration. JNJ 7777120 (100 mg/kg p.o., b.i.d.) likewise reduced the macroscopic injury and the increases in colonic myeloperoxidase and TNF-α levels. These findings indicate a pro-inflammatory role for the histamine $H_4$ receptor in this model and suggest a novel pharmacological approach to the treatment of colitis.


*Keywords:* Histamine $H_4$ receptor antagonist; JNJ 7777120; JNJ 10191584; Colitis; Inflammatory bowel disease; Neutrophil; Tumour necrosis factor-α

## 1. Introduction

The histamine $H_4$ receptor is a recently described G-protein coupled receptor, with little homology to the classical pro-inflammatory histamine $H_1$ receptor or the histamine $H_2$ receptor, and some 35% homology with the histamine $H_3$ receptor (Nakamura et al., 2000; Oda et al., 2000; Coge et al., 2001; Morse et al., 2001; Nguyen et al., 2001; Liu et al., 2001a; Zhu et al., 2001). The histamine $H_4$ receptor appears to be exclusively expressed on cells of the immune system with hematopoietic lineage (Oda et al., 2000; Morse et al., 2001; Liu et al., 2001a,b; Zhu et al., 2001). Notably, the receptor has been described on eosinophils, mast cells, basophils, dendritic cells and T cells (Oda et al., 2000; Liu et al., 2001a; Morse et al., 2001; Zhu et al., 2001; Gantner et al., 2002; Hofstra et al., 2003; Ling et al., 2004). As such, it would appear that the histamine $H_4$ receptor is an interesting target for pharmacological modulation (De Esch et al., 2005), with the prospect of ameliorating inflammatory conditions such as inflammatory bowel diseases, where histamine, eosinophils and mast cells may play a role (Bischoff et al., 1996; Lampinen et al., 2001; Winterkamp et al., 2002; Hogan and Rothenberg, 2004).

Activation of the histamine $H_4$ receptor can mediate calcium mobilisation and chemotaxis in mast cells (Hofstra et al., 2003) and induce shape change and promote chemotaxis in eosinophils (O'Reilly et al., 2002; Buckland et al., 2003; Ling et al., 2004). Others have proposed that the histamine $H_4$ receptor along with $H_2$ receptors can modulate histamine-induced interleukin-16 release from $CD8^+$ T cells (Gantner et al., 2002). A role for the histamine $H_4$ receptors in modulating the release of the chemoattractant, leukotriene $B_4$, and mast-cell dependent neutrophil recruitment into the pleural cavity has also been suggested from the use of the non-selective histamine $H_3/H_4$ receptor antagonist, thioperamide (Takeshita et al., 2003).

Recently, novel potent and selective histamine $H_4$ antagonists with oral bioavailability have been described. Thus, JNJ 7777120 and JNJ 10191584 which are indole and

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doi:10.1016/j.ejphar.2005.08.045

benzimidazole amides, respectively, have high affinity for the $H_4$ receptor and greater than 1000-fold selectivity for the histamine $H_4$ receptor compared with the other known histamine receptors, while having little action on a range of other receptor types (Jablonowski et al., 2003, 2004; Thurmond et al., 2004; Terzioglu et al., 2004; Venable et al., in press). Supporting the immune function of the histamine $H_4$ receptor, its proposed role in histamine-dependent chemotaxis in both mast cells and eosinophils has been confirmed in vitro using JNJ 7777120 (Thurmond et al., 2004; Ling et al., 2004). Moreover, this compound has shown efficacy in reducing histamine-induced mast cell infiltration in mouse trachea and zymosan-induced neutrophil influx into the peritoneum following oral or subcutaneous administration, while not affecting histamine $H_1$-receptor dependent paw oedema induced by histamine (Thurmond et al., 2004).

The pharmacological actions of selective histamine $H_4$ receptor antagonists have now been evaluated in a widely used experimental model of inflammatory bowel disease provoked by colonic instillation of the hapten, trinitrobenzene sulphonic acid (Boughton-Smith et al., 1988a; Morris et al., 1989; Whittle et al., 2003) In this work, the effects of the recently described potent histamine $H_4$ receptor antagonist, JNJ 10191584 (Venable et al., in press) following oral administration in the rat, have been investigated on the colitis provoked by trinitrobenzene sulphonic acid over a 3 day period. The effects of histamine $H_4$ receptor antagonism in this colitis model have also been confirmed using the other well-characterised histamine $H_4$ antagonist, JNJ 7777120 (Jablonowski et al., 2003; Thurmond et al., 2004, Ling et al., 2004).

## 2. Materials and methods

### 2.1. Induction of colitis

Male Wistar rats (200–240 g) were randomised before commencement of the study and housed in groups, with their body weight being measured daily. The investigation was performed in accordance with the United Kingdom Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, published by HMSO, London. Food was withdrawn for 12 h overnight before trinitrobenzene sulphonic acid (TNBS) administration, but the rats were allowed free access to drinking water.

In these studies, a submaximal dose of 10 mg of TNBS was used to provoke a reproducible yet not unduly severe acute mucosal injury in the colon. This was determined after 3 days, a time when plateau levels of both neutrophil infiltration and acute tissue injury is observed (Boughton-Smith et al., 1988a,b). On the morning of the day of challenge, day 0, the rats were transiently anaesthetised with ether and TNBS (10 mg in 0.25 ml of 50% ethanol) was instilled into the colon using a soft plastic catheter inserted 8 cm in the rat rectum. The rats were allowed to recover with free access to food and drinking water. At the end of the experiment, 72 h after TNBS administration (i.e., on the morning of day 3), the distal colon was exposed and the terminal 8 cm dissected, photographed and stored appropriately for subsequent analyses.

The primary parameters measured were the area of macroscopic injury and its severity score, myeloperoxidase levels and tumour necrosis factor-α (TNF-α) levels in segments of distal 8 cm of colon. In addition, the weight of the whole 8 cm colonic segment was measured as an indirect and non-specific marker of oedema, while the body weight of the animals was determined each day of the study as an indicator of general health.

The experimental compounds prepared in the vehicle, 20% hydroxypropyl β cyclodextrin (HPCD) were administered orally, twice daily (2 ml/kg) commencing 24 h before TNBS administration, on the day of TNBS administration and on days 1 and 2 after challenge. The doses of JNJ 10191584 were 10, 30 and 100 mg/kg, p.o., twice a day, which being the maleate salt, reflect doses of 7, 20 and 70 mg/kg, twice a day of the active free base. The dose of JNJ 7777120 was 100 mg/kg, p.o., twice a day, and again being a maleate salt, was equivalent to 70 mg/kg twice a day of the free base. A further group of rats that was challenged with TNBS received the HPCD vehicle alone, twice a day (2 ml/kg p.o.), while another group had no challenge or drug treatment but did receive isotonic saline twice a day (2 ml/kg p.o.) and was used for base-line measurements.

### 2.2. Macroscopic analysis

The distal 8 cm portion of the colon (measured from the rectum) was removed, opened longitudinally and gently rinsed with ice-cold phosphate buffer (PBS; pH 7.4), blotted, weighed (Scaltec, Germany) and photographed (Samsung, Digimax 340, digital camera). The extent of macroscopically apparent damage and haemorrhagic necrosis in the 8 cm segment was determined in a randomised manner from the colour images via computerised planimetry (Scion Image B4.02 version; Scion Corp.). The area of macroscopically visible mucosal damage was calculated and expressed as the percentage of the total colonic segment area under study.

The tissue was subsequently cut into longitudinal strips, each strip being thus 8 cm long and included the whole of the zone of injury. This tissue was weighed, processed and the resulting supernatant stored at −20 °C for the subsequent determination of myeloperoxidase activity, protein levels and for the assay for TNF-α.

#### 2.2.1. Damage score

In addition to the quantitative measurement of area of damage, the degree of colonic damage was also assessed in a randomised blinded fashion using a Damage Score, utilizing a 1–5 scale than has been adapted from that used previously (Boughton-Smith et al., 1988a):

0 No damage.
1 One region of localized inflammation or thickening. No ulcers.
2 Linear ration, but no significant inflammation.
3 Linear ulceration with inflammation at one site.

4 Two or more sites of ulceration and/or inflammation. Ulcers present in at least one site.
5 Two or more sites of ulceration and inflammation or one major site of ulceration and inflammation extending >1 cm along the length of the colon.

### 2.3. Myeloperoxidase activity

The myeloperoxidase activity was determined in colonic tissue as previously described (Kiss et al., 1997). The 8 cm longitudinal strips of the colon were weighed, homogenised (Ultra turrax, T25, 13,500 rev/min; 2×30 s; 250 mg colon/1 ml buffer) in ice-cold phosphate buffer (50 mM, pH 6.0), freeze-thawed three times and then centrifuged twice (each time at 15,000 ×*g* for 15 min at 4 °C). Then, a 12 μl aliquot of the supernatant was mixed with 280 μl phosphate buffer (50 mM, pH 6) containing 0.167 mg/ml of *O*-adenosine dihydrochloride and the reaction started with 10 μl 0.03% hydrogen peroxide and assayed spectrophotometrically at 490 nm (Benchmark Microplate reader, Bio-Rad Labs) after 90 s of shaking. Myeloperoxidase activity was expressed as mU/mg protein.

### 2.4. Tumour necrosis factor-α levels

The colonic tissue samples were thawed, weighed and homogenized (Ultra-turrax, T25, 2×30 s on ice; 250 mg colon/ml buffer) of a modified a Greenburg buffer (300 mmol/l NaCl, 15 mmol/l Tris, 2 mmol/l $MgCl_2$, 2 mmol/l Triton X-100, 20 ng/ml pepstatin A, 20 ng/ml leupeptin, 20 ng/ml aprotonine; pH: 7.4). Tissue homogenates were lysed for 30 min on ice, and then centrifuged twice (10 min, 14,000 ×*g*). The aliquots of the supernatant were stored at −20 °C until use (Ten Hove et al., 2001).

The TNF-α levels were determined with quantitative TNF-α solid-phase Enzyme Linked ImmunoSorbent Assay (ELISA). The samples were measured spectrophotometrically at 450 nm and were diluted 2 or 4 times with the buffer included in the kit. This commercially available kit used had a range of the standard curve of 0–2000 pg/ml with minimum detection level of 10 pg/ml of TNF-α. The TNF-α values were expressed as pg/mg protein.

### 2.5. Protein determination

Using a commercial protein assay kit, aliquots (20 μl) of the diluted samples (25× or 50× with distilled water) was mixed with 980 μl distilled water and 200 μl Bradford reagent added to each sample. After mixing and a 10 min incubation, the samples were assayed spectrophotometrically at 595 nm. Protein level was expressed as mg protein/ml.

### 2.6. Histological studies

For light microscopy, tissue samples from the distal 8 cm (measured from the rectum) of the colon of each animal from 4 separate groups of animals were collected: the vehicle HPCD, JNJ 10191584 (100 mg/kg p.o., twice a day) and the control non-challenged group. Tissue samples were taken from each colon, 1.5–2.5 cm from the rectum. The samples were fixed for 4 h in 4% buffered paraformaldehyde (40 g paraformaldehyde dissolved in 500 ml distilled water plus 500 ml 0.1 M phosphate buffered saline; pH 7.38), washed (3×30 min), dehydrated in increasing concentrations of ethanol (30, 50, 75, 96 and 100% ethanol 5–5 min, respectively; 2×5 min in xylol), and embedded in paraffin (12 h, 54 °C).

Thereafter, 8 μm sections of tissue were cut using a rotary microtome, mounted on gelatine glass slides, and were cleared with acetic acid:absolute ethanol (1:1 solution), placed in gelatine solution and dried for 2 h at 37 °C. The sections were cleared, hydrated and stained with hematoxylin and eosin or with toluidine blue using standard procedures for histological evaluation of colon damage. The slides were coded to prevent observer bias during evaluation. All tissue sections were examined with an Olympus microscope and digital imaging system (Olympus BX60 microscope, DP50 digital camera and Viewfinder Lite version 1.0 software) for characterisation of the histopathological changes.

### 2.7. Morphometric analysis

Photographs (4× and 10× magnification) of colon samples were analyzed with Scion image software analysis program (B4.02. version, Scion Corp.). The depth of the mucosa and submucosa were measured in several sections from each tissue sample and expressed in μm.

The high power field method was used for cell counting; images (40×) of sections of the submucosa were taken and the number of the neutrophils, as assessed by their morphology on the stained slides, were counted. This latter analysis was performed only on the submucosa where the neutrophils could be readily measured using this approach.

### 2.8. Reagents and materials

The investigational drugs, JNJ 10191584 (5-chlorobenzimidazolyl *N*-methylpiperazine carboxamide) and JNJ 7777120 (5-chloroindolyl *N*-methylpiperazine carboxamide) were synthesized as maleate salts in the laboratories of Johnson and Johnson Pharmaceutical Research and Development, L.L.C. as described before (Jablonowski et al., 2003; Venable et al., in press). These were prepared freshly on each day of use in the vehicle, 20% hydroxypropyl β cyclodextrin (HPCD, Sigma Chemical Company).

The 2,4,6, trinitrobenzene sulphonic acid was obtained from Fluka Chemie AG (Buchs, Switzerland). The Bradford protein assay was from Bio-Rad. All other assay reagents were from the Sigma Chemical Company. The TNF-α ELISA assay kit was obtained from Hycult Biotechnology b.v. 5405 Uden, The Netherlands.

### 2.9. Statistical evaluation

Results shown in the figures are expressed as mean±S.E.M. from (*n*) rats per experimental group. For statistical comparisons,

the two-tailed Student's *t*-test and the analysis of variance with the Bonferoni test were used where appropriate. $P<0.05$ was taken as significant. In the graphs and tables, statistical comparison is made against the values for the HPCD vehicle group.

## 3. Results

### 3.1. Effects on macroscopic colonic injury

Following intracolonic instillation of TNBS (10 mg), the area of colonic injury, determined 72 h after challenge in the control group of rats that had only received the vehicle involved 46±4% ($n=16$) of the total colonic area of the segment studied (Fig. 1). This macroscopic injury consisted of broad areas of haemorrhagic necrosis, with evidence of tissue inflammation and hyperaemia, and was similar in area and nature to that induced by TNBS in a group of saline-treated rats (48±3%; $n=9$). There was no detectable macroscopic injury in the colons from the non-challenged group of rats receiving saline p.o. (Fig. 1).

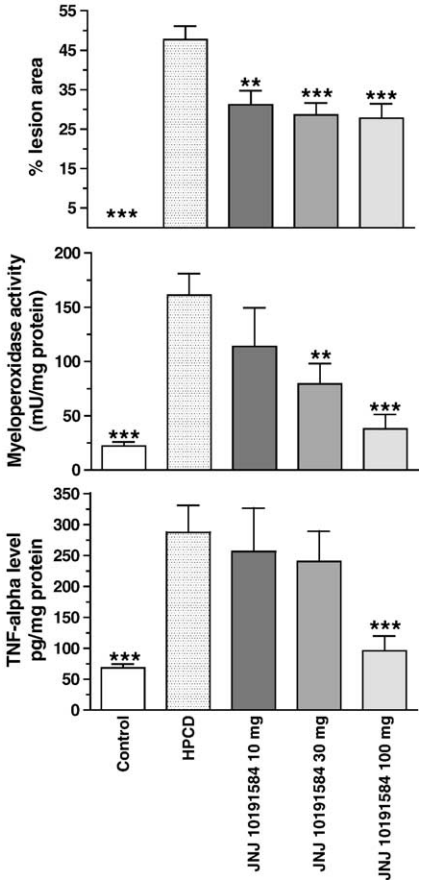


Fig. 1. Effects of the vehicle HPCD, or JNJ 10191584 (10, 30, 100 mg/kg b.i.d. p.o), on the area of colonic injury, expressed as % of the total colonic area of the segment (upper panel), colonic myeloperoxidase activity, expressed as mU/mg protein (middle panel) and colonic TNF-α levels expressed as pg/mg protein (lower panel). Measurements were taken 3 days after intracolonic challenge with TNBS. The values from the non-challenged control group are also shown. Results are expressed as mean±S.E.M.; $n=8$–16; ⋆⋆$P<0.01$, ⋆⋆⋆$P<0.001$ compared with the HPCD group.

Table 1
Comparison of the effects of JNJ 10191584 or JNJ 7777120 (both at 100 mg/kg b.i.d. p.o) on the area of colonic injury, increase in colon wet weight, colonic myeloperoxidase activity and colonic TNF-α levels

| | % inhibition | |
|---|---|---|
| | JNJ 10191584 | JNJ 7777120 |
| Macroscopic damage area | 40±8[c] | 44±10[a] |
| Colon weight increase | 23±6[a] | 24±6[a] |
| Myeloperoxidase | 79±6[c] | 35±6[b] |
| TNF-α | 67±6[c] | 56±12[b] |

Measurements were taken 3 days after intracolonic challenge with TNBS. Results are expressed as % inhibition of the respective values in the HPCD vehicle group, mean±S.E.M.; $n=8$–16; [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ compared with HPCD group.

Treatment with JNJ 10191584 (10, 30 and 100 mg/kg p.o., twice a day, equivalent to doses of 7, 20 and 70 mg/kg b.i.d. of the free base) commencing 24 h prior to challenge, caused a dose-dependent reduction in the area of TNBS-induced colonic injury, being statistically significantly different from the damage in the HPCD vehicle group at all doses (Fig. 1). However, there was a shallow dose–response relationship for this parameter, with the higher dose of JNJ 10191584 (100 mg/kg p.o., twice a day) causing a 40±8% inhibition ($P<0.001$; $n=16$) of this macroscopic injury, as shown in Table 1.

A confirmatory study was conducted to evaluate the actions of a further $H_4$-receptor antagonist, JNJ 7777120, where the area of colonic injury, determined 72 h after TNBS challenge involved 52±6% ($n=8$) of the total colonic area of the segment studied. Treatment with JNJ 7777120 (100 mg/kg p.o. twice a day, equivalent to 70 mg/kg b.i.d of the free base) caused similar degree of inhibition of the area of macroscopic injury as did JNJ 10191584 (Table 1).

When the severity of the colonic damage was evaluated using a 1–5 macroscopic score, comparable findings to those from the determination of damage area were noted. Thus, the marked a near-maximal score that was achieved following challenge in the HPCD vehicle group, which was dose-dependently reduced by treatment with JNJ 10191584 (10–100 mg/kg, b.i.d), as shown in Table 2. Likewise, treatment with JNJ 7777120 (100 mg/kg, b.i.d) reduced the macroscopic score (Table 2).

Table 2
The effects of JNJ 10191584 (10, 30 and 100 mg/kg b.i.d. p.o) or JNJ 7777120 (100 mg/kg b.i.d. p.o), on the macroscopic score assigned to the colonic injury, 3 days after intracolonic challenge with TNBS, compared to that in the HPCD vehicle group and the non-challenged control group

| | mg/kg | Macroscopic score |
|---|---|---|
| Control | – | 0 |
| HPCD | – | 4.7±0.1 |
| JNJ 10191584 | 10 | 4.2±0.4 |
| JNJ 10191584 | 30 | 3.7±0.4[a] |
| JNJ 10191584 | 100 | 3.3±0.4[a] |
| JNJ 7777120 | 100 | 4.0±0.2[a] |

Results are expressed as the score on a 1–5 scale, mean±S.E.M.; $n=8$–16; [a]$P<0.05$, compared with the HPCD vehicle group.

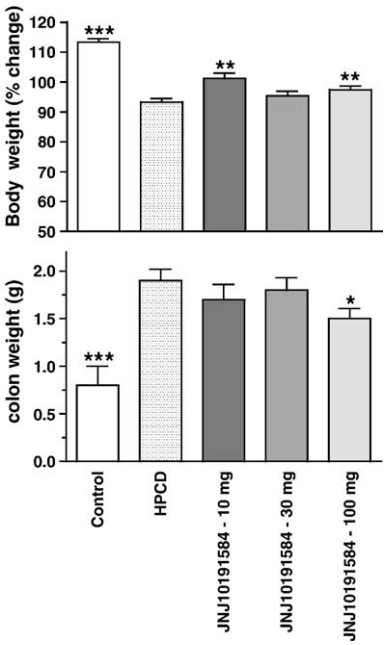


Fig. 2. Effects of the vehicle HPCD, or JNJ 10191584 (10, 30, 100 mg/kg b.i.d. p.o), on the change in body weight, expressed a % change in body weight prior to challenge (upper panel) and the weight of the colon segment expressed as g (lower panel) 3 days after intracolonic challenge with TNBS. The values from the non-challenged control group are also shown. Results are expressed as mean±S.E.M.; $n=8$–16; ⋆$P<0.05$, ⋆⋆$P<0.01$, ⋆⋆⋆$P<0.001$ compared with the HPCD group.

### 3.2. Effects on body and colon weight

Following challenge with TNBS, there was a progressive fall in body weight over the 3 day treatment period in the HPCD vehicle-treated group, being reduced to 93±1% of the pre-challenged weight, while the non-challenged group gained weight over the 4 day experimental period (Fig. 2). The fall in body weight following challenge with TNBS was attenuated by JNJ 10191584 (10–100 mg/kg p.o., twice a day), the effects being significant at both the lower and higher doses (Fig. 2).

Treatment with JNJ 7777120 (100 mg/kg p.o. twice a day) significantly ($P<0.01$) attenuated the fall in body weight determined 3 day after TNBS challenge, being 103±3% ($n=10$) of the weight prior to challenge compared with 94±2% ($n=8$) of that weight in the corresponding HPCD vehicle group.

As an indirect index of inflammatory oedema in the colonic tissue, the weight of the colonic segments was determined at the end of the treatment period. As shown in Fig. 2, the colonic weight in the groups receiving HPCD and challenged with intracolonic TNBS was significantly higher than that of non-challenged colon for a comparable tissue segment. Treatment with JNJ 10191584 at the higher dose (100 mg/kg p.o., twice a day) significantly reduced the elevated colonic weight (Fig. 2).

Treatment with JNJ 7777120 (100 mg/kg p.o. twice a day) also reduced the increase in colon weight, and this was by a comparable degree as with JNJ 10191584 (Table 1).

### 3.3. Effects on colonic myeloperoxidase levels

The level of myeloperoxidase activity in the colonic tissue from rats in the non-challenged control group was substantially increased in the HPCD vehicle group following TNBS challenge, as shown in Fig. 1. Treatment with JNJ 10191584 (10–100 mg/kg p.o., twice a day) caused a dose-dependent reduction in the TNBS-elevated myeloperoxidase levels, being significant at the intermediate and higher doses (Fig. 1).

Treatment with JNJ 7777120 (100 mg/kg p.o., twice a day) likewise caused a significant reduction in the elevated myeloperoxidase activity, although not to the same extent as did JNJ 10191584 (Table 1).

### 3.4. Effects on colonic TNF-α levels

The levels of TNF-α in the control colonic tissue from unchallenged rats was significantly increased some 4-fold following intrarectal TNBS, determined after 3 days in the HPCD vehicle as shown in Fig. 1. These elevated TNF-α levels

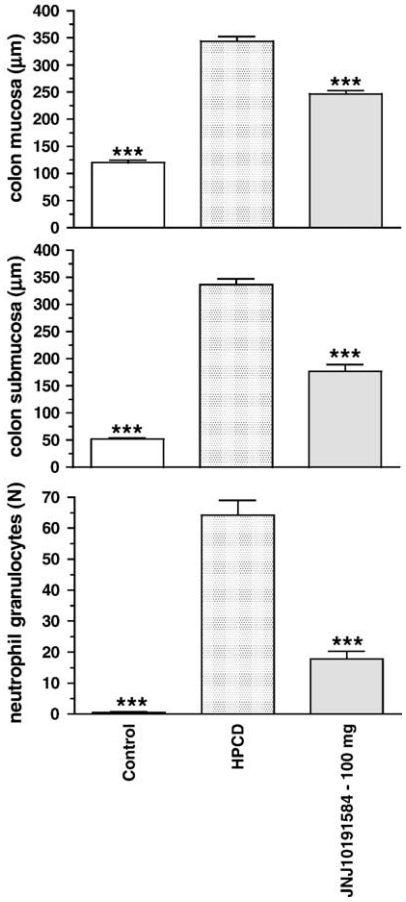


Fig. 3. Effects of the vehicle HPCD or JNJ 10191584 (10, 30, 100 mg/kg b.i.d. p.o), on TNBS-induced change in the depth of the distal colonic mucosa (upper panel), the submucosa (middle panel) and the neutrophil infiltration into the submucosa of the colon 3 days after intracolonic challenge with TNBS, as determined by microscopic evaluation. The values from the non-challenged control group are also shown. Results are expressed as mean±S.E.M.; $n=12$ where statistical difference is shown as ⋆⋆⋆$P<0.001$ compared with the HPCD group.

were significantly reduced following administration of JNJ 10191584 (100 mg/kg p.o., twice a day) as shown on Fig. 1.

Likewise, treatment with JNJ 7777120 (100 mg/kg p.o. twice a day) caused a similar reduction in TNF-α levels in the inflamed colon (Table 1).

### 3.5. Histological evaluation of effects of JNJ 10191584

TNBS challenge caused a significant increase in the measurable depth of the sections of the colonic mucosa and submucosa compared with the non-challenged control, reflecting the tissue disruption and oedema accompanied by cellular infiltration (Fig. 3). Treatment with JNJ 10191584 (100 mg/kg p.o., twice a day) caused a significant reduction in this elevated depth of tissue in both the mucosa and submucosa (Fig. 3).

Challenge with TNBS also caused a substantial neutrophil infiltration in the colonic tissue compared with the unchallenged control group (Fig. 3). This cellular influx was significantly ($P<0.001$) reduced by treatment with JNJ 10191584 (100 mg/kg p.o., twice a day) as shown in Fig. 3.

## 4. Discussion

The TNBS-model of colitis is widely used and well characterised (Boughton-Smith et al., 1988a,b; Morris et al., 1989; Reuter et al., 1996; Kiss et al., 1997; Galvez et al., 2000; Whittle et al., 2003). The inflammatory response provoked by TNBS is considered to reproduce many of the macroscopic, histological and immunological hallmarks of clinical colitis. Thus, open ulceration may be produced, with transmural inflammation and thickening of the bowel wall. Histological features include distorted crypt architecture, crypt atrophy, granulomata, giant cells, basal lymphoid aggregates, with an inflammatory infiltrate with neutrophilic and eosinophilic involvement (Morris et al., 1989; Yamada et al., 1992; Hoffmann et al., 1997; Torres et al., 1999; Neurath et al., 2000; Whittle et al., 2003). In the present study, the intra-colonic instillation of TNBS (10 mg) caused a subchronic colitis in the rat. This macroscopic injury in the colon, determined 72 h after challenge, consisted of broad areas of haemorrhagic necrosis, with evidence of tissue inflammation and hyperaemia.

Oral administration of JNJ 10191584 produced a significant reduction in the extent of macroscopic injury, both in terms of the involved area and the severity of the damage at all the doses evaluated, although the dose–response relationship was somewhat shallow. The elevated weight of the colonic segments following TNBS challenge was also significantly reduced by JNJ 10191584 at the highest dose evaluated. Moreover, histological analysis of the colonic tissue indicated that the increase in the measurable depth of the colonic mucosa and submucosa following TNBS challenge was reduced by JNJ 10191584, presumably reflecting attenuation of the tissue disruption and oedema. The fall in body weight seen in the vehicle-treated groups over the 3 days following TNBS challenge was also reduced by JNJ 10191584, which likely reflects the reduction in colonic tissue injury by this agent and abrogation of any subsequent stress to the animal or the amelioration of any appetite loss. To confirm that these effects did reflect actions on the histamine $H_4$ receptor, further studies with JNJ 7777120 were conducted, which likewise showed a significant reduction in macroscopic injury and score, and in colonic weight, while attenuating the TNBS-induced fall in body weight.

The macroscopic injury caused by TNBS challenge was accompanied by a substantial increase in the levels of myeloperoxidase, an index of neutrophil infiltration, in the colonic tissue that was confirmed by the histological analysis. Studies in both this experimental model of colitis (Boughton-Smith et al., 1988a,b; Morris et al., 1989; Guo et al., 1999; Sun et al., 2001) and in clinical studies (Raab et al., 1993; Kristjansson et al., 2004) indicate the important involvement of neutrophils in these inflammatory processes in the colon. JNJ 10191584 dose-dependently reduced the elevated colonic myeloperoxidase levels and also inhibited the elevated neutrophil influx seen by histological evaluation in the colonic submucosa following TNBS challenge. JNJ 7777120 likewise reduced the elevation of myeloperoxidase in the challenged colonic tissue. Other in vivo studies using histamine $H_4$ antagonists have demonstrated actions on neutrophils, with the neutrophil influx into the peritoneal cavity provoked by zymosan being inhibited by JNJ 7777120 following oral or subcutaneous administration (Thurmond et al., 2004). Previous work on neutrophil infiltration into the pleural cavity has also shown that it can be reduced by the non-selective histamine $H_3$/$H_4$ receptor antagonist, thioperamide, thought to be acting on the histamine $H_4$ receptor, which also reduced histamine-dependent mobilization of neutrophils from bone marrow (Takeshita et al., 2003, 2004).

Effects on other cell types acting in concert, are also likely be involved in the beneficial actions of these histamine $H_4$ antagonists in colitis. Thus, JNJ 7777120 has previously been shown to affect mast cell function in vitro, to inhibit mast cell migration in mouse trachea in vivo and to ameliorate the mast-cell dependent peritonitis induced by zymosan in vivo (Thurmond et al., 2004). The degranulation of mast cells occurs in colonic tissue from colitic patients (Bischoff et al., 1996). Moreover, the urinary excretion of the histamine metabolite, *N*-methyl histamine, increased some two-fold in patients with active ulcerative colitis, with its output correlating with the disease severity, as also found in Crohn's disease patients (Winterkamp et al., 2002). Mast cells have been implicated in the fibrosis provoked by TNBS (Xu et al., 2002), as well as in the epithelial barrier dysfunction that accompanies the colitis in this model (Stein et al., 1998; Barreau et al., 2004). In studies in the pleural cavity, it has been suggested that the production of the chemotactic eicosanoid, leukotriene $B_4$ is an underlying mechanism of the histamine $H_4$-receptor- and mast cell-dependent neutrophilic inflammation evoked by zymosan (Takeshita et al., 2003). It is therefore relevant that leukotriene $B_4$ is considered as an inflammatory mediator involved with TNBS colitis (Boughton-Smith et al., 1988a,b; Wallace et al., 1989; Rachmilewitz et al., 1989). In addition, mast cells are considered to be an important source of the cytokine, TNF-α in human intestinal tissue (Bischoff et al., 1999).

Eosinophils are also considered to be involved in the inflammatory process in clinical colitis (Raab et al., 1998; Bischoff et al., 1996; Hogan and Rothenberg, 2004; Lampinen et al., 2001, 2004) and such cells, like mast cells, express the histamine $H_4$ receptor (see Fung-Leung et al., 2004). The functional responses of these cells in terms of shape change, chemotaxis and adhesion molecule expression is down-regulated by JNJ 7777120 (Ling et al., 2004). The potential effect of such actions on eosinophilic function in vivo on the progress of the colitis in the present model requires exploration.

The release of pro-inflammatory cytokines is considered to play a pivotal pathological role in the inflammatory events underlying colitis (Papadakis and Targan, 2000) and the therapeutic use of anti-TNF-α approaches have proved very beneficial (Rutgeerts et al., 2004). Colonic TNF-α levels are increased in TNBS-induced colitis (Ameho et al., 1997; Ribbons et al., 1997; Sykes et al., 1999; Villegas et al., 2003) and inhibitors of its synthesis or administration of the TNF-α antibody, infliximab can reduce the damage (Ten Hove et al., 2001; Bobin-Dubigeon et al., 2001; Woodruff et al., 2003). In the present study, the elevated levels of TNF-α in the colonic tissue were significantly reduced by treatment with either JNJ 10191584 or JNJ 7777120. It is possible that the inhibition of the TNF-α levels reflects an action of the histamine $H_4$ receptor antagonists on the cellular transduction mechanisms promoting cytokine biosynthesis. However, it is also likely that the effect is to some extent, the consequence of the reduced area of tissue injury and inflammation brought about by the compounds, with a resulting reduced number of cytokine-producing inflammatory cells infiltrating into the colonic tissue.

The present study thus indicates that JNJ 10191584, as well as JNJ 7777120 when given twice a day by oral gavage, has an effective therapeutic activity in this rat model of colitis. These agents thus significantly reduced both macroscopic colonic injury, attenuated the increase in tissue oedema and inhibited the rise in myeloperoxidase levels and neutrophil influx, as well as reduced the increase in TNF-α levels in colonic tissue that follow the challenge by TNBS. The mechanisms and cellular events underlying the anti-inflammatory effects of these selective histamine $H_4$ receptor antagonists have not yet been fully identified. Indeed, whether such beneficial effects on colitis reflect the modulatory actions on a local mast cell-driven inflammatory response involving neutrophils, along with the known actions of the histamine $H_4$ receptor antagonists on eosinophil function, warrants further exploration. The present findings with histamine $H_4$ antagonists could point to a novel pharmacological approach to the therapy of gut inflammation.

## Acknowledgments

BJRW is supported by a grant from the William Harvey Research Foundation; C V is supported by a Janos Bolyai Research Scholarship.

## References

Ameho, C.K., Adjei, A.A., Harrison, E.K., Takeshita, K., Morioka, T., Arakaki, Y., Ito, E., Suzuki, I., Kulkarni, A.D., Kawajiri, A., Yamamoto, S., 1997. Prophylactic effect of dietary glutamine supplementation on interleukin 8 and tumour necrosis factor alpha production in trinitrobenzene sulphonic acid induced colitis. Gut 41, 487–493.

Barreau, F., Ferrier, L., Fioramonti, J., Bueno, L., 2004. Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier and mucosal immunity in rats. Gut 53, 501–506.

Bischoff, S.C., Wedemeyer, J., Herrmann, A., Meier, P.N., Trautwein, C., Cetin, Y., Maschek, H., Stolte, M., Gebel, M., Manns, M.P., 1996. Quantitative assessment of intestinal eosinophils and mast cells in inflammatory bowel disease. Histopathology 28, 1–13.

Bischoff, S.C., Lorentz, A., Schwengberg, S., Weier, G., Raab, R., Manns, M.P., 1999. Mast cells are an important cellular source of tumour necrosis factor alpha in human intestinal tissue. Gut 44, 643–652.

Bobin-Dubigeon, C., Collin, X., Grimaud, N., Robert, J.M., Le Baut, G., Petit, J.Y., 2001. Effects of tumour necrosis factor-alpha synthesis inhibitors on rat trinitrobenzene sulphonic acid-induced chronic colitis. Eur. J. Pharmacol. 431, 103–110.

Boughton-Smith, N.K., Wallace, J.L., Morris, G.P., Whittle, B.J.R., 1988a. The effect of anti-inflammatory drugs on eicosanoid formation in a chronic model of inflammatory bowel disease in the rat. Br. J. Pharmacol. 94, 65–72.

Boughton-Smith, N.K., Wallace, J.L., Whittle, B.J.R., 1988b. Relationship between arachidonic acid metabolism, myeloperoxidase activity and leukocyte infiltration in a rat model of inflammatory bowel disease. Agents Actions 25, 115–123.

Buckland, K.F., Williams, T.J., Conroy, D.M., 2003. Histamine induces cytoskeletal changes in human eosinophils via the H(4) receptor. Br. J. Pharmacol. 140, 1117–1127.

Coge, F., Guenin, S.P., Rique, H., Boutin, J.A., Galizzi, J.P., 2001. Structure and expression of the human histamine H4-receptor gene. Biochem. Biophys. Res. Commun. 284, 301–309.

De Esch, I.J., Thurmond, R.L., Jongejan, A., Leurs, R., 2005. The histamine H(4) receptor as a new therapeutic target for inflammation. Trends Pharmacol. Sci. 26, 462–469.

Fung-Leung, W.-P., Thurmond, R.L., Ling, P., Karlsson, L., 2004. $H_4$ receptor antagonist—the new antihistamines? Curr. Opin. Investig. Drugs 5, 1174–1183.

Galvez, J., Garrido, M., Merlos, M., Torres, M.I., Zarzuelo, A., 2000. Intestinal anti-inflammatory activity of UR-12746, a novel 5-ASA conjugate, on acute and chronic experimental colitis in the rat. Br. J. Pharmacol. 130, 1949–1959.

Gantner, F., Sakai, K., Tusche, M.W., Cruikshank, W.W., Center, D.M., Bacon, K.B., 2002. Histamine $H_4$ and $H_2$ receptors control histamine-induced interleukin-16 release from human CD8+ T cells. J. Pharmacol. Exp. Ther. 303, 300–307.

Guo, X., Wang, W.P., Ko, J.K., Cho, C.H., 1999. Involvement of neutrophils and free radicals in the potentiating effects of passive cigarette smoking on inflammatory bowel disease in rats. Gastroenterology 117, 884–892.

Hoffmann, P., Zeeh, J.M., Lakshmanan, J., Wu, V.S., Procaccino, F., Reinshagen, M., McRoberts, J.A., Eysselein, V.E., 1997. Increased expression of transforming growth factor alpha precursors in acute experimental colitis in rats. Gut 41, 195–202.

Hofstra, C.L., Desai, P.J., Thurmond, R.L., Fung-Leung, W.-P., 2003. Histamine $H_4$ receptor mediates chemotaxis and calcium mobilization of mast cells. J. Pharmacol. Exp. Ther. 305, 1212–1221.

Hogan, S.P., Rothenberg, M.E., 2004. Review article: the eosinophil as a therapeutic target in gastrointestinal disease. Aliment. Pharmacol. Ther. 20, 1231–1240.

Jablonowski, J.A., Grice, C.A., Chai, W., Dvorak, C.A., Venable, J.D., Kwok, A.K., Ly, K.S., Wei, J., Baker, S.M., Desai, P.J., Jiang, W., Wilson, S.J., Thurmond, R.L., Karlsson, L., Edwards, J.P., Lovenberg, T.W., Carruthers, N.I., 2003. The first potent and selective non-imidazole human histamine $H_4$ receptor antagonists. J. Med. Chem. 46, 3957–3960.

Jablonowski, J.A., Carruthers, N.I., Thurmond, R.L., 2004. Histamine $H_4$ receptor ligands and their potential therapeutic uses. Mini Rev. Med. Chem. 4, 993–1000.

Kiss, J., Lamarque, D., Delchier, J.C., Whittle, B.J.R., 1997. Time-dependent actions of nitric oxide synthase inhibition on colonic inflammation induced by trinitrobenzene sulphonic acid in rats. Eur. J. Pharmacol. 336, 219–224.

Kristjansson, G., Venge, P., Wanders, A., Loof, L., Hallgren, R., 2004. Clinical and subclinical intestinal inflammation assessed by the mucosal patch technique: studies of mucosal neutrophil and eosinophil activation in inflammatory bowel diseases and irritable bowel syndrome. Gut 53, 1806–1812.

Lampinen, M., Carlson, M., Sangfelt, P., Taha, Y., Thorn, M., Loof, L., Raab, Y., Venge, P., 2001. IL-5 and TNF-alpha participate in recruitment of eosinophils to intestinal mucosa in ulcerative colitis. Dig. Dis. Sci. 46, 2004–2009.

Lampinen, M., Carlson, M., Hakansson, L.D., Venge, P., 2004. Cytokine-regulated accumulation of eosinophils in inflammatory disease. Allergy 59, 793–805.

Ling, P., Ngo, K., Nguyen, S., Thurmond, R.L., Edwards, J.P., Karlsson, L., Fung-Leung, W.P., 2004. Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. Br. J. Pharmacol. 142, 161–171 (Erratum in: Br J Pharmacol. 2004,142,1052).

Liu, C., Ma, X.-J., Jiang, X., Wilson, S.J., Hofstra, C.L., Blevitt, J., Pyati, J., Li, X., Chai, W., Carruthers, N., Lovenberg, T.W., 2001a. Cloning and pharmacological characterization of a fourth histamine receptor ($H_4$) expressed in bone marrow. Mol. Pharmacol. 59, 420–426.

Liu, C., Wilson, S.J., Kuei, C., Lovenberg, T.W., 2001b. Comparison of human, mouse, rat, and guinea pig histamine $H_4$ receptors reveals substantial pharmacological species variation. J. Pharmacol. Exp. Ther. 299, 121–130.

Morse, K.L., Behan, J., Laz, T.M., West Jr., R.E., Greenfeder, S.A., Anthes, J.C., Umland, S., Wan, Y., Hipkin, R.W., Gonsiorek, W., Shin, N., Gustafson, E.L., Qiao, X., Wang, S., Hedrick, J.A., Greene, J., Bayne, M., Monsma Jr., F.J., 2001. Cloning and characterization of a novel human histamine receptor. J. Pharmacol. Exp. Ther. 296, 1058–1066.

Morris, G.P., Beck, P.L., Herridge, M.S., Depew, W.T., Szewzcuk, M.R., Wallace, J.L., 1989. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96, 795–803.

Nakamura, T., Itadani, H., Hidaka, Y., Ohta, M., Tanaka, K., 2000. Molecular cloning and characterization of a new human histamine receptor, HH4R. Biochem. Biophys. Res. Commun. 279, 615–620.

Neurath, M., Fuss, I., Strober, W., 2000. TNBS-colitis. Int. Rev. Immunol. 19, 51–62.

Nguyen, T., Shapiro, D.A., George, S.R., Setola, V., Lee, D.K., Cheng, R., Rauser, L., Lee, S.P., Lynch, K.R., Roth, B.L., O'Dowd, B.F., 2001. Discovery of a novel member of the histamine receptor family. Mol. Pharmacol. 59, 427–433.

Oda, T., Morikawa, N., Saito, Y., Masuho, Y., Matsumoto, S.-I., 2000. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. J. Biol. Chem. 275, 36781–36786.

O'Reilly, M., Alpert, R., Jenkinson, S., Gladue, R.P., Foo, S., Trim, S., Peter, B., Trevethick, M., Fidock, M., 2002. Identification of a histamine $H_4$ receptor on human eosinophils-role in eosinophil chemotaxis. J. Recept. Signal Transduct. Res. 22, 431–448.

Papadakis, K.A., Targan, S.R., 2000. Role of cytokines in the pathogenesis of inflammatory bowel disease. Annu. Rev. Med. 51, 289–298.

Raab, Y., Gerdin, B., Ahlstedt, S., Hallgren, R., 1993. Neutrophil mucosal involvement is accompanied by enhanced local production of interleukin-8 in ulcerative colitis. Gut 34, 1203–1206.

Raab, Y., Fredens, K., Gerdin, B., Hallgren, R., 1998. Eosinophil activation in ulcerative colitis: studies on mucosal release and localization of eosinophil granule constituents. Dig. Dis. Sci. 43, 1061–1070.

Rachmilewitz, D., Simon, P.L., Schwartz, L.W., Griswold, D.E., Fondacaro, J. D., Wasserman, M.A., 1989. Inflammatory mediators of experimental colitis in rats. Gastroenterology 97, 326–337.

Reuter, B.K., Asfaha, S., Buret, A., Sharkey, K.A., Wallace, J.L., 1996. Exacerbation of inflammation-associated colonic injury through inhibition of cyclooxygenase-2. J. Clin. Invest. 98, 2076–2085.

Ribbons, K.A., Thompson, J.H., Liu, X., Pennline, K., Clark, D.A., Miller, M.J., 1997. Anti-inflammatory properties of interleukin-10 administration in hapten-induced colitis. Eur. J. Pharmacol. 323, 245–254.

Rutgeerts, P., Van Assche, G., Vermeire, S., 2004. Optimizing anti-TNF treatment in inflammatory bowel disease. Gastroenterology 126, 1593–1610.

Stein, J., Ries, J., Barrett, K.E., 1998. Disruption of intestinal barrier function associated with experimental colitis: possible role of mast cells. Am. J. Physiol. 274, G203–G209.

Sun, F.F., Lai, P.S., Yue, G., Yin, K., Nagele, R.G., Tong, D.M., Krzesicki, R.F., Chin, J.E., Wong, P.Y., 2001. Pattern of cytokine and adhesion molecule mRNA in hapten-induced relapsing colon inflammation in the rat. Inflammation 25, 33–45.

Sykes, A.P., Bhogal, R., Brampton, C., Chander, C., Whelan, C., Parsons, M.E., Bird, J., 1999. The effect of an inhibitor of matrix metalloproteinases on colonic inflammation in a trinitrobenzenesulphonic acid rat model of inflammatory bowel disease. Aliment. Pharmacol. Ther. 13, 1535–1542.

Takeshita, K., Sakai, K., Bacon, K.B., Gantner, F., 2003. Critical role of histamine H4 receptor in leukotriene B4 production and mast cell-dependent neutrophil recruitment induced by zymosan in vivo. J. Pharmacol. Exp. Ther. 307, 1072–1078.

Takeshita, K., Bacon, K.B., Gantner, F., 2004. Critical role of L-selectin and histamine H4 receptor in zymosan-induced neutrophil recruitment from the bone marrow: comparison with carrageenan. J. Pharmacol. Exp. Ther. 310, 272–280.

Ten Hove, T., Corbaz, A., Amitai, H., Aloni, S., Belzer, I., Graber, P., Drillenburg, P., van Deventer, S.J., Chvatchko, Y., Te Velde, A.A., 2001. Blockade of endogenous IL-18 ameliorates TNBS-induced colitis by decreasing local TNF-alpha production in mice. Gastroenterology 121, 1372–1379.

Terzioglu, N., van Rijn, R.M., Bakker, R.A., De Esch, I.J., Leurs, R., 2004. Synthesis and structure-activity relationships of indole and benzimidazole piperazines as histamine H(4) receptor antagonists. Bioorg. Med. Chem. Lett. 14, 5251–5256.

Thurmond, R.L., Desai, P.J., Dunford, P.J., Fung-Leung, W.P., Hofstra, C.L., Jiang, W., Nguyen, S., Riley, J.P., Sun, S., Williams, K.N., Edwards, J.P., Karlsson, L., 2004. A potent and selective histamine $H_4$ receptor antagonist with anti-inflammatory properties. J. Pharmacol. Exp. Ther. 309, 404–413.

Torres, M.I., Garcia-Martin, M., Fernandez, M.I., Nieto, N., Gil, A., Rios, A., 1999. Experimental colitis induced by trinitrobenzenesulfonic acid: an ultrastructural and histochemical study. Dig. Dis. Sci. 44, 2523–2529.

Venable, J.D., Cai, H., Chai, W., Dvorak, C.A., Grice,C.A., Jablonowski, J.A., Kwok, A.K., Ly, S.K., Pio, B., Wei, J.,Desai, P.J., Wen Jiang, Nguyen, S., Wilson, S.J.,Dunford, P.J., Thurmond, R.L., Lovenberg, T.W., Karlsson, L., Carruthers, N.I., Edwards, J.P., in press. Preparation and biological evaluation of indole, benzimidazole, and thienopyrrole piperazine carboxamides: potent human histamine $H_4$ antagonists. J. Med. Chem.

Villegas, I., La Casa, C., Orjales, A., Alarcon de la Lastra, C., 2003. Effects of dosmalfate, a new cytoprotective agent, on acute and chronic trinitrobenzene sulphonic acid-induced colitis in rats. Eur. J. Pharmacol. 460, 209–218.

Wallace, J.L., MacNaughton, W.K., Morris, G.P., Beck, P.L., 1989. Inhibition of leukotriene synthesis markedly accelerates healing in a rat model of inflammatory bowel disease. Gastroenterology 96, 29–36.

Winterkamp, S., Weidenhiller, M., Otte, P., Stolper, J., Schwab, D., Hahn, E.G., Raithel, M., 2002. Urinary excretion of *N*-methylhistamine as a marker of disease activity in inflammatory bowel disease. Am. J. Gastroenterol. 97, 3071–3077.

Whittle, B.J.R., Cavicchi, M., Lamarque, D., 2003. Assessment of anticolitic drugs in the trinitrobenzene sulfonic acid (TNBS) rat model of inflammatory bowel disease. In: Winyard, P.G., Willoughby, D.A. (Eds.), Inflammation Protocols: Methods in Molecular Biology, vol. 225. Humana Press, New Jersey, pp. 209–222.

Woodruff, T.M., Arumugam, T.V., Shiels, I.A., Reid, R.C., Fairlie, D.P., Taylor, S.M., 2003. A potent human C5a receptor antagonist protects against disease

pathology in a rat model of inflammatory bowel disease. J. Immunol. 171, 5514–5520.

Xu, X., Weskler-Zangen, S., Pikarsky, A., Pappo, O., Wengrower, D., Bischoff, S.C., Pines, M., Rivkind, A., Goldin, E., Levi-Schaffer, F., 2002. Mast cells involvement in the inflammation and fibrosis development of the TNBS-induced rat model of colitis. Scand. J. Gastroenterol. 37, 330–337.

Yamada, Y., Marshall, S., Specian, R.D., Grisham, M.B., 1992. A comparative analysis of two models of colitis in rats. Gastroenterology 102, 524–534.

Zhu, Y., Michalovich, D., Wu, H.-L., Tan, K.B., Dytko, G.M., Mannan, I.J., Boyce, R., Alston, J., Tierney, L.A., Li, X., 2001. Cloning, expression and pharmacological characterization of a novel human histamine receptor. Mol. Pharmacol. 59, 434–441.