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Short communication

Antiinflammatory and antinociceptive effects of the selective histamine H_4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced acute inflammation

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Abstract

The effects of the highly selective histamine H_4 receptor antagonists JNJ7777120 and VUF6002 were investigated on the carrageenan-induced inflammation and thermal hyperalgesia in rats. JNJ7777120 (10 and 30 mg/kg, s.c.) and VUF6002 (10 mg/kg, s.c.) significantly reduced paw edema and hyperalgesia provoked by subplantar injection of carrageenan; the effect was evident against the early (2 h) phase of inflammation. An inactive analog of VUF6002, VUF6007 (10 mg/kg, s.c.) slightly aggravated paw edema, while leaving unaltered carrageenan-induced nociception. These findings indicate that histamine H_4 receptors participate in the early phase of acute inflammation induced by carrageenan in rats, influencing both edema and thermal hyperalgesia.

Keywords: Histamine H_4 receptor antagonists; JNJ7777120; VUF6002; Paw edema; Thermal hyperalgesia; Carrageenan

1. Introduction

The histamine H_4 receptor is a G-protein coupled receptor recently cloned by several independent groups following the elucidation of the human genome (for review see Hough, 2001; de Esch et al., 2005; Lim et al., 2006). The high expression of the histamine H_4 receptor in cells of the hemopoietic lineage and immune cells (eosinophils, leukocytes, mast cells, T cells and dendritic cells) suggests that this new histamine receptor plays a role in inflammatory and immune responses (Jablonowski et al., 2004; de Esch et al., 2005). Indeed, *in vitro* data have unravelled a functional role for histamine H_4 receptors in the control of chemotactic response and cytoskeletal changes of human eosinophils (O'Reilly et al., 2002; Buckland et al., 2003; Ling et al., 2004), murine mast cell chemotaxis (Hofstra et al., 2003), up-regulation of the adhesion molecules CD11b and CD54 (Ling et al., 2004) and release of interleukin-16 from human $CD8^+$ T lymphocytes (Gantner et al., 2002).

Few studies have examined the biological role of histamine H_4 receptors in intact animals by using the first selective histamine H_4 receptor antagonist, JNJ7777120 (Jablonowski et al., 2004) or the mixed H_3/H_4 receptor antagonist, thioperamide. Most findings were obtained in mice and showed that histamine H_4 receptor antagonists were effective in mast cell-dependent models, including LTB_4 release (Takeshita et al., 2003; Thurmond et al., 2004), zymosan-induced pleurisy (Takeshita et al., 2004) or peritonitis (Thurmond et al., 2004), but not in mast cell-independent models, such as thioglycollate-induced peritonitis, histamine-induced paw edema in mice (Thurmond et al., 2004) or carrageenan-induced neutrophilia (Takeshita et al., 2003). However, an inhibitory effect of histamine H_4 receptor antagonists was observed on allergic airway inflammation in mast cell deficient mice (Dunford et al., 2006). In rats, a protective effect of JNJ7777120 was reported in a model of intestinal inflammation (Varga et al., 2005).

The inflammatory response to a local injection of carrageenan into a rat paw was introduced by Winter et al. (1962) and has become a widely used model of acute inflammation, involving mast cell mediators, prostaglandins and the kinin

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system (Vinegar et al., 1969; Di Rosa et al., 1971; Damas and Remacle-Volon, 1986; Kulkarni et al., 1986).

In the present study we evaluated the anti-inflammatory and antinociceptive effects of selective histamine H_4 receptor antagonists against carrageenan-induced acute inflammation in the rat paw, at selected time points (2, 4 and 6 h) after induction of the inflammatory reaction. In particular, the first selective histamine H_4 receptor antagonist JNJ7777120 (Jablonowski et al., 2004) and its benzimidazole derivative VUF6002 (Terzioglu et al., 2004) were used, together with a related analog, compound VUF6007, which binds to histamine H_4 receptors with a 100-fold lower affinity and acts as a chemical control for VUF6002 (Terzioglu et al., 2004) (Fig. 1).

2. Materials and methods

2.1. Animals

Male Wistar rats (180–250 g; Harlan-Italy, Milan) were housed under controlled standard conditions (23 °C temperature, 12 h light/dark cycle and 65% humidity). Food and water were provided *ad libitum*. The experiments received the approval of the local Animal Ethics Committee of the University of Parma, Italy.

2.2. Induction of acute inflammation

Paw edema was induced by subplantar injection of 0.1 ml of carrageenan (suspended in 1% carboxymethylcellulose, CMC) into the left hind paw.

Paw volume was measured with a plethysmometer (Basile, Comerio, Italy) immediately prior to the injection of carrageenan and thereafter at 2, 4 and 6 h. For each animal edema was expressed as % increase in paw volume after carrageenan injection relative to the pre-injection value, considered as 100.

2.3. Evaluation of thermal nociception

The hyperalgesic response to thermal stimuli was determined by using a plantar test apparatus (Ugo Basile, Comerio, Italy). Rats were placed individually in plexiglas chambers and allowed to acclimatize for 20 to 30 min before testing. The radiant heat was positioned under the chamber floor directly beneath the hind paw and the latency to paw withdrawal was automatically recorded by a photocell and an electronic timer. The intensity of the radiant heat was adjusted to achieve baseline latencies of 10–15 s and a cut-off time of 30 s was preset in order to avoid tissue damage. Unresponsive animals after 30 s (cut-off time) were discarded. Two subsequent applications of heating stimulus were done, separated by 1- to 2-min intervals, and the mean of the two measures was taken. For each animal paw withdrawal latencies were recorded before carrageenan administration and 2, 4, 6 h afterwards; responses to carrageenan were expressed as % values relative to the pre-injection value, considered as 100.

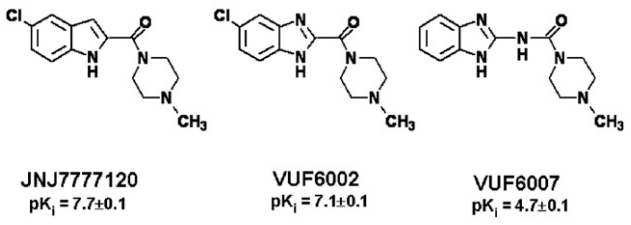

Fig. 1. Chemical structures and pharmacological properties of histamine H_4 receptor antagonists (from Terzioglu et al., 2004).

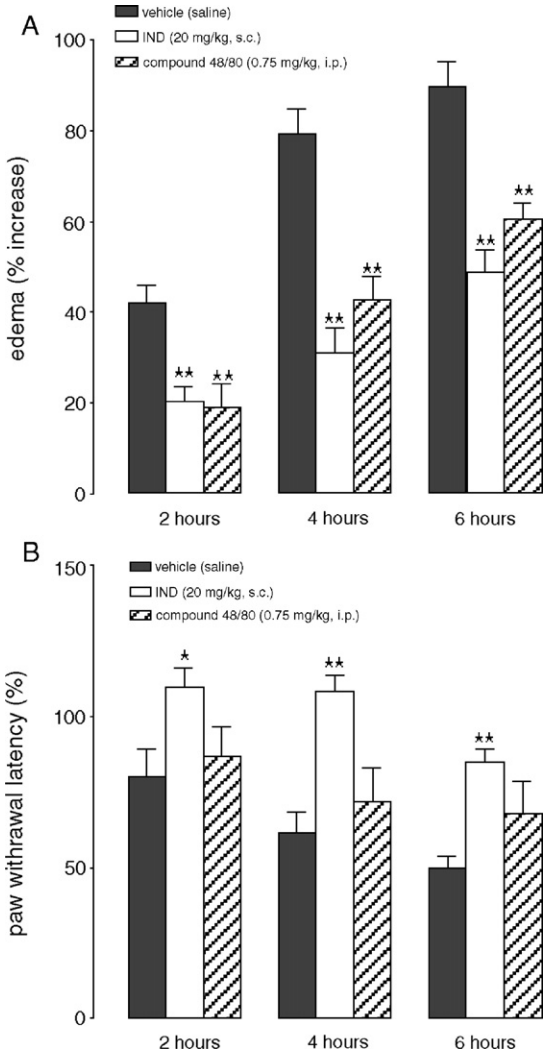

Fig. 2. Time course of increase in paw volume (A) and thermal hyperalgesia (B) induced by subplantar injection of carrageenan (1% in CMC) in vehicle-treated rats or in rats pretreated with indomethacin (IND) or compound 48/80, administered immediately prior to carrageenan injection. Data are expressed as mean±S.E.M. $N=8–10$ rats per group. *$P<0.05$ and **$P<0.01$ compared to vehicle-treated animals.

2.4. Drug administration

Equivalent volumes (0.1 ml per 100 g) of test drugs or vehicle were administered subcutaneously (s.c.) in separate

groups of rats immediately prior to carrageenan injection. In separate experiments, JNJ7777120 was injected s.c. 2 h after carrageenan administration and edema and thermal hyperalgesia were evaluated 2 h thereafter. The involvement of prostaglandins or mast cell mediators in the responses to carrageenan was assessed by the use of indomethacin (20 mg/kg, s.c.) or of the mast cell degranulator, compound 48/80 (0.75 mg/kg, intraperitoneally, i.p.), respectively.

2.5. Data analysis

Data are expressed as mean±S.E.M. Unless otherwise specified, differences between groups were assessed by using one-way ANOVA, followed by Dunnett's test. A value of $P<0.05$ was considered statistically significant. The software package Prism GraphPad 3.0 (GraphPad Software Inc., San Diego, CA) was used to process data.

2.6. Drugs

JNJ7777120, VUF6002, VUF6007 were synthetized by one of us (I.J.P de Esch). λ-carrageenan and compound 48/80 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Indomethacin was a gift from Chiesi Farmaceutici (Italy). Histamine H_4 receptor antagonists were dissolved, immediately before use, in 100% dimethylsulfoxide (DMSO); indomethacin and 48/80 were dissolved in 0.9% NaCl.

3. Results

3.1. Development of edema and thermal hyperalgesia in carrageenan-treated rats

As expected, the subplantar injection of carrageenan increased the volume of the injected hind paw (edema) and reduced latency to paw withdrawal in the presence of thermal stimuli; both effects followed a similar time-course, developing slowly and reaching a maximal intensity 5–6 h after challenge (Fig. 2). Carrageenan-induced edema was significantly reduced by indomethacin (20 mg/kg, s.c.) or compound 48/80 (0.75 mg/kg, i.p.) (Fig. 2A). By contrast, only indomethacin significantly increased paw withdrawal latency to thermal stimuli (Fig. 2B).

3.2. Effects of histamine H_4 receptor antagonists on carrageenan-induced inflammation and thermal hyperalgesia

JNJ7777120 (10 and 30 mg/kg, s.c.) significantly inhibited paw edema and increased paw withdrawal latency 2 h after carrageenan injection (Fig. 3A and C); at this time point VUF6002 (10 mg/kg, s.c.) induced a less pronounced inhibition of paw edema and nociceptive response (Fig. 3B and D). By contrast, compound VUF6007 (10 mg/kg, s.c.) significantly enhanced paw edema at 2 h (Fig. 3B), while leaving unaltered the paw withdrawal latency (Fig. 3D). When injected 2 h after carrageenan JNJ7777120 (10 mg/kg, s.c.) did not modify paw

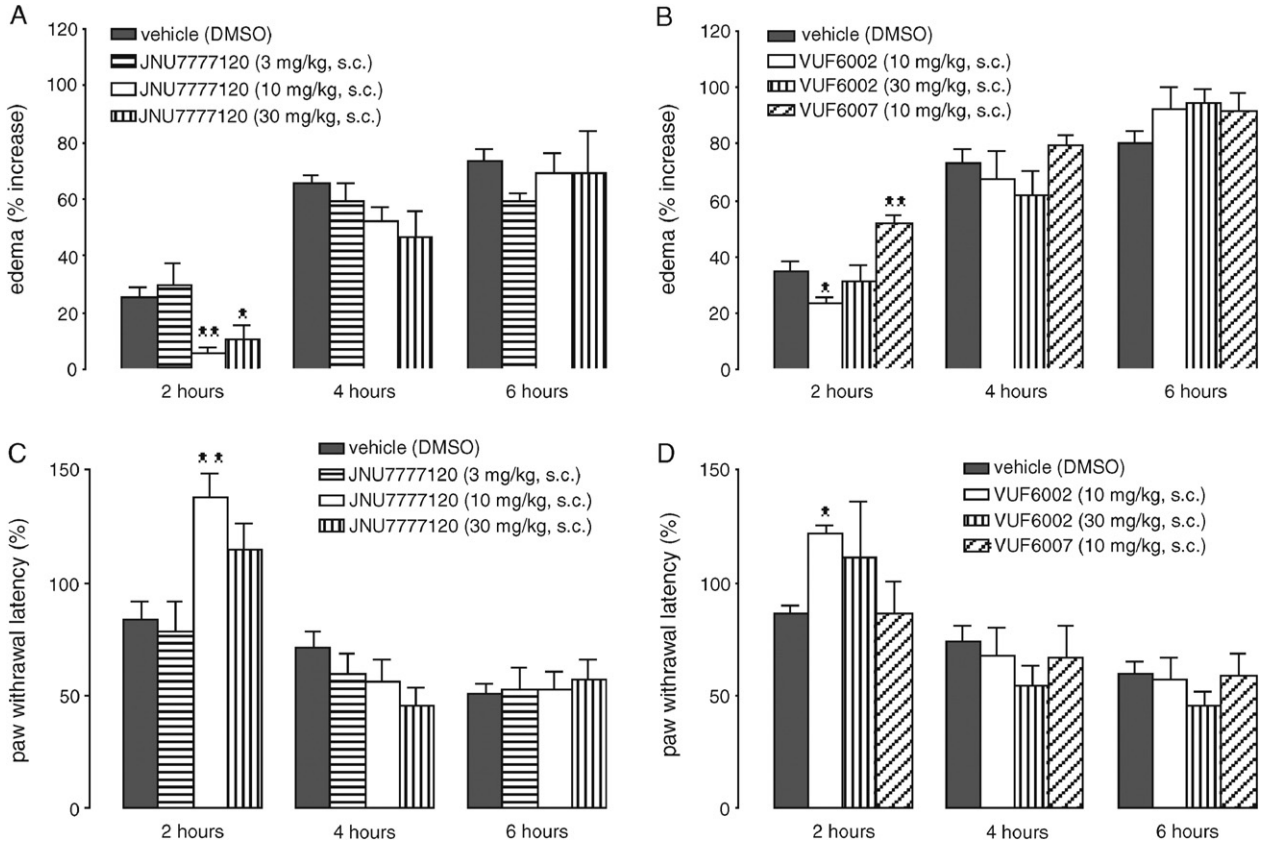

Fig. 3. Effects of histamine H_4 receptor antagonists on paw edema (A and B) and thermal hyperalgesia (C and D) induced by subplantar injection of carrageenan (1% in CMC) in rats. Data are expressed as mean±S.E.M. $N=8–10$ rats per group. *$P<0.05$ and **$P<0.01$ compared to vehicle-treated animals.

edema (% increase were: 59.83±8.39, $n=6$ and 64.06±5.01, $n=9$ for vehicle- and JNJ7777120-treated rats, respectively; $P>0.05$, Student's t test) nor thermal hyperalgesia (% reduction in paw withdrawal threshold were: 57.08±10.21, $n=6$ and 66.37±7.29, $n=9$ for vehicle- and JNJ7777120-treated rats, respectively; $P>0.05$, Student's t test).

4. Discussion

The present study investigated the anti-inflammatory and antinociceptive effects of histamine H_4 receptor antagonists in a rat model of acute inflammation and pain induced by carrageenan. The time-dependent increase in paw edema induced by carrageenan was significantly reduced, at any time point considered (2, 4 and 6 h after challenge), by parenteral administration of both indomethacin and the mast cell degranulator, compound 48/80, clearly indicating that both mast cell mediators and prostaglandins contribute to carrageenan-induced inflammation in rats.

In this model the selective histamine H_4 receptor antagonists JNJ7777120 and VUF6002, but not VUF6007, significantly reduced carrageenan-induced edema. Since both JNJ7777120 and VUF6002 have a selectivity for the histamine H_4 receptor greater than 300–1000-fold over the histamine H_1, H_2 and H_3 receptors (Jablonowski et al., 2004; Terzioglu et al., 2004), whereas the related analog VUF6007 has a 100-fold lower affinity for histamine H_4 receptors (Terzioglu et al., 2004), these data suggest that the histamine H_4 receptor has a role in the acute inflammatory response to carrageenan in rats. Histamine H_4 receptor antagonists reduced only the edema developing at 2 h after challenge, whereas late inflammatory responses (at 4 and 6 h) to carrageenan were not affected. A possible explanation could be related to the very short half life of JNJ7777120 and VUF6002 in rodents (Thurmond et al., 2004); however, the ineffectiveness of JNJ7777120, administered 2 h after carrageenan injection, minimizes a role for the unfavourable kinetics of JNJ7777120 and points toward the hypothesis that histamine H_4 receptors are preferentially involved in the early component of the inflammatory response to carrageenan. However, the precise mechanism underlying the anti-inflammatory effect of histamine H_4 receptor antagonists needs further studies.

In the present study carrageenan-induced thermal hyperalgesia was reduced by indomethacin but not by the mast cell degranulator, compound 48/80, confirming that carrageenan-induced hyperalgesia is totally dependent on prostaglandin production (Seibert et al., 1994; Jett et al., 1999). It is of interest that JNJ7777120 and VUF6002 significantly attenuated the hyperalgesic response to thermal stimuli in carrageenan-treated rats, suggesting a mast cell-independent mechanism. This is in contrast with other studies carried out in mice, which failed to detect anti-inflammatory effects of histamine H_4 receptor blockers in mast cell-independent models, such as thioglycollate-induced peritonitis (Thurmond et al., 2004), histamine-induced paw edema (Thurmond et al., 2004) or carrageenan-induced neutrophil recruitment into pleural cavity (Takeshita et al., 2003). On the other hand, in the same species histamine H_4 receptor antagonists were able to reduce histamine-induced scratching (Bell et al., 2004) and the activation of T cells in mast cell-deficient animals (Dunford et al., 2006).

Data on the role of histamine receptors in nociception are intriguing; whereas central activation of histamine H_1 and H_2 receptors induces antinociception (Thoburn et al., 1994), peripheral histamine was found to stimulate nociceptive fibers in rodents, through activation of histamine H_1 (Malmberg-Aiello et al., 1998; Parada et al., 2001) and H_3 receptors (Girard et al., 2004). Recent studies, aimed at elucidating the role of histamine H_3 receptors, gave inconsistent results, due to the use of mixed H_3/H_4 ligands, such as (*R*)-α-methylhistamine, immepip, clobenpropit or thioperamide (Girard et al., 2004; Cannon and Hough, 2005; Farzin and Nosrati, 2007). The low level of histamine H_4 receptor expression in the central nervous system (Hough, 2001; Lozada et al., 2004) and the hydrophilic nature of the histamine H_4 receptor antagonists (Thurmond et al., 2004) rule out the possibility that their antinociceptive effects are due to central mechanisms. Therefore it might be hypothesized that peripheral histamine H_4 receptors have a role in the modulation of sensitivity to noxious stimuli.

In conclusion, this study suggests that histamine H_4 receptors may be involved in the early phase of acute inflammation induced by carrageenan in the rat. However, the limited efficacy of histamine H_4 receptor antagonists in this model makes it premature to hypothesize potential benefits from the use of these drugs in the treatment of inflammatory human disease.

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