Biochemical Pharmacology 98 (2015) 102–109

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Proinflammatory role of the histamine H_4 receptor in dextrane sodium sulfate-induced acute colitis

Bastian Schirmer*[,1], Thomas Rezniczek[1], Roland Seifert, Detlef Neumann

Institute of Pharmacology, Hannover Medical School, Hannover, Germany

ARTICLE INFO

Article history:
Received 20 July 2015
Accepted 8 September 2015
Available online 10 September 2015

Keywords:
Experimental colitis
Histamine H_4 receptor
Histamine
Cytokines
Inflammatory bowel diseases

ABSTRACT

Millions of people worldwide are suffering from inflammatory bowel disease (IBD), which severely affects patients' life qualities and even life expectancies. The cause of the ailment is unknown and a profound understanding of the underlying pathogenetic mechanisms is still lacking. The biogenic amine histamine is one of several inflammatory mediators, to which a pathogenetic role in IBD has been attributed. Out of the four known histamine receptors, the histamine H_4 receptor (H_4R) has been demonstrated to act proinflammatory in experimental models of several inflammatory diseases.

In order to evaluate a potential involvement of H_4R in IBD we investigated the effect of genetic or pharmacological blockade of H_4R-signaling in the model of dextran sodium sulfate (DSS)-induced colitis in mice. We analysed severity and progression of clinical signs of colitis, as well as histopathologic alterations in the colons and systemic or local cytokine concentrations.

Both genetic deficiency and pharmacological blockade of H_4R with the selective antagonist JNJ7777120 improved clinical and histological signs of colitis and dampened the inflammatory cytokine response.

Our results indicate a proinflammatory role of histamine *via* H_4R in IBD, thus extending the current pathophysiological understanding of IBD and demonstrating the therapeutic potential of selective H_4R-antagonists for patients suffering from IBD.

1. Introduction

The biogenic amine histamine (2-(4-imidazolyl)-ethylamine) is involved in a broad variety of (patho-) physiological processes. Currently, there are four histamine receptors known, which all belong to the class of G-protein coupled receptors: histamine H_1 receptor (H_1R), H_2R, H_3R and H_4R [1]. The H_4R has only been discovered in the beginning of the 21st century and shares the highest homology (37%) with the predominantly neuromodulatory H_3R [2]. The H_4R is expressed mainly on immune cells such as mast cells, dendritic cells, T-cells and eosinophils [3–7], and receptor stimulation leads to a G_i-mediated activation of these cells *via* phospholipase C activation and subsequent intracellular calcium mobilization and inhibition of membrane-bound adenylyl cyclase [8–13]. The exact function of H_4R still remains unclear, but several studies indicate a proinflammatory role of H_4R in a number of (auto-) inflammatory diseases like allergic asthma, atopic dermatitis and arthritis [14–19].

Inflammatory bowel disease (IBD) is an idiopathic, chronic-recurring disease of the gut. The two main forms of IBD are ulcerative colitis (UC) and Crohn's disease (CD), differing in their clinical, endoscopic and histologic appearance. Both ailments severely affect the quality of patients' lives and eventually limit their life expectancy through complications like colon cancer and extraintestinal manifestations such as arthritis and keratoconjunctivitis [20,21]. Currently, IBD patients are treated with immunosuppressive agents such as 5-aminosalicylic acid or corticosteroids [22]. Unfortunately, remission rates are only 50% and long-term treatment can lead to immunosuppression-related cancers and infections. Since there are no effective and specific therapeutics available for the treatment of IBD, it is of economical and scientific interest to discover new specific drug targets.

Numerous murine models of IBD are available, but the chemically induced dextrane sodium sulfate (DSS)-colitis and 2,4,5-trinitrobenzenesulfonic acid (TNBS)-colitis are the two best characterized and most widely used models of experimental colitis [23]. The immunopathology after DSS ingestion is thought to be dominated by mechanisms of innate immunity, whereas the

* Corresponding author at: Institute of Pharmacology OE 5320, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany. Fax: +49 511 532 4081.
E-mail address: schirmer.bastian@mh-hannover.de (B. Schirmer).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bcp.2015.09.006
0006-2952/

influence of T-cell-dependent immunity prevails in TNBS-induced colitis [24].

Histamine is known to act as proinflammatory mediator in IBD [25–27] and H_4R blockade was already shown to be beneficial in TNBS-induced colitis in rats [28]. Since a broad variety of genetic models is available in mice, but not in rats [29], the present study aims at elucidating the role of H_4R in an acute DSS-induced colitis model in mice. DSS chemically destroys the epithelial barrier, resulting in an increased antigenic load in the subepithelial tissue, thereby initiating the colonic inflammation. Mice deficient in expression of functional H_4R ($H_4R^{-/-}$) were used to address the question, whether inhibition of H_4R function modifies this severe disease. In order to prove these data by an independent approach, the DSS-induced colitis model was applied to wild-type animals, which were treated with the selective H_4R antagonist JNJ7777120 (1-[(5-chloro-1*H*-indol-2-yl)carbonyl]-4-methylpiperazine) [30,31]. To our knowledge, this is the first study to demonstrate, that H_4R exerts proinflammatory effects in acute colitis in mice.

2. Materials and methods

2.1. Chemicals and reagents

If not stated otherwise, all chemicals and reagents were obtained from Sigma–Aldrich, DE. DSS (36,000–50,000 M.Wt.) was purchased from MP Biomedicals, US. Phosphate buffered saline (PBS) and RPMI1640 were purchased from Gibco invitrogen, US. αCD3 antibody was purchased from EXBIO Praha, CZ. JNJ7777120 was kindly provided by Armin Buschauer (University of Regensburg, DE). Ketamin was obtained from Dr. E. Gräub GmbH, CH, and xylazine was obtained from Bayer, DE.

2.2. Animals

Eight–ten-week old BALB/cJRj mice were purchased from Janvier Labs and housed in the animal facility of Hannover Medical School. H_4R knockout mice ($H_4R^{-/-}$; strain: C.129HrH_4^{tm1Lex}) as described earlier by Hofstra et al. [7] were generated by Lexicon Genetics (Woodlands, TX) and backcrossed onto the above mentioned BALB/cJRj strain in our facility.

2.3. Induction of colitis by DSS and animal dissection

For induction of acute colitis mice were fed with water charged with 3% (w/v) DSS from day 0 to day 7. Water-fed mice served as control. On day 7, the animals were euthanized with carbon dioxide and subsequent heart puncture, the sera were collected and the ceca and colons were removed. Colons were washed with PBS to remove remaining feces, fixed in 4% (v/ v) formaldehyde, embedded in paraffin, and further processed for hematoxylin/eosin (H/E) staining. Mesenterial lymph nodes were removed and a lymphocyte enriched single cell suspension was generated by passing the tissue through a sterile 100 μm mesh with 5 mL RMPI1640 medium.

2.4. Cytokine measurements

Mesenterial lymph node cells were seeded at 2×10^6 cells/well on a 96 well plate (Greiner Bio-One, DE), and incubated at 37 °C/5% (v/v) CO_2 for 24 h either with or without immobilized CD3-antibody (αCD3) stimulation. Concentration of interleukin (IL)-4, IL-5, IL-6, IL-10, IL-17, and interferon (IFN)-γ was measured in serum samples and cell-free supernatants of αCD3-stimulated lymphocytes with a customized multiplex magnetic Luminex Kit (R&D Systems, US).

2.5. Treatment with JNJ7777120

JNJ7777120 was dissolved at 20 mg/ml in dimethylsulfoxide (DMSO) and subsequently diluted two-fold with PBS. A solution of 50% (v/v) DMSO in PBS served as vehicle control. Osmotic pumps (Model 1007D, Alzet, US) were filled with JNJ7777120 or vehicle solutions and assembled according to manufacturer's instructions, then primed by incubation in isotonic saline solution at 37 °C for 12 h. Mice were anaesthetized by intraperitoneal injection with 80 mg/kg ketamine and 10 mg/kg xylazine in isotonic salt solution. The pumps were implanted subcutaneously through a midscapular incision behind the neck which was sealed with sterile wound clips. The pumps delivered the agent continuously for a one-week period at a rate of 360 nmol/day.

2.6. Evaluation of disease activity

Mice were examined daily and scored according to a disease activity index (DAI, adopted from Ref. [32]) ranging from 0 to 12. The DAI is based on total body weight loss (0: no weight loss, 1: 1–5%, 2: 5–10%, 3: 10–15%, 4: >15%), stool consistency (0: normal, 2: soft, 4: diarrhea) and peranal bleeding (0: no bleeding, 2: little bleeding, 4: massive bleeding).

2.7. Histological analysis

H/E-stained tissue slices of proximal, medial and distal colon segments were analyzed in a blinded fashion by two independent pathologists. A histological severity score was calculated for each segment by evaluating overall severity of inflammation (0: normal,

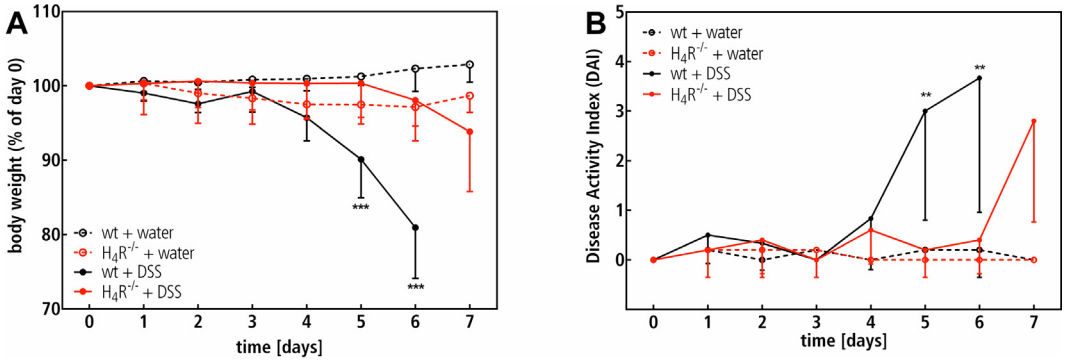

Fig. 1. Influence of H_4R-deficiency on clinical signs of DSS-induced colitis. Wild-type or $H_4R^{-/-}$ mice were fed with 3% DSS from day 0 to 7. Mice fed with pure water served as control. Body weight loss (A) and disease activity indices (B) were assessed daily. Wild-type mice had to be withdrawn from the experiment at day 6 because of their severely compromised health state (data shown are the means ± 95% CI of 5 experiments; $^{***}p < 0.001$, one-way ANOVA with Tukey's *post-hoc* test).