e-SPEN Journal 7 (2012) e16–e22

Contents lists available at SciVerse ScienceDirect

e-SPEN Journal

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Lithium induces intestinothrophic effects in the healthy colon, but does not ameliorate dextran sulfate sodium-induced colitis in mice☆

Elise M.J. van der Logt [a,*], Tjasso Blokzijl [a], Arjan Diepstra [b], Maikel P. Peppelenbosch [c], Gerwin Huls [d], Klaas Nico Faber [a], Gerard Dijkstra [a]

[a] Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Internal Postal Code BB41, Groningen, The Netherlands
[b] Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[c] Department of Gastroenterology and Hepatology, Erasmus Medical Center, Rotterdam, The Netherlands
[d] Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 25 March 2011
Accepted 14 December 2011

Keywords:
Lithium
DSS-induced colitis
Ulcerative colitis
Glycogen synthase kinase 3β
Colon length

SUMMARY

Background & aims: Ulcerative colitis is characterized by severe damage of the colon epithelium. Wnt-signaling is important for repair and regeneration of the intestinal epithelium. Lithium activates Wnt-signaling through inhibition of Glycogen Synthase Kinase 3β. Lithium induced prolonged remission in a patient with a bipolar disorder and ulcerative colitis, suggesting a therapeutic potential for ulcerative colitis.
Methods: Here, we investigated the effect of lithium (4 mg/day via a subcutaneous osmotic pump) on 5% dextran sulfate sodium-induced colitis in female Balb/c mice.
Results: At day 7, colon length was significantly increased in lithium-treated compared to untreated mice (8.6 cm [7.0–9.5] versus 7.6 cm [6.7–8.0], $p < 0.05$). As expected, dextran sulfate sodium treatment reduced colon length (5.9 cm [5.1–6.5], $p < 0.001$), but this was not altered by lithium (6.0 cm [5.5–7.0]). No significant differences were detected in bodyweight, histology, inflammatory (myeloperoxidase, iNOS, cytokines) and Wnt-pathway (β-catenin, p-Glycogen Synthase Kinase 3β) markers between dextran sulfate sodium- and lithium/dextran sulfate sodium-treated mice.
Conclusions: Lithium has no therapeutic effect on dextran sulfate sodium-induced colitis in mice. However, in the healthy intestine it shows intestinothrophic potential that might be beneficial for short bowel patients.
© 2011 European Society for Clinical Nutrition and Metabolism. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are two types of inflammatory bowel diseases (IBDs). Usually CD and UC start in childhood or youth with a peak between 20 and 30 years of age. CD is characterized by inflammation with a discontinuous pattern, potentially affecting the whole gastrointestinal tract. The inflammation can be transmural with large ulcerations and granuloma.[1] UC, however, is characterized by inflammation with superficial ulcerations limited to the mucosa of the colon. It normally starts in the rectum and continuously spreads throughout the colon.[1] Prolonged inflammation leads to severe damage of the colon epithelium of UC patients. The balance between epithelial cell growth and cell death is disturbed.

Epithelial cell turn-over is regulated by the Wnt-pathway. Active Wnt increases cell proliferation, while inactive Wnt induces apoptosis.[2] In the absence of Wnt-signaling, Glycogen Synthase Kinase 3β (GSK3β) forms a degradation complex with Axin and adenomatosis polyposis coli (APC) proteins and phosphorylates β-catenin, which promotes its proteolysis. So, GSK3β is a suppressor of the Wnt-pathway. Inhibition of GSK3β reduces β-catenin phosphorylation and activates Wnt-signaling, which is

Abbreviations: CD, Crohn's disease; GSK3β, glycogen synthase kinase 3β; APC, adenomatosis polyposis coli; DKK1, Dickkopf1; IBD, inflammatory bowel diseases; MPO, myeloperoxidase; Rspo1, R-spondin 1; RT-PCR, reverse transcription polymerase chain reaction; qPCR, quantitative PCR; UC, ulcerative colitis.

☆ Conference presentation. Dutch Experimental Gastroenterology and Hepatology Meeting, Veldhoven, The Netherlands (March 2010) and United European Gastroenterology Week, London, United Kingdom (November 2009).

* Corresponding author. Tel.: +31 50 3619564; fax: +31 50 3619306.
E-mail address: e.m.j.van.der.logt01@umcg.nl (E.M.J. van der Logt).

2212-8263/$36.00 © 2011 European Society for Clinical Nutrition and Metabolism. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.eclnm.2011.12.006

characterized by elevated cytoplasmic β-catenin levels and finally leads to translocation of β-catenin to the nucleus. This results in transcription of target genes, which encode proteins that are anti-apoptotic and stimulate cell proliferation.[3]

An active Wnt-pathway helps to repair and regenerate the damaged intestinal epithelium. This self-renewing capacity of the intestine starts with proliferation of stem cells in the bottom of the crypts. A process that is entirely dependent on continual stimulation of the Wnt-pathway in these cells and is characterized by accumulation of nuclear β-catenin. Therefore, pharmacological activation of Wnt-signaling might prevent or reduce epithelial damage during intestinal inflammation. Indeed, treatment with R-spondin 1 (Rspo1), an activator of Wnt, was recently shown to restore damaged intestinal- and/or oral epithelium in mice models for colitis,[4] as well as 5-fluorouracil (5-FU)- or radiation-induced mucositis.[5,6]

The Wnt-signaling suppressor GSK3β does not only inhibit epithelial cell turn-over. An important discovery was that GSK3β inhibition shifts the balance from pro-inflammatory to anti-inflammatory cytokines.[7] This observation rapidly expanded the application of GSK3β inhibitors to control inflammation in animal models. Anti-inflammatory effects were seen for collagen-induced arthritis, endotoxemia, and asthma.[8] Treatment with the selective inhibitors of GSK3β, TDZD-8 and SB415286, substantially reduced the inflammation and tissue injury in a rat model of acute TNBS colitis.[9] In more detail, treatment with these inhibitors decreased body weight loss and colonic inflammation markers, like myeloperoxidase and TNF-α, which occurred after a single intra-colonic challenge with TNBS.

Lithium, as simple cation, is the simplest drug in the modern pharmacopoeia.[10] Lithium has been shown to also be a direct, reversible inhibitor of GSK3β[11,12] and has anti-inflammatory properties. Lithium (4 mg/day) reduced chronic DSS-induced colitis, characterized by decreased histological scores and increased production of IL-10 by mesenteric lymph node cells.[13] Furthermore, treatment with lithium (20 mg/kg i.p.) ameliorated colitis 24 h after induction with acetic acid. Lithium improved macroscopic and histological scores of colitis and diminished the elevation of myeloperoxidase and TNF-α.[14] Interestingly, its therapeutic potential for IBD was reported once in 1972. Lithium (900 mg/day) induced prolonged remission after treatment of a patient with a bipolar disorder and active UC.[15]

Here, we hypothesize that lithium has a beneficial effect on UC, because it stimulates regeneration of the intestinal epithelium through the Wnt-pathway and shifts the production of cytokines from pro- to anti-inflammatory. However, although lithium induced a significant intestinothrophic effect in the healthy mouse colon, it did not ameliorate dextran sulfate sodium-induced colitis in mice.

2. Materials and methods

2.1. Lithium-containing osmotic pumps

Lithium chloride (JT Baker (4002-01)) was dissolved in sterile water and filtered (0.2 μm). ALZET mini-osmotic pumps (model 2002) were obtained from DURECT (Cupertino, CA, USA). Osmotic pumps were filled with LiCl according to the instruction of the manufacturer. To stimulate their function, pumps were incubated in 0.9% saline at 37 °C, o/n, prior to implantation. Each pump will deliver 4 mg LiCl/day. Plasma levels of lithium were measured by a standard Inductively Coupled Plasma-Mass Spectrometry procedure on a Varian 820 Mass Spectrometer at the department of Hospital and Clinical Pharmacy of the University Medical Center Groningen.

2.2. Ethical considerations

The animal welfare committee of the University Medical Center Groningen approved the study, with the limitation that mice that lose >10% of their bodyweight and are in a bad condition should be euthanized to protect them from severe discomfort.

2.3. Animals

Mice (16–18 g; Harlan, The Netherlands) were housed in groups under standard laboratory conditions, were fed standard chow diet and had free access to tap water.

We investigated the effect of lithium on Dextran Sulfate Sodium (DSS, MW 36.000–50.000; MP biochemicals, Illkirch, France)-induced colitis in female Balb/c mice. Four groups were studied ($n = 10$ each): untreated mice were compared to lithium-, DSS- and lithium/DSS-treated mice. One day before the induction of colitis, lithium-containing pumps were placed subcutaneously on the back of the mice. The other mice underwent a sham-operation. Colitis was induced by adding 5% DSS to the drinking water for 6 days. Mice were monitored daily for signs of colitis and bodyweight. Colon length and weight were measured directly after the mice were sacrificed. In addition, colon histology, inflammatory- (myeloperoxidase, iNOS, cytokines) and Wnt-pathway (β-catenin, p-GSK3β) markers were studied. Mice showing loss of >10% bodyweight and/or a bad condition, including signs of bloody diarrhea and dehydration were euthanized and only data on bodyweight and colon histology were included in the results.

2.4. Histology

Colon tissue was fixed in 4% formaldehyde, embedded in paraffin, cut into slides of 4 μm and stained with haemotoxylin–eosin (H&E) for morphology. Slides were evaluated by an experienced pathologist in a blinded fashion for epithelial damage (E) and inflammation (I) according to Obermeier et al.[16]

2.5. Myeloperoxidase (MPO) ELISA kit

MPO activity in colon tissue was measured using a mouse MPO ELISA kit (HK210; Hycult biotechnology, Uden, The Netherlands). Colon tissue was homogenized in MPO lysis buffer (200 mM NaCl, 5 mM EDTA, 10 mM Tris–HCl, 10% glycerol, 1 mM PMSF, 1 μg/ml leupeptide, 28 μg/ml aprotinine, pH 7.4; 20 μl/mg) by 25 strokes with a plastic pestle. Supernatant was collected after two centrifugation steps of 10 min, 1500*g*, at 4 °C, and stored at −80 °C until use. Each sample was diluted 10 times in dilution buffer. The ELISA was performed according to the suppliers' protocol, except that after addition of the substrate the reaction was stopped after 15 min.

2.6. Reverse transcription polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR)

RNA isolation was done using Trizol (Sigma Aldrich). RNA concentrations were determined using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). RT-PCR was carried out as described by Blokzijl et al.,[17] but on 12.5 μg RNA in the presence of 0.5 μg random nanomers. We performed qPCR for iNOS, TNF-α, IL-1β, GSK3β, CD44 and c-Met. Primers (Invitrogen) and probes (Eurogentec, Maastricht, The Netherlands) were designed using Primer Express 2.0 software (Applied Biosystems). Details of primers and probes are listed in Table 1. The qPCR conditions were according to Blokzijl et al.,[17] except that we used 1 ng undiluted cDNA. Fluorescence was

Download English Version:

https://daneshyari.com/en/article/2690280

Download Persian Version:

https://daneshyari.com/article/2690280

Daneshyari.com