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Original article

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

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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.

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.

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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

Table 1
Mice primer and probe sequences for quantitative PCR.

iNOS	Forward	5′-CTA TCT CCA TTC TAC TAC TAC CAG ATC GA-3′
	Reverse	5′-CCT GGG CCT CAG CTT CTC AT-3′
	Probe	5′-FAM-CCC TGG AAG ACC CAC ATC TGG CAG-TAMRA-3′
TNF-α	Forward	5′-CAT CTT CTC AAA ATT CGA GTG ACA A-3′
	Reverse	5′-CCA GCT GCT CCT CCA CTT G-3′
	Probe	5′-FAM-CCT GTA GCC CAC GTC GTA GCA AAC CA-TAMRA-3′
IL-1β	Forward	5′-ACC CTG CAG CTG GAG AGT GT-3′
	Reverse	5′-TTG ACT TCT ATC TTG TTG AAG ACA AAC C-3′
	Probe	5′-FAM-CCC AAG CAA TAC CCA AAG AAG AAG ATG GAA-TAMRA-3′
GSK3β	Forward	5′-CTG AAT TGT TGC TAG GAC AAC CAA-3′
	Reverse	5′-TTG TTG GTG TTC CTA GGA CCT TAA-3′
	Probe	5′-FAM-CCA CCA ACT GAT CCA CAC CAC TGT CC -TAMRA-3′
CD44	Forward	5′-CCC CCC TAC CCC AAG TGA-3′
	Reverse	5′-TCT GTG TTG TTA TTC TTT GAC TTG GAT-3′
	Probe	5′-FAM-ACA GAA GGG ACA ACT GCT TCG GCC C-TAMRA-3′
c-Met	Forward	5′-AAC GAG AGC TGT ACC TTG ACC TTA A-3′
	Reverse	5′-CTC ATC GCG GGA CCA ACT-3′
	Probe	5′-FAM-CGA GAG CAC GAC AAA TAC GTT GAA ATG CA-TAMRA-3′
18S	Forward	5′-CGG CTA CCA CAT CCA AGG A-3′
	Reverse	5′-CCA ATT ACA GGG CCT CGA AA-3′
	Probe	5′-FAM-CGC GCA AAT TAC CCA CTC CCG A-TAMRA-3′

measured using 7900 HT Fast Real-Time PCR system (Applied Biosystems). Each sample was analyzed in duplicate by ABI PRISM Sequence Detector software, version 2.1. Finally, the gene of interest was normalized to 18S ($2^{-\Delta Ct}$ method).

2.7. Protein assay

Protein concentration of colon tissue lysates (see MPO activity assay) was determined using the BioRad D_C protein assay (BioRad, CA, USA). Absorbance was measured at 750 nm on an EL800 universal microplate reader (Bio-Tek instruments).

2.8. Western-blotting

The colon tissue lysates (20 μg) were fractionated on a 10% sodium dodecyl sulfate polyacrylamide gel and transferred to nitrocellulose (GE Healthcare Europe, Diegem, Belgium), using a semidry-blotting system according to manufacturer's instructions (Pharmacia, Uppsala, Sweden). Blots were blocked with PBS containing 2% skim milk powder, 0.5% BSA and 0.1% Tween-20 for 1 h at RT. Then blots were incubated with the monoclonal antibody for β-catenin (1:500; #610153, BD Biosciences, Breda, The Netherlands) or the polyclonal antibody for p-GSK3β (1:1000, #9336, Cell Signaling, Boston, MA, USA) in PBS containing 0.5% BSA and 0.1% Tween-20, o/n at 4°C. Subsequently, blots were incubated with horseradish peroxidase-labeled rabbit anti-mouse or goat anti-rabbit immunoglobulin G (dilution 1:2000; DAKO A/S, Glostrup, Denmark), respectively, for 1 h at RT and finally developed using the enhanced chemiluminescence SuperSignal West Dura detection system (#34076, Pierce, Rockford, IL, USA). GAPDH (#CB1001, Calbiochem) was used as loading control.

2.9. Statistics

Data obtained for each experimental group was depicted as a Box–Whisker plot (minimum – lower quartile – median – upper quartile – maximum) or described as median [range]. To verify statistically significant differences among experimental groups, a Kruskal–Wallis test was performed. When this resulted in a $p < 0.05$, data were analyzed further with a Mann–Whitney *U* test for comparisons between experimental groups. Differences were considered to be statistically significant at $p < 0.05$. All statistical analyses were performed using GraphPad Prism 5.00 software (GraphPad software, CA, USA).

3. Results

To confirm the effective release of lithium from the osmotic pumps, plasma levels of lithium were determined. Plasma levels were 0.50 mEq/l (0.23–0.74) and 0.57 mEq/l (0.47–2.51) in lithium- and lithium/DSS-treated mice, respectively, and were below detection limit (0.1 mEq/l) in mice that were not treated with lithium.

To study the effects of lithium on healthy and inflamed colon, we analyzed the length and weight of the colon from untreated, lithium-, DSS- and lithium/DSS-treated mice (Fig. 1). The colon length was significantly increased in lithium-treated compared to untreated mice (8.6 cm [7.0–9.5] vs. 7.6 cm [6.7–8.0], $p < 0.05$). As expected, DSS treatment was characterized by shortening of the colon (5.9 cm [5.1–6.5], $p < 0.001$), however, this was not altered by lithium (6.0 cm [5.5–7.0]). Also colon weight tended to increase in lithium-treated

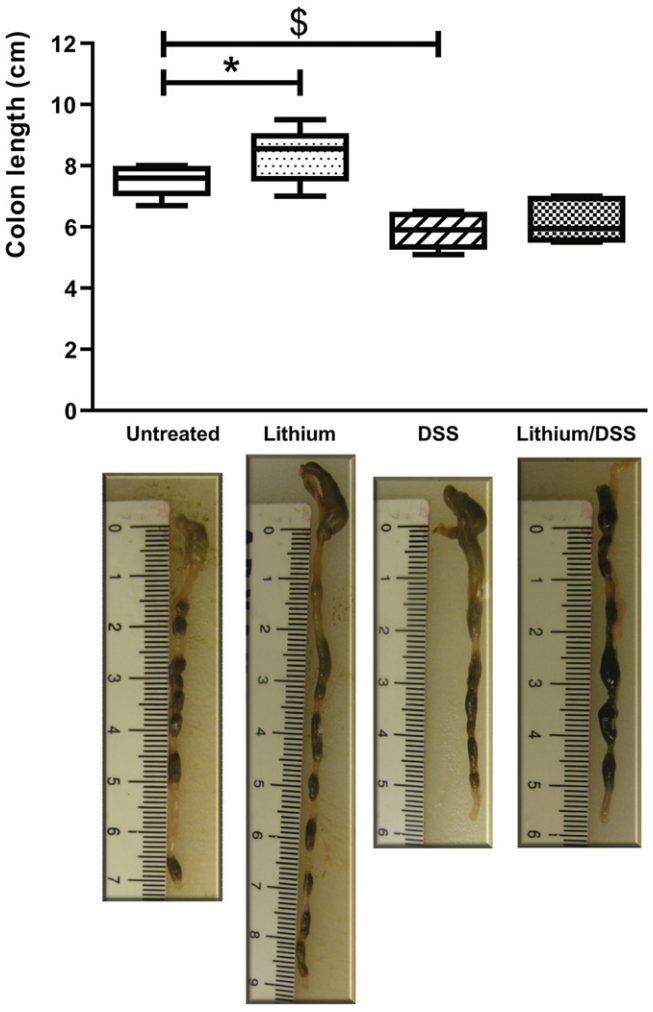

Fig. 1. Effects of lithium on colon length in the healthy colon and Dextran Sulfate Sodium (DSS)-induced colitis. After 7 days lithium increased the colon length compared to untreated mice ($^{*}p < 0.05$). DSS treatment decreased colon length ($^{\$}p < 0.001$), however, this was not changed by lithium ($p > 0.05$). Data from mice euthanized before Day 7 were excluded from analyses.

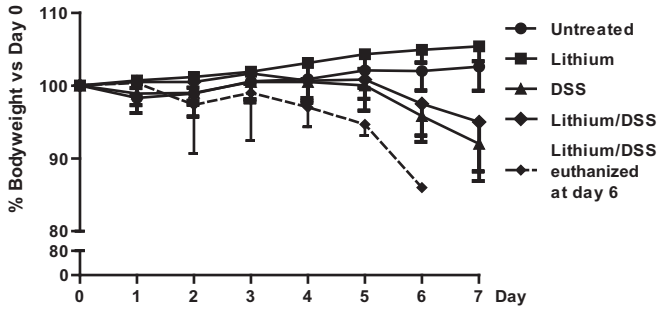

Fig. 2. Therapeutic effect of lithium in treating DSS-induced colitis. Lithium treatment was started at Day 0 and DSS-treatment at Day 1. % Bodyweight as compared with bodyweight on Day 0 of the experiment. From Day 5 the bodyweight was higher of lithium-treated compared to untreated mice ($p < 0.05$ at Days 5 and 6, $p = 0.11$ at Day 7). After 7 days lithium increased the bodyweight ($p = 0.11$). DSS-treated mice had a significantly lower body weight than untreated mice ($p < 0.001$ at Days 6 and 7). This was aggravated in combination with lithium for three mice (DSS vs. lithium/DSS euthanized at Day 6: $p < 0.05$ at Days 5 and 6), but not for the other six mice (DSS vs. lithium/DSS: $p > 0.05$ at Days 5–7). In total four lithium/DSS-treated mice were euthanized because they exceeded the limit of 10% weight loss and had a bad condition. One mouse was euthanized at Day 2, because of severe weight loss (19%) and three mice were euthanized at Day 6, because of bloody diarrhea and signs of dehydration combined with 14% weight loss.

compared to untreated mice, although this was not statistically significant (262 mg [189–328] vs. 306 mg [225–415], $p = 0.09$).

To study the effect of lithium on DSS-induced colitis, we monitored morbidity after the start of DSS exposure. Unexpectedly, lithium/DSS treatment caused more severe morbidity than DSS treatment. In one mouse of the lithium/DSS group significant bodyweight loss was detected one day after placement of the osmotic pump, and dropped to 19% after one day of DSS exposure. Therefore, this mouse was euthanized at day 2 and not included for further analysis. On day 6 three more lithium/DSS-treated mice had to be euthanized (5 days of DSS exposure; lithium: 0.52 mEq/l [0.4–0.76]), because of severe discomfort, characterized by >10% bodyweight loss (Fig. 2), bloody diarrhea and signs of dehydration, compared to none in the DSS group. All other mice were sacrificed at day 7.

In Fig. 2, the courses of bodyweight in time of all groups investigated are shown as percentage of day 0. From the start, lithium treatment tended to increase bodyweight compared to untreated mice, but this was not statistically significant at day 7 ($p = 0.11$). At day 5, mice exposed to DSS started to lose weight compared to untreated mice. This continued until day 7, when the mice were sacrificed, and was highly significant ($p < 0.001$). The bodyweight course of lithium/DSS-treated mice did not differ significantly from DSS-treated mice. However, as already mentioned, 3 mice of the lithium/DSS group exceeded the limit of 10% weight loss at day 6 (=14% vs. 4% in DSS-treated mice, $p < 0.05$).

To assess whether lithium affected DSS-induced colon damage, we scored colon morphology (Fig. 3A) for crypt changes (Fig. 3B) and infiltrate of inflammatory cells (Fig. 3C). DSS-treatment induced crypt damage, characterized by loss of crypts and goblet cells (3 [1–4] points), and infiltration of neutrophils into the (sub)

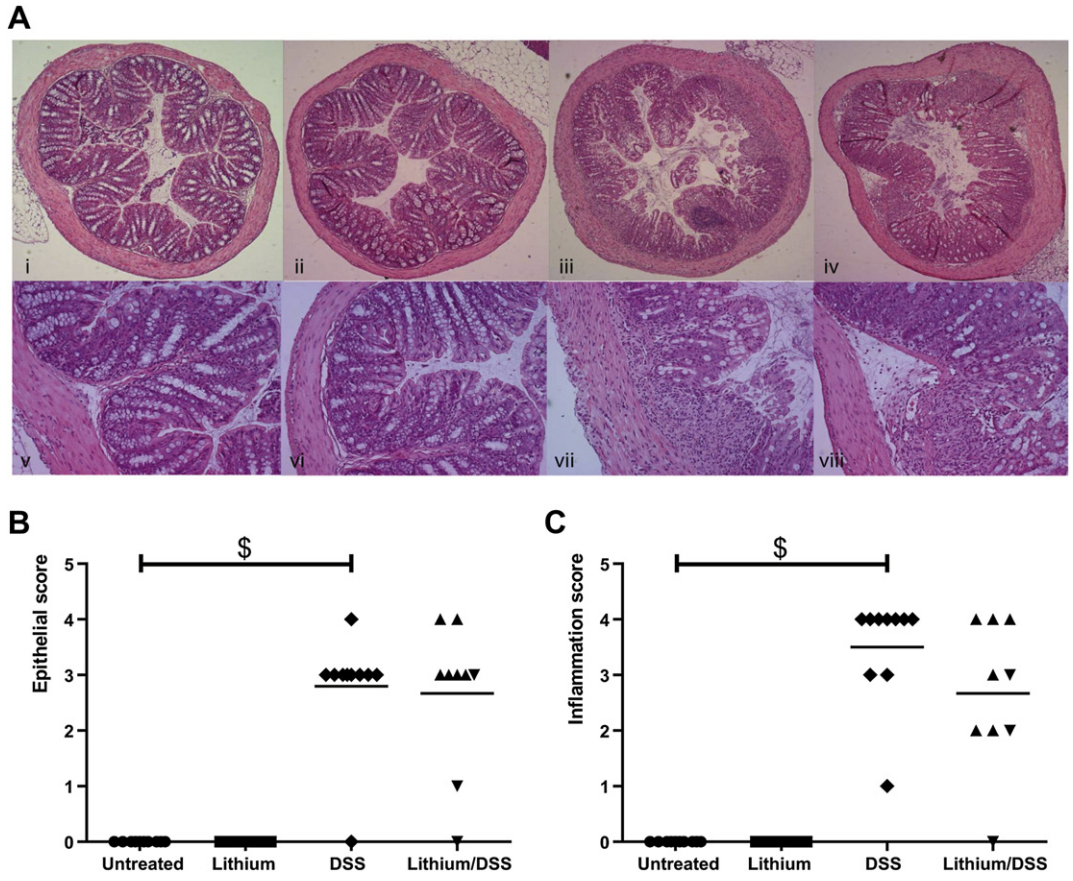

Fig. 3. (A) H&E staining of colon sections: (i/v) Untreated, (ii/vi) lithium-, (iii/vii) DSS- and (iv/viii) lithium/DSS-treated mice (50× and 200× magnification, respectively). DSS-induced epithelial damage and lithium treatment did not change this. (B) Histology epithelial damage score. Lithium did not affect DSS-induced epithelial damage that was characterized by loss of crypts and goblet cells. (C) Histology inflammation score. Colon section of lithium/DSS-treated mice showed less infiltrates of neutrophils into the (sub) mucosa compared to DSS-treated mice, however this was mainly caused by the score of the 3 mice that were euthanized at Day 6 (▼). $^{\$}p < 0.001$.

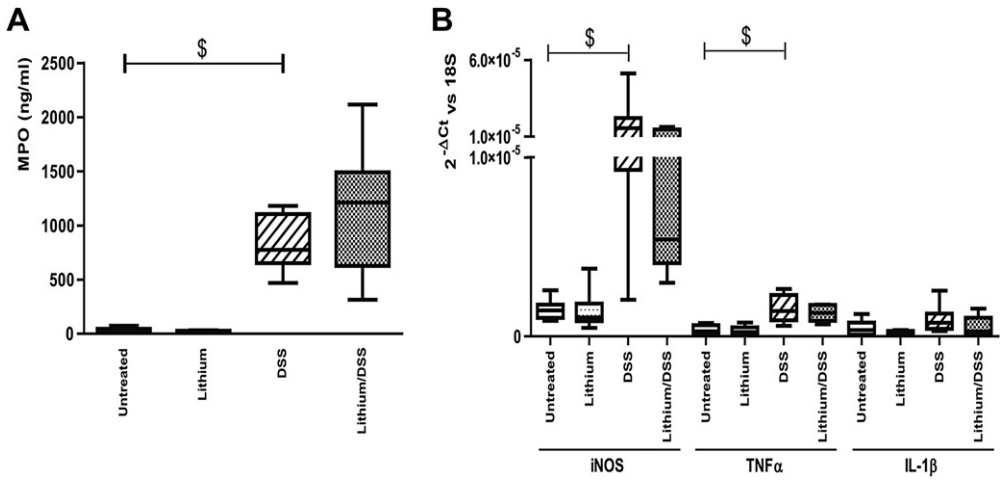

Fig. 4. Effect of lithium on inflammatory markers in DSS-treated mice. (A) Myeloperoxidase (MPO) activity in colonic tissue. DSS-induced overproduction of MPO ($^{\$}p < 0.001$) was not changed by lithium ($p > 0.05$). (B) Quantitative PCR of iNOS, TNF-α, IL-1β in colonic tissue corrected for 18S. DSS-induced expression levels of iNOS ($p < 0.001$), TNF-α ($p < 0.001$), IL-1β ($p = 0.075$) were not affected by lithium treatment ($p > 0.05$). Data from mice euthanized before Day 7 were excluded from analyses.

mucosa (4 [1–4] points). In lithium/DSS-treated mice we found a slightly better histology score than in DSS-treated mice; 5/9 vs. 8/10 times a score of 3 for crypt loss, and 3/9 vs. 7/10 times a score of 4 for infiltration of inflammatory cells. However, this was mainly caused by the lower score of the 3 mice that were euthanized at day 6, and therefore had a shorter exposure to DSS. Untreated and lithium-treated mice had a normal appearing colon, without infiltration of inflammatory cells.

To determine whether lithium modulated DSS-induced inflammation, we analyzed colon tissue from all experimental groups for MPO activity (Fig. 4A), and mRNA levels of iNOS, TNF-α and IL-1β (Fig. 4B). An increased myeloperoxidase activity was measured in DSS-treated compared to untreated mice ($p < 0.001$). Furthermore, DSS-treated mice had a higher mRNA expression of iNOS ($p < 0.001$), TNF-α ($p < 0.001$) and IL-1β ($p = 0.075$) compared to untreated mice. However, no statistically significant differences were detected in these inflammatory markers between DSS- and DSS/lithium-treated mice.

To investigate the effects of lithium on the Wnt-pathway, mRNA levels of GSK3β and the Wnt-target genes CD44 and c-Met were determined. Also protein levels of β-catenin and p-GSK3β were measured. Lithium had no effect on any of the markers (Fig. 5).

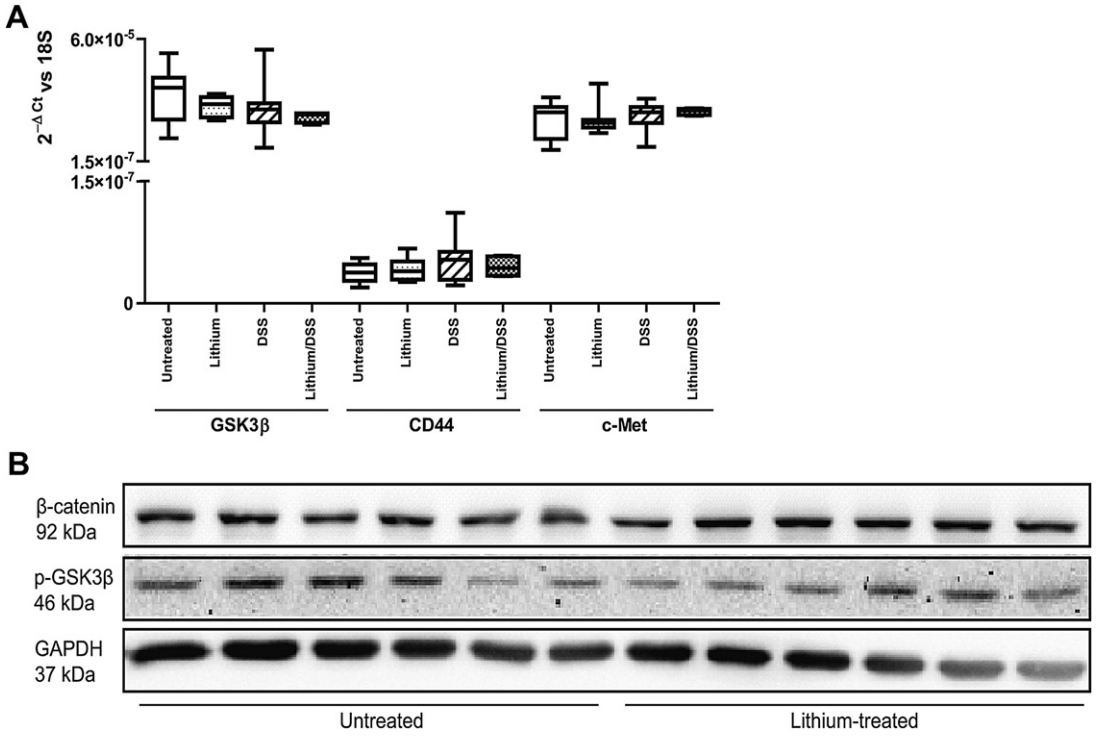

Fig. 5. Effect of lithium on Wnt-pathway markers in DSS-treated mice. (A) Quantitative PCR of GSK3β, c-Met, CD44 in colonic tissue corrected for 18S. No differences were observed between all investigated groups. Data from mice euthanized before Day 7 were excluded from analyses. (B) Western blot of protein expression of β-catenin and p-GSK3β with GAPDH as loading control of untreated and lithium-treated mice. Lithium did not change β-catenin and p-GSK3β protein levels.

4. Discussion

In UC patients prolonged inflammation leads to severe damage of the colon epithelium. Recently, a number of animal studies have shown that epithelial damage is attenuated by activation of the Wnt-pathway. Treatment with Wnt-signaling activators resulted in intestinotrophic effects[4,5] and amelioration of colitis[4] or mucositis[5,6] in various mouse models of inflammatory disease and chemotherapy/radiation cancer treatment, respectively. The aim of the present study was to extend these findings by treating DSS colitis through activating Wnt with lithium. A clinically approved drug widely used in patients with bipolar disorders for more than 60 years.[10] However, its application and therapeutic potential for UC was reported only once in a case report in 1972.[15]

In our study we observed intestinotrophic properties of lithium. In other words, lithium treatment (0.50 mEq/l [0.23–0.74]) significantly increased the colon length and tended to increase the weight of the healthy colon of lithium compared to untreated mice. This is in agreement with earlier reports, which found a significantly increased weight of the gastrointestinal tract of rats that were treated with lithium (0.14–0.21 mEq/l).[18,19] In studies using another activator of Wnt, Rspo1, an elongation of the intestine and an increase in intestinal weight was observed in female Balb/c mice.[4,5]

At the same time, Rspo1 treatment increased the crypt depth of the colon, goblet cell number (both at day 7)[4] and cytosolic β-catenin staining (6 h after injection),[5] indicating active Wnt-signaling, compared to controls. Although lithium, like Rspo1, showed to act through activation of the Wnt-pathway,[3] we were not able to detect the effect of lithium on this pathway. No stabilization of cytosolic β-catenin was seen, no change in phosphorylation of GSK3β was observed and no difference in the expression of Wnt-target genes could be detected. Also the number of goblet cells was unchanged (data not shown). The discrepancy of our findings with those of Kim et al.[5]could be explained by methodological differences. The time-point of analyzing the colon tissue and the way mice were exposed to the Wnt-pathway activators were different in these studies. We checked for modulation of the β-catenin after 7 days of treatment, while they already looked 6 h after injection.[5] This suggests that Wnt-signaling is only temporarily activated. Next, in our study lithium was released constantly from an osmotic pump for 7 days, whereas Kim et al. injected R-spondin 1 once daily for maximally 7 days.

Recently, the importance of Wnt-β-catenin signaling in homeostasis of the gut was reported again. Manicassamy et al.[20] showed that Wnt-β-catenin signaling in intestinal dendritic cells regulates the balance between inflammatory versus regulatory responses in the gut. β-catenin was required for the expression of anti-inflammatory mediators, and the stimulation of regulatory T cell induction while suppressing inflammatory effector T cells. Two hours of lithium treatment activated β-catenin in dendritic cells and induced greater frequencies of regulatory T cells. Ablation of β-catenin in dendritic cells induced inflammatory responses and disease upon DSS treatment in mice. However, the effects of lithium treatment of dendritic cells on the DSS model were not documented.

In our study, administration of 5% DSS to the drinking water for 6 days caused severe colitis, characterized by significant body-weight loss and bloody diarrhea. This correlated with severe damage of the crypts in the colon and massive infiltration of neutrophils, indicated by an increased morphology score and induction of MPO activity. Treatment with lithium did not have any beneficial effect on the disease course of DSS-induced colitis. Furthermore, no differences were detected in histology and inflammation markers. In contrast, lithium was recently shown to ameliorate colitis in a chronic DDS model in mice (4 mg/day)[13] and an acetic acid model in rats (20 mg/kg i.p.).[14] Moreover, lithium (900 mg/day) induced prolonged remission after treatment of a patient with a bipolar disorder and active UC.[15]

Hofmann et al.[13] investigated the effect of lithium in a chronic model of DSS-induced colitis, while we studied this in an acute model. In the chronic model lithium improved the morphology of the colon, but like in the acute model did not change the levels of pro-inflammatory cytokines. Important differences between our study and that from Daneshmand and co-workers[14] are that we used a UC-like model in mice and analyzed the colon after 6 days of DSS exposure, whereas they tested lithium in a CD-like model in rats and examined the colon 24 h after acetic acid enema. Therefore, the choice of colitis model, animal species and inflammatory status at time of analysis may be of influence in studying the therapeutic potential of a new compound. The mouse strain Balb/c is used commonly for induction of DSS colitis. Administration of DSS causes epithelial damage in the colon and disrupts the gut wall. The exposure of the body to pathogens leads to an inflammatory reaction in which cytokines involved in ulcerative colitis are increased. Therefore, DSS-induced colitis is a good model for ulcerative colitis. Because we were interested in modulation of epithelial recovery by lithium, we chose the DSS-epithelial damage model. Lithium can modulate GSK3β, which plays a role in epithelial cell homeostasis, and therefore might affect the process of epithelial recovery after epithelial damage occurs.

Lithium has a very small therapeutic range when prescribed to patients with bipolar disorders. Plasma levels of $<$0.4 mEq/l have no effect and levels $>$1.2 mEq/l likely cause adverse effects. During exacerbation periods of a bipolar disorder lithium plasma levels of 0.8–1.1 mEq/l are advised, while in remission levels should reach 0.6–0.8 mEq/l.[21] The therapeutic range of lithium as treatment for UC is unknown. In our study, lithium levels were approximately 0.50 mEq/l. This is comparable to the effective concentration of 0.60 mEq/l found by Daneshmand et al.[14] in the acetic acid colitis model. Notably, lithium doses that yielded 0.20 and 0.34 mEq/l did not affect acetic acid-induced colitis. Therefore, it seems that lithium levels $<$0.60 mEq/l can increase length and weight of the healthy colon, but do not influence colitis, while lithium concentration $\geq$0.60 mEq/l could ameliorate colitis. However, pilot studies in our lab revealed that treatment with lithium $\geq$8 mg per day ($>$0.7 mEq/l) had adverse effects. Mice showed signs of dehydration, because lithium had a diuretic effect. In the present study, signs of dehydration were also observed in some lithium/DSS-treated mice, but not in mice treated with lithium alone. The combination with DSS-induced diarrhea may explain the bad condition of these mice, because diarrhea is also characterized by loss of fluid. DSS-water consumption was the same in DSS- and lithium/DSS-treated mice.

Under DSS conditions treatment with another activator of the Wnt-pathway, RSpo1, showed contrasting results compared to our lithium treatment. Rspo1 treatment resulted in decreased epithelial damage, protection from DSS-induced reductions in colon length and crypt depth, and suppression of DSS-induced colonic MPO- and cytokine production. Both Rspo1 prophylaxis and Rspo1 treatment after colitis onset had beneficial effects.[4] A factor that might explain the difference in outcome after lithium treatment compared to Rspo1 could be the fact that lithium and Rspo1 act on Wnt-signaling in different ways. Lithium is an inhibitor of GSK3β and results in a decreased degradation of β-catenin, whereas Rspo1 antagonizes the inhibitory function of Dickkopf1 (DKK1).[22,23] DKK1 inhibits Wnt-signaling by binding to the Wnt co-receptor LRP5/6, leading to the internalization of LRP5/6. Another factor that might explain why Rpso1 ameliorated DSS-induced colitis, and lithium did not, could be that lithium and Rspo1 have a different affinity for Wnt (when used in the same dosage). When Rspo1 has a higher affinity than lithium, this could explain the different outcome.

We conclude that lithium does not have a therapeutic activity in a mice model of acute UC. However, in the healthy intestine lithium showed intestinotrophic effect. Therefore, lithium has probably more therapeutic potential in short bowel patients than in UC patients. Because short bowel patients miss a part of their small bowel the capacity to take up nutrients is decreased in these patients compared to a healthy colon. In this study, we observed that lithium is able to increase the length of the colon and Zhao[4] and Kim[5] also observed this phenomenon in the small intestine. A bigger surface could result in a higher capacity for nutrient uptake. Therefore, we propose that short bowel patients could benefit from lithium treatment due to its intestinal elongating effect.

Conflict of interest

Each author states that there is no financial or personal interest in any company or organization sponsoring the research.

Statement of authorship

The contributions of the authors to the manuscript are as follows: EL carried out the study, collected the data, performed data analyses and drafted the manuscript. TB did the samples analyses. AD evaluated the H&E slides and reviewed of the manuscript. MP, GH and KN participated in the design of the study, data interpretation and writing of the manuscript. GD conceived of the study, coordinated the study and wrote the manuscript. All authors read and approved the final manuscript.

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