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Therapeutic effects of an azaphenothiazine derivative in mouse experimental colitis

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Summary. Phenothiazines represent a class of compounds of potential therapeutic utility. In this report we evaluated therapeutic value of an azaphenothiazine derivative, 6-acetylaminobutyl-9-chloroquino[3,2-b]benzo[1,4]thiazine (QBT), given intragastrically, in the model of dextran sodium sulfate-induced colitis in C57BL/6 mice using 5-aminosalicylic acid (5-ASA) as a reference drug. Colitis symptoms such as body weight loss, diarrhea and hematochezia (blood in stool) were observed and registered and disease activity index (DAI) was calculated. In addition, weight and cell numbers in the lymphatic organs and histological parameters of the colon wall were analyzed. The effects of QBT on viability of colon epithelial cell lines were also determined. We showed that weight and cell number of draining mesenteric lymph nodes were lower in mice treated with QBT in comparison to their control counterparts. The number of thymocytes, drastically reduced in control mice, was elevated in mice treated with the compounds with a significant effect of 5-ASA. In addition, an abnormal composition of blood cell types was partially corrected in these groups. Histological analysis of the colon revealed that the pathological changes were partially normalized by QBT and even to a higher degree by 5-ASA. In conclusion we demonstrated a therapeutic efficacy of the compound in amelioration of local and systemic pathological changes associated with chemically-induced colitis in mice. A possible mechanism of action of the compound is discussed.

Key words: Azaphenothiazines, 5-ASA, Colitis, Dextran sodium sulfate, Mice

Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease of undefined etiology and remains a worldwide health problem (Gajendran et al., 2018). Despite the introduction of new therapeutic approaches, such drugs as corticosteroids, 5-aminosalicylate (5-ASA), azathioprine (Troncone and Monteleone, 2017), anti-diarrheal agents and nutritional supplements have already been in general use for a few decades (Jacknowitz, 1980).

New approaches in the treatment of UC include biological drugs that might inhibit cytokines and signaling molecules relevant in the pathogenesis of UC. This strategy includes development of compounds targeting the action of TNF α, IL-23, Janus kinases, sphingoside-1-phosphate and Smad7 (an inhibitor of the TGF-β1). The utility of these preparations has serious limitations, as quite a large proportion of patients are not responsive to such a therapy and are at risk of developing a number of adverse effects (Argollo et al.,

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Abbreviations. 5-ASA, 5-aminosalicylic acid; DAI, Disease Activity Index; DSS, dextran sodium sulfate; QBT, 6-acetylaminobutyl-9-chloroquino[3,2-b]benzo[1,4]thiazine; UC, ulcerative colitis

2017). Other strategies aimed at amelioration of symptoms of UC comprise the application of various compounds isolated from plants or milk (Kanwar et al., 2016; Wang et al., 2016; Salaritabar et al., 2017), infection with helminths (Summers et al., 2005) and transplantation of fecal microbiota (Narula et al., 2017).

Phenothiazines belong to a class of compounds demonstrating various biological activities, such as antipsychotic, antihistaminic, antitussive and antiemetic, and are a source of valuable drugs (Gupta and Kumar, 1988). Modifications of phenothiazines with azine rings originated azaphenothiazines (reviewed in Pluta et al., 2017). Recently we evaluated the immunosuppressive potential of several azaphenothiazines in delayed type hypersensitivity and carrageenan-induced inflammation (Artym et al., 2016). Subsequently, two active immunosuppressive compounds, 6-chloroethylureido-ethyldiquino[3,2-b;2',3'-e][1,4]thiazine and 6-acetylaminobutyl-9-chloroquino[3,2-b]benzo[1,4]thiazine, were selected and shown to effectively suppress contact sensitivity to oxazolone in mice (Artym et al., 2018a). In addition, the compounds inhibited chemically induced psoriatic changes in mice (Artym et al., 2018b).

The model of ulcerative colitis induced by dextran sodium sulfate (DSS) in rodents is a basic and well described test, suitable for evaluation of the therapeutic efficacy of various compounds (Melgar et al., 2005; Park et al., 2015). The outcome of application of the DSS-induced mouse model of colitis may by affected by several factors, among others by genetic background and gut microflora (Perse and Cerar, 2012). C57BL/6 mice, used in this work, are more susceptible than BALB/c due to a different cytokine profile, in DSS-induced colitis (Melgar et al., 2005). The aim of this investigation was to determine the therapeutic efficacy of 6-acetylaminobutyl-9-chloroquino[3,2-b]benzo[1,4] thiazine (QBT), applied intragastrically, in amelioration of DSS-induced colitis in C57BL/6 mice, and to elucidate its mechanism of action. QBT compound was selected for this investigation on the basis of its inhibitory action in both nonspecific (Artym et al., 2016) and antigen-specific (Artym et al., 2018a) immune reactions, its ability to inhibit TNF α production and lack of proapoptotic action on colon epithelial cell lines (Artym et al., 2018b).

Materials and methods

Mice

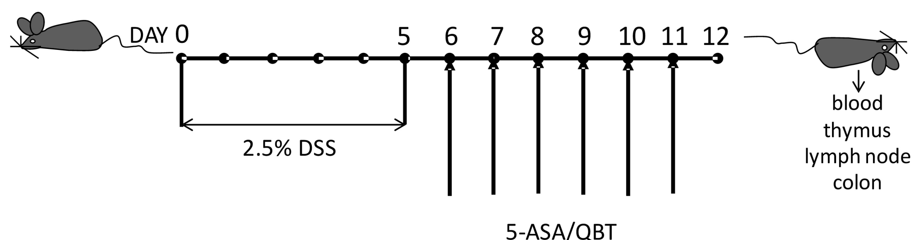
C57BL/6 female mice, 8-10 weeks old, delivered by the Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, were used for the study. The mice were housed in standard conditions and were fed commercial food and water *ad libitum*. The Local Ethics Committee at the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland, approved the study (permission # 64/2015).

Reagents

6-Acetylaminobutyl-9-chloroquino[3,2-b]benzo[1,4]thiazine was obtained via the procedure described previously (Jeleń et al., 2013) and was denoted in this article as QBT and represents compound #4 described in the preceding articles (Artym et al., 2016, 2018a,b). Hank's balanced salt solution (HBSS) and cell culture media (EMEM, DMEM) were from Institute of Immunology and Experimental Therapy, Wrocław (Poland). Fetal bovine serum (FBS) was from Gibco, sodium pyruvate from Serva, D-glucose from POCH (Poland), glutamate, HEPES, 2-mercaptoethanol, dimethylsulfoxide (DMSO), May-Grünwald and Giemsa reagents, toluidine blue, hematoxylin and eosin were from Sigma-Aldrich. Dextran sodium sulfate (DSS; MW 40 kDa) was from TdB Consultancy AB, Uppsala (Sweden), 5-aminosalicylic acid (5-ASA; Pentasa®) was from Ferring-Leciva (Czech Republik), buprenorphine (Vetergesic Vet®) was from Reckitt Benckiser Healthcare Ltd. (UK), and isoflurane (Forane®) was from AbbVie (Poland).

Treatment of mice with dextran sodium sulfate and the preparations

The DSS-induced mouse colitis procedure was performed as previously described (Melgar et al., 2005) with minor modifications. The mice drank a 2.5% solution of DSS in tap water acidified to pH 3.5 (with hydrochloric acid to limit growth of *Pseudomonas* species) for 5 days (days 0-5 of the experiment). The



Scheme 1. Experimental protocol.

DSS solution was freshly prepared each day and the bottles were also replaced each day. Then, the mice drank only acidified water (days 6-12 of the experiment). QBT (a hydrophobic compound) was initially dissolved in DMSO (5 mg in 0.5 ml DMSO), then in 0.9% NaCl to obtain a final volume of 0.2 ml and a dose of 25 μg/mouse (1.25 mg/kg b.w.). The dose of QBT was chosen based on our previous experience with the compound in other experimental models (Artym et al., 2018a,b). The compound was administered through a stomach tube, on days 6-11 of the experiment. 5-ASA was administered in the same way, in a dose of 1.5 mg (in 0.15 ml)/mouse during the same period. The control groups were given 0.9% NaCl or DMSO (the solvent) diluted in 0.9% NaCl in a dilution corresponding to that in the QBT solution, on days 6-11 of the experiment (positive control and DMSO control, respectively). "Background" (BG) mice drank only acidified water (days 0-12 of the experiment) and were treated only with 0.9% NaCl by a stomach tube (days 6-11 of the experiment). For the experimental design and description of the treatment see Table 1.

From day 0 to day 12 of the experiment, the mice were labeled and weighed, consistency of stool and appearance of blood in stool (hematochezia) were registered every day and scored for the Disease Activity Index (DAI) according to Park et al. (2015). All mice were subjected to standard analgesic procedure using buprenorphine.

Isolation of the blood and organs

On day 12 of the experiment the mice were subjected to anesthesia in isoflurane and bled via the retroorbital plexus, followed by cervical dislocation. The spleens, draining mesenteric lymph nodes, thymuses and colon were isolated, the lymph nodes and spleens were weighed, and spleens, lymph nodes and thymuses were placed in ice cold HBSS for subsequent cell enumeration.

Enumeration of thymocytes and lymph node cells

The organs were pressed against a plastic screen into 2 ml cold HBSS and counted in a hemocytometer (in 0.2% trypan blue dye for visualization of viable cells). The results were shown as the number of viable cells per organ (×10^6) as mean values ± standard error (SE).

Analysis of the circulation blood cell composition

Blood smears were stained with Giemsa and May-Grünwald reagents and up to 100 cells were counted (×1000 magnification in immersion oil). The results were presented as a percentage (mean values) of respective blood cell types: band forms of neutrophils (immature neutrophils), segment forms of neutrophils (mature neutrophils), eosinophils and lymphocytes.

Cell culture and treatment with QBT

HCT-116 (ATCC #CCL-247) and LoVo (ATCC #CCL-229™) colon epithelial cell lines were cultured in DMEM with 4.5 g/l D-glucose supplemented with 2 mM glutamate, 10 mM HEPES, 40 μM 2-mercaptoethanol and 10% FBS. The RKO (ATCC #CRL-2577) colon epidermal cell line was cultured in EMEM supplemented with 1 mM sodium pyruvate, 10 mM HEPES, 40 μM 2-mercaptoethanol and 10% FBS. The cell lines were cultured in a cell incubator at standard conditions. The cells were seeded at densities of 4×10^3 cells/0.1 ml (0.32 cm^2) for the cell viability assay. The QBT compound was added to the cultures for a 24 h incubation period at a concentration range of 1-10 μg/ml. The cells, incubated in the culture media containing 0.5% DMSO, served as an adequate control.

Cell viability assay

Cell viability was assessed by CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) according to the manufacturer's protocol. Each treatment within a single experiment was performed in triplicate. Absorbance at 490 nm was recorded by a Wallac 1420 VICTOR^2 plate reader (PerkinElmer, Waltham, USA). Data were normalized to untreated control cultures with 0.5% DMSO and presented as the percentage cell viability at a given compound concentration in

Table 1. The description of treatment of the experimental groups.

Experimental group	n	Treatment of mice
Background	4	On days 0-12 acidified water for drinking *ad libitum*, on days 6-11 0.9% NaCl once daily by a stomach tube
Positive control	8	On days 0-5 2.5% solution of DSS in acidified water for drinking *ad libitum*, then only acidified water (to day 12), on days 6-11 0.9% NaCl once daily by a stomach tube
DMSO control	8	On days 0-5 2.5% solution of DSS in acidified water for drinking *ad libitum*, then only acidified water (to day 12), on days 6-11 DMSO once daily by a stomach tube
5-ASA	8	On days 0-5 2.5% solution of DSS in acidified water for drinking *ad libitum*, then only acidified water (to day 12), on days 6-11 5-ASA (1.5 mg/mouse, once daily) by a stomach tube
QBT	8	On days 0-5 2.5% solution of DSS in acidified water for drinking *ad libitum*, then only acidified water (to day 12), on days 6-11 QBT (25 μg/mouse, once daily) by a stomach tube

comparison to the DMSO control culture (mean values ±SE).

Preparation of colon tissue samples and H&E staining

Colon samples were fixed in 10% neutral buffered formalin, dehydrated in an alcohol series, cleared in xylene and embedded in paraffin. The paraffin blocks were sliced into 5 µm sections. The paraffin sections were stained with hematoxylin and eosin (H&E). Additionally, an immunohistochemical analysis was carried out. The histological analysis was performed in the light microscope Nikon Eclipse 80i with the aid of imagine software NIS-Elements (Nikon).

Immunohistochemical analysis

In order to identify the cellular infiltrates in H&E stained sections, the immunohistochemical analysis was carried out. Neutrophils were visualized with rat anti-mouse Ly6G antibody (GR-1, clone RB6-8C5, Thermo Fisher Scientific), T lymphocytes with rabbit anti-mouse CD3 antibody (clone SP7, Thermo Fisher Scientific) and B lymphocytes with rat anti-mouse CD19 antibody. The primary antibodies were detected using ImmPRESS REAGENT KIT anti-Rabbit Ig (Vector) and ImmPRESS REAGENT KIT anti-Rat IgG, mouse adsorbed (Vector) according to manufacturer instructions. Antigen retrieval was performed using sodium citrate buffer pH-6 for 20 minutes at 97°C in a water bath. Slides were incubated in a 3% H_2O_2 solution to quench endogenous peroxidase activity and blocked in a 2.5% normal goat blocking serum. Sections were incubated for 1 h at room temperature with antibodies at final dilutions: Ly6G 1:800, CD3 1:500, CD19 1:1000. ImmPACT™ DAB (Vector) and ImmPACT™ NovaRED™ (Vector) were used as chromogen. The sections were counterstained with Meyer's Hematoxylin (Sigma). The quantitative analysis of labeled cells (neutrophils, B cells and T cells) was achieved by counting cells in 3 random fields of view (original magnification ×200, surface area 290 000 μm^2) for each animal. From every examined group, 4 animals were analyzed. The immunohistochemical analyses were performed in a light microscope Nikon Eclipse 80i with the aid of imagine software NIS-Elements (Nikon).

Statistics

The results are presented as mean values ±SE. Brown-Forsyth's test was used to determine the homogeneity of variance between groups. When the variance was homogenous, analysis of variance (one-way ANOVA) was applied, followed by post hoc comparisons with the Tukey's test to estimate the significance of the difference between groups. Nonparametric data were evaluated with Kruskal-Wallis' analysis of variance. Significance was determined at $p<0.05$. Statistical analysis was performed using STATISTICA 7.0 for Windows.

Results

Effects of the compound on survival, severity of colitis, body and organ weight, cell number in lymphoid organs and blood cell composition

All mice were daily monitored from the start of the experiment for registration of symptoms of inflammation in the colon. Severity of colitis was assessed with a Disease Activity Index (DAI) (Park et al., 2015). The results are presented as a median cumulative score for day 12 of the experiment and median number of days with score 3 and above 3 (Table 2). The evaluation of the overall mouse condition revealed that it was best in the QBT group. The regaining of weight during the experiment, assuming the initial weight as 100%, was achieved on day 12 in one mouse in the positive control group (0.9% NaCl), two mice in the DMSO control group, none in the 5-ASA-treated mice and four mice treated with QBT. One mouse died on day eight in the

Table 2. Severity of colitis (DAI index).

Group	Cumulative score for day 12 of the experiment		No. of days with score 3 and above 3	
	Median	Q1-Q3	Median	Q1-Q3
BG	4.0	1.0-6.0	0.0	0.0-0.0
Positive control	21.0	16.0-26.0	3.0	1.0-5.0
DMSO control	22.0	12.0-27.0	4.0	1.5-5.0
5-ASA	17.0	12.0-25.0	3.0	0.5-5.0
QBT	13.0	11.5-28.5	1.5	1.0-6.0

The symptoms of colitis were assessed by DAI. The results are presented as a cumulative score for day 12 of the experiment (for every individual mouse, a cumulative score was calculated by adding all given scores in the evaluated time frame: day 0-12 of the experiment) and median number of days with a score equal to and above 3. The results from mice euthanized on day 12 of the experiment are presented.

Table 3. The weight and cell number of draining mesenteric lymph nodes.

Group	The lymph node weight		The lymph cell number	
	Weight (mg/organ)	± SE	Cell number $\times 10^6$	± SE
BG	23.08	4.03	15.10	3.34
Positive control	31.50	3.10	24.00	3.35
DMSO control	37.18	4.35	29.30	3.07
5-ASA	34.33	4.85	16.40	1.37
QBT	26.81	2.41	11.25	1.09

The draining mesenteric lymph nodes were isolated on day 12 of the experiment, weighed and homogenized to obtain single cell suspension. The cells were stained with 0.2% trypan blue and viable cells were counted. The results are shown as the mean number of cells per organ ($\times 10^6$) ±SE.

positive control group, one on days eight and two and one on days 9 and 10, respectively, in the 5-ASA group, and none in the QBT group.

The reduction in weight and number of cells in mesenteric draining lymph nodes was marked (but not statistically significant) in mice treated with the studied compound, in comparison to the DMSO control group (Table 3). We observed a similar effect of QBT in relation to the spleen and thymus weight (data not shown).

Since the involution of thymus in experimental colitis has been reported (Fritsch Fredin et al., 2007), we also evaluated this phenomenon, revealing a profound loss of thymocytes in both control groups. A best (about 50%) recovery of the thymocyte number was registered in mice treated with 5-ASA, QBT was less effective (Fig. 1).

The content of the myelocytic cell types in the circulating blood was greatly increased in the positive control group but was lowered in the DMSO control

Table 4. Immunohistochemical analysis identifying cell types in the cellular infiltrates in colon sections.

Group	Neutrophils		T lymphocytes		B lymphocytes	
	Number of cells in field of view	± SE	Number of cells in field of view	± SE	Number of cells in field of view	± SE
BG	0.00	0.00	1.6	0.3	1.1	0.61
Positive control	62.4	15.8	20.2	2.7	76.5	15.5
DMSO control	48.6	23.3	25.5	2.6	66.4	23.7
5-ASA	*0.0	0.0	*#11.6	2.1	*19.6	8.3
QBT	41.3	19.0	#10.2	1.8	47.2	5.7

The mean numbers ±SE of neutrophils (staining with anti-Ly6G antibody), T cells (staining with anti-CD3 antibody) and B cells (staining with anti-CD19 antibody) from 3 random fields of view are depicted. Statistics: $p<0.05$, *: vs Positive control, #: vs DMSO control.

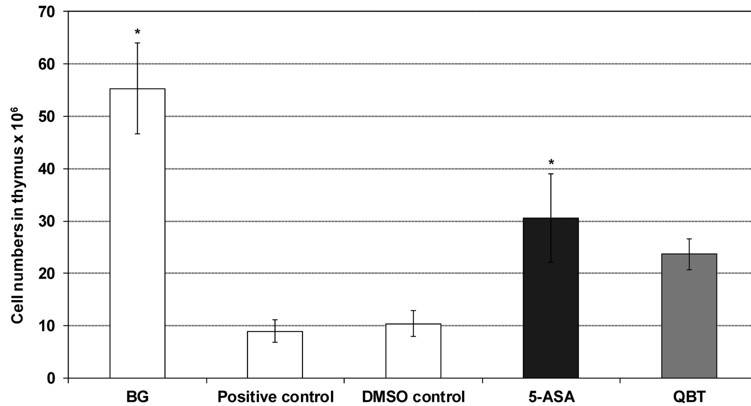


Fig. 1. The changes in the number of thymocytes within the experimental groups. The thymuses were isolated on day 12 of the experiment and homogenized to obtain single cell suspension. The cells were stained with 0.2% trypan blue and viable cells were counted. The results are shown as the mean number of cells per organ ($\times 10^6$) ±SE. Statistics: *, $p<0.05$ versus DMSO control mice.

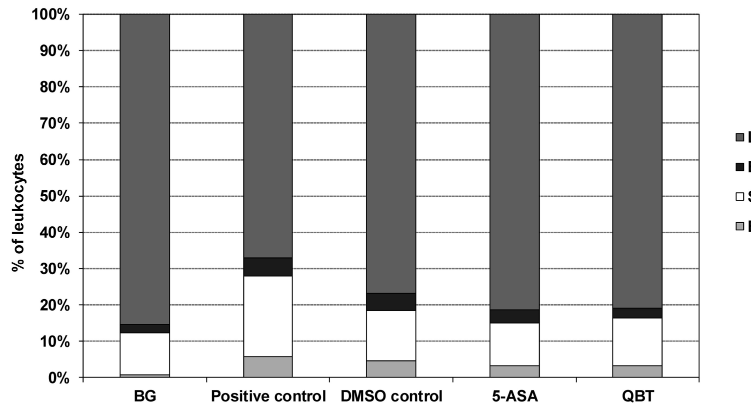


Fig. 2. The composition of circulating blood cell types in the experimental mice. Mice were bled on day 12 of the experiment and blood cell types counted in blood smears stained with Giemsa and May-Grünwald reagents; up to 100 cells were counted (1000× in immersion oil). The results are presented as a percentage (mean values) of blood cell types: band forms of neutrophils (immature neutrophils, B), segment forms of neutrophils (mature neutrophils, S), eosinophils (E) and lymphocytes (L).

group (Fig. 2). Both 5-ASA and QBT further decreased the contribution of the myelocytic cells in the blood reflecting their anti-inflammatory actions.

Histological examination of the colon

The histological examinations (Fig. 3A-E) revealed

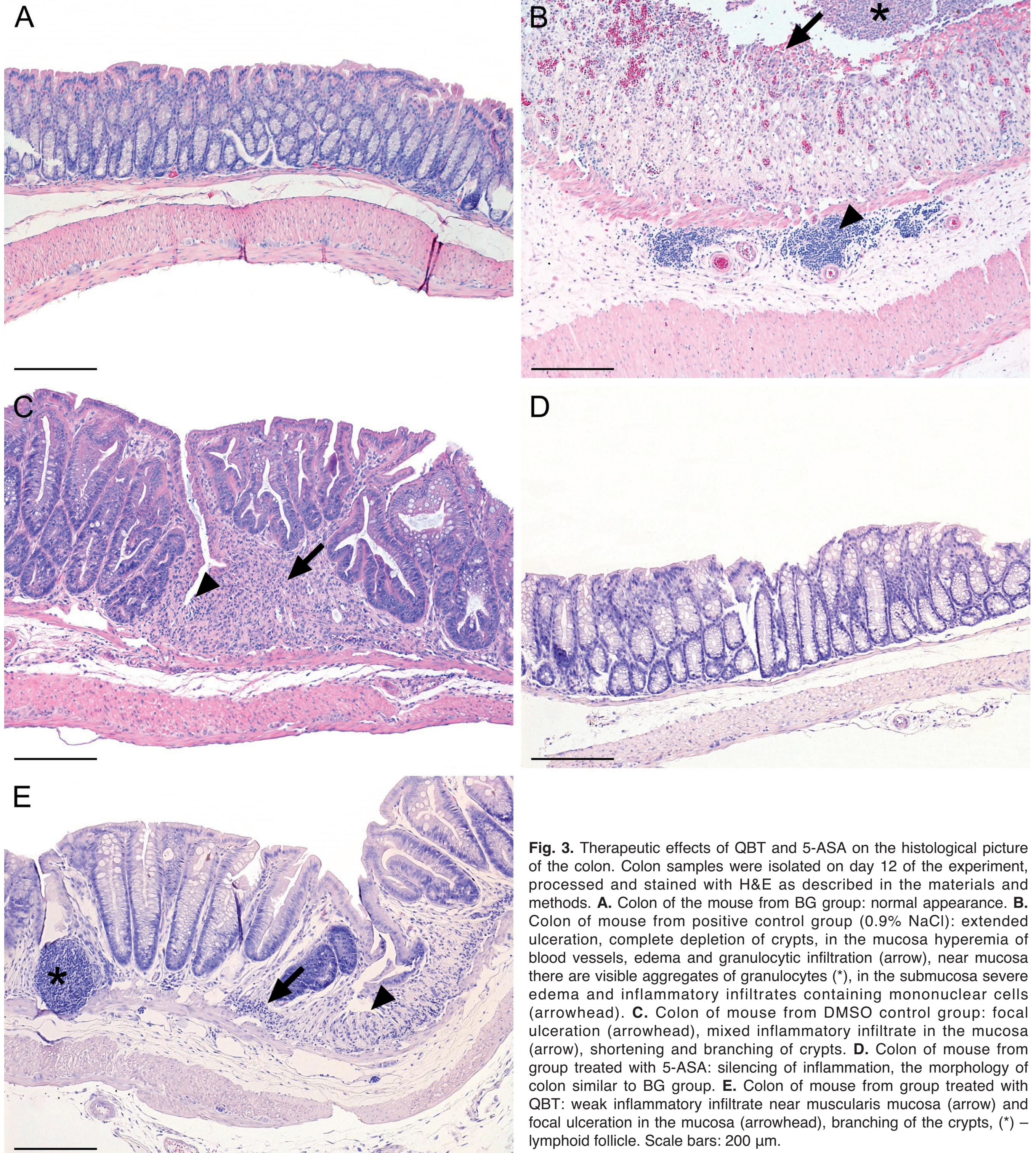


Fig. 3. Therapeutic effects of QBT and 5-ASA on the histological picture of the colon. Colon samples were isolated on day 12 of the experiment, processed and stained with H&E as described in the materials and methods. **A.** Colon of the mouse from BG group: normal appearance. **B.** Colon of mouse from positive control group (0.9% NaCl): extended ulceration, complete depletion of crypts, in the mucosa hyperemia of blood vessels, edema and granulocytic infiltration (arrow), near mucosa there are visible aggregates of granulocytes (*), in the submucosa severe edema and inflammatory infiltrates containing mononuclear cells (arrowhead). **C.** Colon of mouse from DMSO control group: focal ulceration (arrowhead), mixed inflammatory infiltrate in the mucosa (arrow), shortening and branching of crypts. **D.** Colon of mouse from group treated with 5-ASA: silencing of inflammation, the morphology of colon similar to BG group. **E.** Colon of mouse from group treated with QBT: weak inflammatory infiltrate near muscularis mucosa (arrow) and focal ulceration in the mucosa (arrowhead), branching of the crypts, (*) – lymphoid follicle. Scale bars: 200 µm.

Table 5. The effect of QBT on viability of colon epithelial cell lines.

Concentration	HCT 116 cell line		RKO cell line		LoVo cell line	
of QBT (µg/ml)	% of cell viability	± SE	% of cell viability	± SE	% of cell viability	± SE
1.0	#113.19	1.68	103.52	2.92	109.93	3.97
2.5	108.97	3.06	104.93	1.64	103.01	2.30
5.0	108.23	3.08	92.43	1.27	106.30	2.46
10.0	104.23	3.12	*87.28	1.47	97.20	2.31

HCT-116, LoVo and RKO cell lines were cultured at densities of 4×10^3 cells/0.1 ml and were analysed for cell viability as described in the materials and methods. QBT compound was added to the cultures for a 24 h incubation period at 1-10 µg/ml. The cells, incubated in the culture media with 0.5% DMSO, served as an adequate control and the results are presented as the percentage cell viability at a given compound concentration in comparison to DMSO control culture (mean values ±SE). Statistics: * $p<0.05$ (inhibition), # $p<0.05$ (stimulation of cell viability).

severe pathological changes in the colon wall of the positive control mice, such as extended ulcerations, complete depletion of crypts, hyperemia of blood vessels, edema and granulocytic infiltration in the mucosa and furthermore severe edema, inflammatory infiltrates containing mononuclear cells in the submucosa. Smaller pathological changes in DMSO-treated mice included focal ulcerations, mixed inflammatory infiltrates in the mucosa and shortening and branching of crypts. In the group administered 5-ASA, a silencing of inflammation was observed with the morphology of the colon similar to the BG group. In mice treated with QBT, weak inflammatory infiltrate near muscularis mucosa, focal ulceration in the mucosa and occasional branching of the crypts were observed. The pathological changes induced by DSS lead to an enormous enlargement of the colonic mucosa thickness so a reader may have an impression that the magnification scale of the epithelium for naive mice is smaller. In fact, all photographs have ×100 magnification.

The results of the immunostaining, presented as quantitative parameters, are shown in Table 4. Neutrophils were absent in background and 5-ASA-treated mice, but a marked infiltrate in the positive control and moderate infiltrates in DMSO control and QBT-treated mice were found. An increase of T cell ($CD3^+$) number was registered in the positive control and DMSO control groups, in comparison to the background mice. However, the number of T cells significantly decreased in the groups treated with QBT and 5-ASA as compared to the positive control group. The staining with anti-CD19 antibody revealed an increase in the content of B cells in the positive control and DMSO control groups and decreases in mice treated with 5-ASA and QBT.

Effects of the compound on the viability of colonic cell lines

Previously our investigations revealed that QBT upregulated expression of signaling molecules involved in apoptosis in Jurkat cell line (human T cells), inhibited growth of KERTr cell line (human keratinocytes) and inhibited LPS-induced TNF α production (Artym et al., 2018b).

Here we evaluated the effect of the compound at a concentration range of 1-10 µg/ml on viability of epithelial colon cell lines: HCT-116, LoVo and RKO (Table 5). No significant inhibition by QBT was observed up to 10 µg/ml for HCT116 and LoVo cell lines but a significant inhibition was registered for RKO cells at a 10 µg/ml. Previous (Artym et al., 2018b) and present experiments aimed at determination of the possible mechanism of QBT action.

Discussion

In this study we demonstrated a therapeutic usefulness of the azaphenothiazine derivative in experimentally induced colitis in mice as evaluated by histological analysis and generalized effects on the blood composition and the lymphoid organs. 5-ASA was chosen as a reference drug in ulcerative colitis (Punchard et al., 1992). The histological examinations demonstrated protective actions of QBT and 5-ASA, with regard to the intestinal mucosa and submucosa. Possible mechanisms of local and generalized protective properties of QBT and its solvent DMSO and side effects of 5-ASA are discussed below.

The immunostaining procedures revealed the involvement of respective cell types corresponding to the severity of pathological changes in the colon wall (Table 4). Neutrophils, as indicators of the inflammatory processes, were absent in the BG and 5-ASA groups. The T cell content was increased in both controls (0.9% NaCl and DMSO), in comparison to the BG mice, and decreased in QBT and 5-ASA-treated mice. B cell numbers were higher in both control groups and significantly lower in mice treated with 5-ASA. Although the presence of neutrophils in the pathogenesis of ulcerative colitis is evident (Yasukawa et al., 2012), these cells may also play a protective role in this model (Zindl et al., 2013). Actually, development of DSS-

induced colitis does not require the presence of T and B cells (Dieleman et al., 1994). Nevertheless, in normal mice, B cells may act as regulatory cells, which recruit T regulatory cells promoting B cell differentiation into IgA producing plasma cells (Wang et al., 2015).

Apart from systemic protective effects of QBT on the immune system cells shown above, the mice treated with the compound demonstrated a better physical condition and higher body weight among the studied groups. Also, the severity of colitis, assessed by DAI scoring, although statistically not significant, was best in QBT group (Table 2).

Several results may account for protective properties of the QBT compound. QBT was not toxic to the colon cell lines HCT-116 and LoVo up to a concentration of 10 μg/ml and did not induce apoptosis in HCT-116 and RKO colon epithelial cell lines (Artym et al., 2018b). Physicochemical studies (Jeleń et al., 2015) revealed that the compound should have good oral bioavaiability which is relevant to this study. QBT is also more lipophilic at physiologic pH than classical antipsychotic phenothiazines. Nevertheless, QBT was shown to strongly upregulate expression of signaling molecules, involved in apoptosis in Jurkat cells, and inhibited growth of an epidermal KERTr cell line (Artym et al., 2018b). Thus, the effects of QBT may differ depending on the cell type.

The protective effects of QBT are probably obscured to some degree by DMSO (the solvent). The anti-inflammatory and anti-oxidative properties of DMSO are well known (Ahn et al., 2014; Li et al., 2015) and could account for its stabilizing effect regarding mouse physical condition (body weight), and a normalizing action on the blood picture (Fig. 2).

Most likely, a strong ability of QBT to inhibit LPS-induced TNF α production (Artym et al., 2018b) plays a major role in the therapeutic effects of the compound. Neutralization of TNF α in DSS-induced colitis is protective (Kojouharoff et al., 1997). In addition, a phenomenon of intraluminal release of TNF α in UC has been described (Casellas et al., 1994), followed by a demonstration of efficacy of anti-TNF α treatment in these patients (Argollo et al., 2017).

Although QBT exhibited overall less favorable effects on the structure of the colonic tissue, in comparison to 5-ASA, one has to keep in mind that its daily dose was 60× lower than that of 5-ASA (1.5 mg). In addition, the mechanism of action of 5-ASA differs from that of QBT by inhibition of prostaglandin and leukotriene synthesis and scavenging reactive oxygen species (Punchard and Greenfield, 1992). Recent findings indicate that the mechanism of action of 5-ASA may also involve inhibition of the phosphorylation of JNKs and p38 in mouse macrophages (Qu et al., 2017). Nevertheless, despite the high efficacy of 5-ASA in amelioration of the pathological changes, the drug is not devoid of side-effects (Yigitler et al., 2004; Troncone and Monteleone, 2017).

In conclusion, we demonstrated the therapeutic usefulness of the oral applied azophenothiazine derivative (QBT compound) in amelioration of DSS-induced colitis, which included both local and systemic parameters.

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Conflicts of interest. All authors declare no conflicts of interest.

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