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Evaluation of butyric acid as a potential supportive treatment in anterior uveitis

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ABSTRACT

BACKGROUND: The aim of the study was to evaluate the anti-inflammatory effect of topically administered aqueous sodium butyrate solution in an endotoxin-induced uveitis rat model and compare the results with corticosteroid treatment.

MATERIAL AND METHODS: Forty female Lewis rats were randomly divided into five groups. Uveitis was induced by a single lipopolysaccharide (LPS) injection into each footpad of each LPS+ rat. Group I (naive) received saline injected into the footpad of each rat at a dose of 0.1 mL/each footpad; Group II (LPS+) received saline solution topically. Group III (LPS+ Dex) — an aqueous dexamethasone sodium phosphate solution topically; Group IV (LPS+ But 0.5 mM) — 0.5 mM aqueous sodium butyrate solution topically, Group V (LPS+ But 1 mM) — 1.0 mM aqueous sodium butyrate solution topically. Clinical scoring of inflammation in rat eyes was evaluated before LPS injection and after 24 hours. The iris involvement, posterior synechiae presence, and insight into the eye fundus were clinically assessed. A histopathological examination was also performed. The rats were euthanatized 24 hours after LPS injection, and aqueous humor (AqH) was collected from the eyes by anterior chamber puncture. Levels of inflammatory cytokines and chemokines in the AqH were determined with commercially available Luminex assays.

RESULTS: Development of iris hyperemia associated with miosis and poor visibility of fundus details occurred 24 hours after LPS injection. Compared to the LPS+ group, the clinical scores were strongly suppressed in rats treated with Dexamethasone and moderately diminished in LPS+ But 0.5 mM. These clinical features were not observed in the controls (Group 1 — naive). Data from inflammatory cytokines evaluation indicates no significant differences between the LPS+ group (Group 2) and the LPS+ But groups (Groups 4 and 5). Histopathological examinations suggest that hyperemia, corneal stratification, and lesions were less common in the group of animals treated with BA in a lower concentration.

CONCLUSION: Topical administration of sodium butyrate as a therapeutic agent might alleviate the severity of intraocular inflammation in eyes with uveitis. The effect of sodium butyrate was slight but clinically significant in 0.5 mM dose, so other doses of topically administered sodium butyrate should be considered and evaluated in further research.

KEY WORDS: butyric acid; anterior uveitis; bacterial metabolites; ophthalmology; autoimmune diseases

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INTRODUCTION

Anterior uveitis is a common type of ocular inflammation in developing and developed countries and remains a challenging condition for ophthalmologists. It is a potentially blinding intraocular disease and represents a broad spectrum of disorders characterized by uveal tract inflammation (iris, ciliary body, and choroid) and inflammation of the adjacent structures (vitreous humor, retina, optic nerve, and vessels). According to the criteria of Standardization of Uveitis Nomenclature (SUN), uveitis can be classified (according to the anatomic site) into: anterior (anterior chamber inflammation), intermediate (*pars plana* inflammation), posterior (posterior segment inflammation), or panuveitis (involving both anterior and posterior segment) [1–4]. Despite the vast progress in recognizing and treating uveitis, its complications such as macular edema, glaucoma, cataracts, band keratopathy, or permanent vision loss remain relatively common [5–11]. It can be partially explained by the fact that there are still notable gaps in understanding the multifactorial pathogenesis of uveitis [12]. The first line of treatment remains a combination of topical corticosteroids and mydriatic agents, reserving systemic treatment for patients with refractory and severe involvement [1, 13, 14]. Topical corticosteroids suppress cellular infiltration, capillary dilation, and fibroblast proliferation. The mydriatic drug is usually used concomitantly with topical corticosteroids to reduce or prevent the development of posterior synechiae [15]. Immunomodulators and targeted biological agents are increasingly used as corticosteroid-sparing therapies [16]. Invasive routes of administration of corticosteroids such as intravitreal injections, intraocular implants, and systemic administration could be linked with suboptimal adherence to the therapy. They are sometimes associated with complications, such as increased intraocular pressure [16], systemic adverse effects [17], and related retinal detachment [18, 19]. Moreover, accumulating data suggests that many patients are resistant to these agents or cannot tolerate them. Therefore, novel, effective, safe alternative therapies are still needed [20–22]. Animal models are a possible key to better understanding this disease and finding alternative therapy. Subcutaneous administration of the bacterial endotoxin, lipopolysaccharides (LPS), induces a rapid but short-lived anterior uveitis (EIU, endotoxin-induced uveitis) within 24 h of the injection [23]. Accumulating data suggests that this model has been used for investigating different experimental therapies and numerous features of the acute ocular inflammatory response. Numerous studies report that each person's microbiota composition is unique [24–26]. The interactions between gut microbiota and the immunology system are not sufficiently understood. Some gut microbiota products may play a significant role in developing many immunological diseases [27, 28]. Butyrate is a colorless carboxylic acid produced mainly in the mammalian gut during the fermentation of dietary fiber. Furthermore, the taste of butyrate is characterized as pungent, with a sweetish aftertaste which can be linked with the taste of ether. Butyrate and its salts may affect various physiological functions, including obesity, energy homeostasis, immune system, cancer development, or brain functions. The explanation of the mechanisms is not well understood and may range from metabolic effects on receptor signaling to enzymatic inhibition [29, 30]. Recent studies report that orally administered butyrate may inhibit uveitis [31]. We hypothesized that topical administration of sodium butyrate would allow assessing higher concentrations in the eyeball tissues in a shorter time than through oral administration and exert protective effects in an animal model of uveitis. Our study aimed to investigate the anti-inflammatory effects of the topical administration of BA on the anterior ocular segment in EIU rats.

MATERIAL AND METHODS

Animals

The experiments were performed in accordance with the Directive 2010/63 EU on the protection of animals used for scientific purposes and guidelines of the Association for Research in Vision and Ophthalmology (ARVO). They were also approved by the II Local Ethics Committee in Warsaw (permission no. 653/2018 and WAW2/051/2020). Experiments were performed on 6–8-week-old female Lewis rats (130–200 g; Mossakowski Medical Research Center Polish Academy of Sciences, Warsaw, Poland) fed a sterile laboratory diet (Ssniff GmbH, Lage, Germany), food and water ad libitum. They were housed in groups (2–3) in polypropylene cages with environmental enrichment, 12 h light/12 h dark cycle, temperature 22°C ± 2°C, humidity 55 ± 10%, and randomly taken from cages for experiments. The rats from one cage were assigned to different experimental series. However, there was no specific randomization method.

Induction and treatment of endotoxin-induced uveitis in rats

To induce uveitis, 200 µg of LPS from *Escherichia coli* 055: B6 (Sigma-Aldrich, St. Louis, MO, United States) diluted in 0.2 mL of saline (0.9% NaCl), and 0.1 mL was injected into each footpad of each LPS+ rat at a dose of 100 µg/each footpad [32, 33]. In Group 1 (naive), saline was injected into the footpad of each rat at a dose of 0.1 mL/each footpad. Rats were randomly allocated to the five following groups:

- Group 1 (naive) — consisting of rats that received eye drops containing saline (0.9 % NaCl) applied topically to the eye;
- Group 2 (LPS+) — consisting of rats that received injections of LPS and eye drops containing saline (0.9 % NaCl) applied topically to the eye;
- Group 3 (LPS+ Dex) — consisting of rats that received injections of LPS and eye drops containing dexamethasone phosphate (Dexafree 1 mg/mL, Laboratoires Théa, Clermont-Ferrand, France) applied topically to the eye;
- Group 4 (LPS+ But 0.5 mM) — consisting of rats that received injections of LPS and eye drops containing sodium butyrate (sodium butyrate solution 0.5 mM) applied topically to the eye;
- Group 5 (LPS+ But 1 mM) — consisting of rats that received injections of LPS and eye drops containing sodium butyrate (sodium butyrate solution 1 mM) applied topically to the eye.

The eye drops were applied 2 hours before the LPS injection, and then the eye drops were applied 2, 4, 6, 8, 10, 12, and 14 hours after the LPS injection.

Clinical scoring of inflammation in rat eyes

Clinical scoring of endotoxin-induced uveitis was evaluated using a direct ophthalmoscope (PanOptic, Welch Allyn, Skaneateles Falls, NY) before LPS injection and after 24 hours. The ophthalmoscope was equipped with a customized iPhone adapter; images were captured using the appropriate software (iExaminer App, Welch Allyn, Skaneateles Falls, NY). The images were evaluated by a skilled ophthalmologist (KK-J) and veterinary surgeon (MW-K), unaware of the experimental groups. The scale was based on the scale published by [34], with modifications (Tab. 1).

Collection of aqueous fluid and determination of inflammatory cytokines and chemokines

The rats were euthanized 24 hours after the LPS injection. Aqueous humor (AqH) was collected from the eyes immediately by anterior chamber puncture with a heat-pulled capillary connected to a micro dispenser (Drummond Scientific Co., Broomall, PA, United States) under direct observa-

Table 1. Clinical scale for evaluation of endotoxin-induced uveitis

Investigated part of the eye	Description	Grade
Pupil	Regular	0
	Miosis	1
Iris involvement	Normal iris without any hyperemia of the iris vessels	0
	Minimal injection of secondary vessels but not tertiary	1
	Minimal injection of tertiary vessels and minimal to moderate injection of the secondary vessels	2
	Moderate injection of the secondary and tertiary vessels with a slight swelling of the iris stroma	3
	Marked injection of the secondary and tertiary vessels with marked swelling of the iris stroma	4
Posterior pole	Posterior pole clearly visible	0
	Posterior pole details slightly hazy	1
	Posterior pole details very hazy	2
	Posterior pole details barely visible	3
	Fundus details not visible	4
The effect of the pupil dilatation with tropicamide 1%	Round and regular, properly extended	0
	Irregularly extended to the area of one quarter or less (≤ 1)	1
	Irregularly extended beyond one but less than two quarters, moderately responsive on the mydriatic	2
	Irregularly expanded beyond two but less than three quadrants, poorly responsive to extension	3

Table 2. The levels of inflammatory factors determined in the aqueous humor using Luminex. Values are show as means and standard deviation, *p < 0.05; **p < 0.01; ***p < 0.001

	Naive	LPS+	LPS+ Dex	LPS+ But 0.5 Mm	LPS+ But 1Mm
IL-1α	33.12±25.39 **	199.9±25.91	176±79.49	279±166.5	160.2±67.89
IL-1β	185.6±311.6 **	1268±216.9	78.04±29.87 **	940.2±421.1	760.2±384
IL-2	1885±2366	2185±65.12	2692±977.8	1849±795.1	2091±1342
IL-4	9.01±3.46 *	51.6±4.90	54.72±8.1	43.15±14.37	52.58±18.4
IL-5	60.15±78.23	100.8±36.57	163.9±86.85	76.47±33.89	119.3±59.12
IL-6	10.24±27.08 **	5357±2250	127.8±47.3	8999±6470	5155±4463
IL-7	6.92±11.82	9.23±5	20.4±10.9	17.78±10.56	7.492±3.5
IL-10	13.13±18.25 ***	176.7±38.43	60.51±9.21	150.5±56.07	123±53.45
IL-12	37.23±33.28 *	133.7±8.05	137.4±41.67	145±33.68	108.7±34.69
IL-13	31.36±31.56	39.65±27.48	4.46±9.97	43.44±32.49	23.63±28.47
IL-17	11.45±7.82 **	65.15±28.58	16.9±8.26 *	67.55±34.14	36.97±16.06
IL-18	548.1±337.8 *	6134±8872	856.9±227.8	3856±3159	5438±3191
G-CSF	0.58±0.83	0.91±0.71	1.22±1.21	1.63±1.23	1±0.82
GM-CSF	4.15±8.55	7.53±5.34	1.72±3.24	9.43±9.91	4.34±3.34
INF-γ	8.01±13.79	21.41±9.12	44.09±28.78	33.24±21.18	16.43±10.2
M-CSF	8.21±2.43 ***	42.89±5.92	25.7±3.25	41.82±12.52	33.21±10.89

IL — interleukin; G-CSF — granulocyte colony-stimulating factor; GM-CSF — granulocyte macrophage colony-stimulating factor; INF — interferone; M-CSF — macrophage colony-stimulating factor

tion by a surgical microscope (OPMI FM 1 Pro, Carl Zeiss AG, Oberkochen, Germany). The samples were stored at –80°C until further use. Levels of inflammatory cytokines and chemokines in the AqH were determined by a commercially available LuminexBio-Plex ProTM Rat Cytokine Th1/Th2 Assay (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Equal amounts of AqH obtained from rat eyes were used to determine the inflammatory cytokines and chemokines in AqH. Enucleated eyes were collected, fixed in 4% paraformaldehyde, and stored until further use.

Histopathology

The collected eyeballs were fixed in 4% buffered formaldehyde for at least 48 hours, then cut lengthways, and samples were placed in standard plastic histological cassettes for tissue specimens. Then samples were rinsed in running water, dehydrated in grades of ethanol and xylene, and embedded in paraffin. Paraffin blocks were cut into 4 µm slices and stained with hematoxylin-eosin. The slides were examined by a veterinarian pathologist (RS).

Statistical analysis

Data are presented as the mean ± standard error of the mean. The GraphPad Prism for Windows ver. 6.04 software package (GraphPad Software, San Diego, CA, USA) was used for the statistical assessment of the data. Differences between groups were tested using Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparisons *post hoc* test. Differences between groups were considered significant when $p < 0.05$.

RESULTS

Clinical evaluation

We evaluated the effect of BA eye drops on ocular anterior segment inflammation and compared its effect with dexamethasone solution. The ocular inflammation was assessed by a direct ophthalmoscope (Panoptic, Welch Allyn, Skaneateles Falls, NY) examination 24 hours after LPS injection. The clinical scores were evaluated according to the criteria of EIU (Tab. 1, n = 8). No inflammatory features were observed in normal control rats (Group 1 — naive). Miosis, iris hyperemia, poor visibility of the posterior pole, and problems with tropicamide dilatation of the pupil occurred in Group 2 and Group 5. Inflammatory responses were strongly suppressed in rats treated with dexamethasone and moderately abolished in Group 4 (Fig. 1, 2).

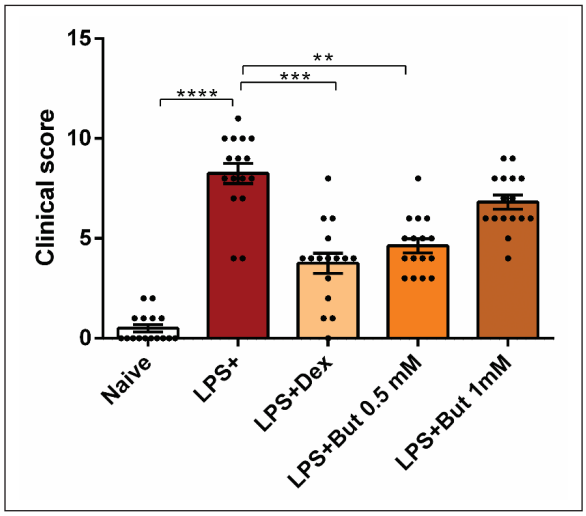

FIGURE 1. Clinical scoring of endotoxin-induced uveitis was assessed with a direct ophthalmoscope 24 hours after lipopolysaccharides (LPS) injection

Evaluation of inflammatory cytokines

Levels of most of the analyzed cytokines were significantly elevated after the LPS treatment, e.g., inteleukins (ILs): IL-1α (Naive: 33.12 ± 9.60 pg/mL *vs.* LPS+: 199.90 ± 11.59 pg/mL, $p < 0.01$), IL-1β (Naive: 185.60 ± 117.80 pg/mL *vs.* LPS+: 1268.00 ± 186.40 pg/mL, $p < 0.01$), IL-4 (Naive: 9.01 ± 3.46 pg/ml *vs.* LPS+: 51.60 ± 2.19 pg/mL, $p < 0.05$), IL-6 (Naive: 10.24 ± 10.24 pg/ml *vs.* LPS+: 5357.00 ± 1006.00 pg/mL, $p < 0.01$), IL-10 (Naive: 13.13 ± 6.90 pg/mL *vs.* LPS+: 176.70 ± 17.19 pg/mL, $p < 0.001$), IL-12 (Naive: 37.23 ± 12.58 pg/ml *vs.* LPS+: 133.7 ± 3.6 pg/mL, $p < 0.05$), IL-17 (Naive: 11.45 ± 2.955 pg/mL *vs.* LPS+: 65.15 ± 12.78 pg/mL, $p < 0.01$), IL-18 (Naive: 548.1 ± 127.7 pg/mL *vs.* LPS+: 6134 ± 3968 pg/mL, $p < 0.05$), macrophage colony-stimulating factor (M-CSF) (Naive: 8.214 ± 0.9171 pg/mL *vs.* LPS+: 42.89 ± 2.648 pg/mL, $p < 0.001$). Com-

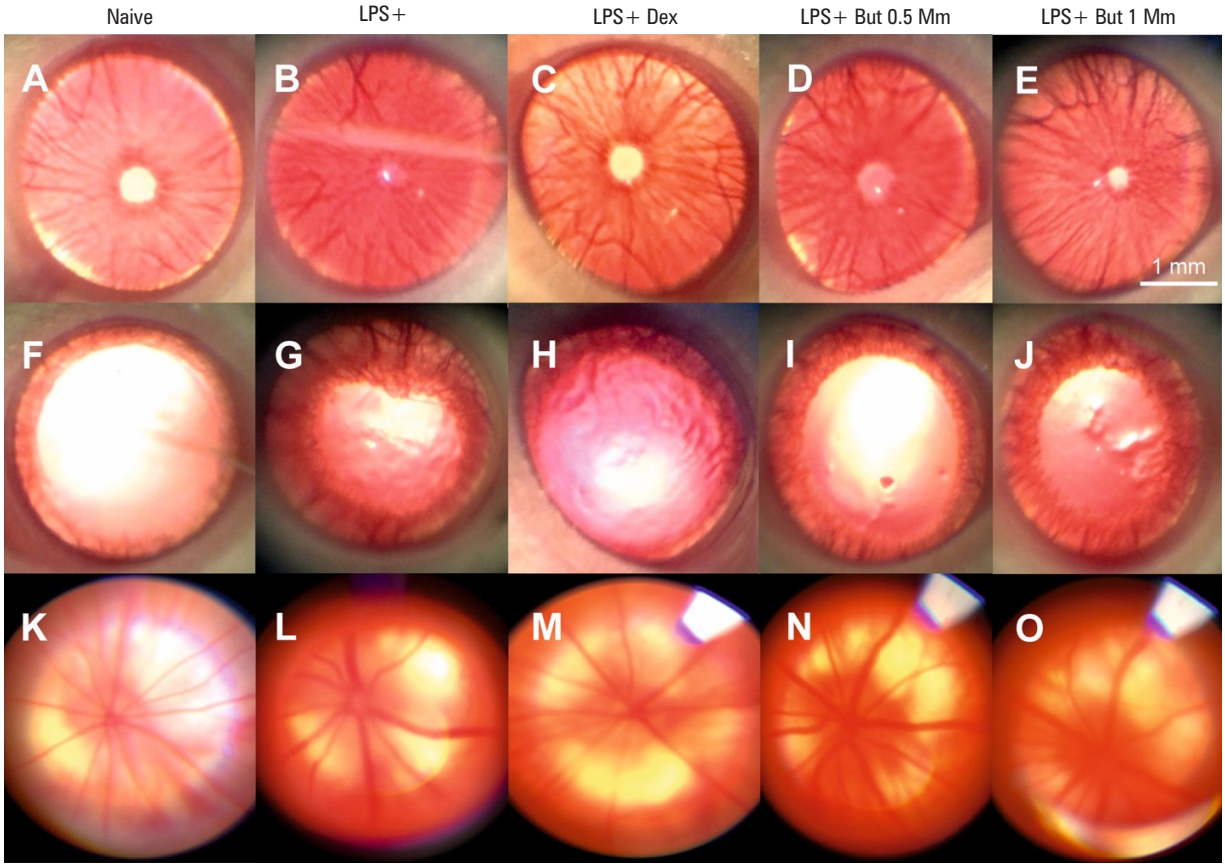

FIGURE 2. Clinical manifestations of ocular inflammation in rat eyes. Ocular inflammation was evaluated by direct ophthalmoscope examination 24 hours after lipopolysaccharides (LPS) injection. No inflammatory features were observed in control rats (Naive group, **A**, **F**, **K**). Miosis, hyperemia, decreased posterior pole visibility, and irregular pupil dilatation after 1% tropicamide administration were observed in LPS-treated rats (**B**, **G**, **L**). The clinical features of inflammation were reduced significantly by dexamethasone treatment (**C**, **H**, **M**) and moderately in LPS+ But 0.5 mM group, consisting of rats that received injections of LPS and eye drops containing sodium butyrate (sodium butyrate solution 0.5 mM) applied topically to the eye (**D**, **I**, **N**) but not in the group treated with 1 mM butyrate (**E**, **J**, **O**)

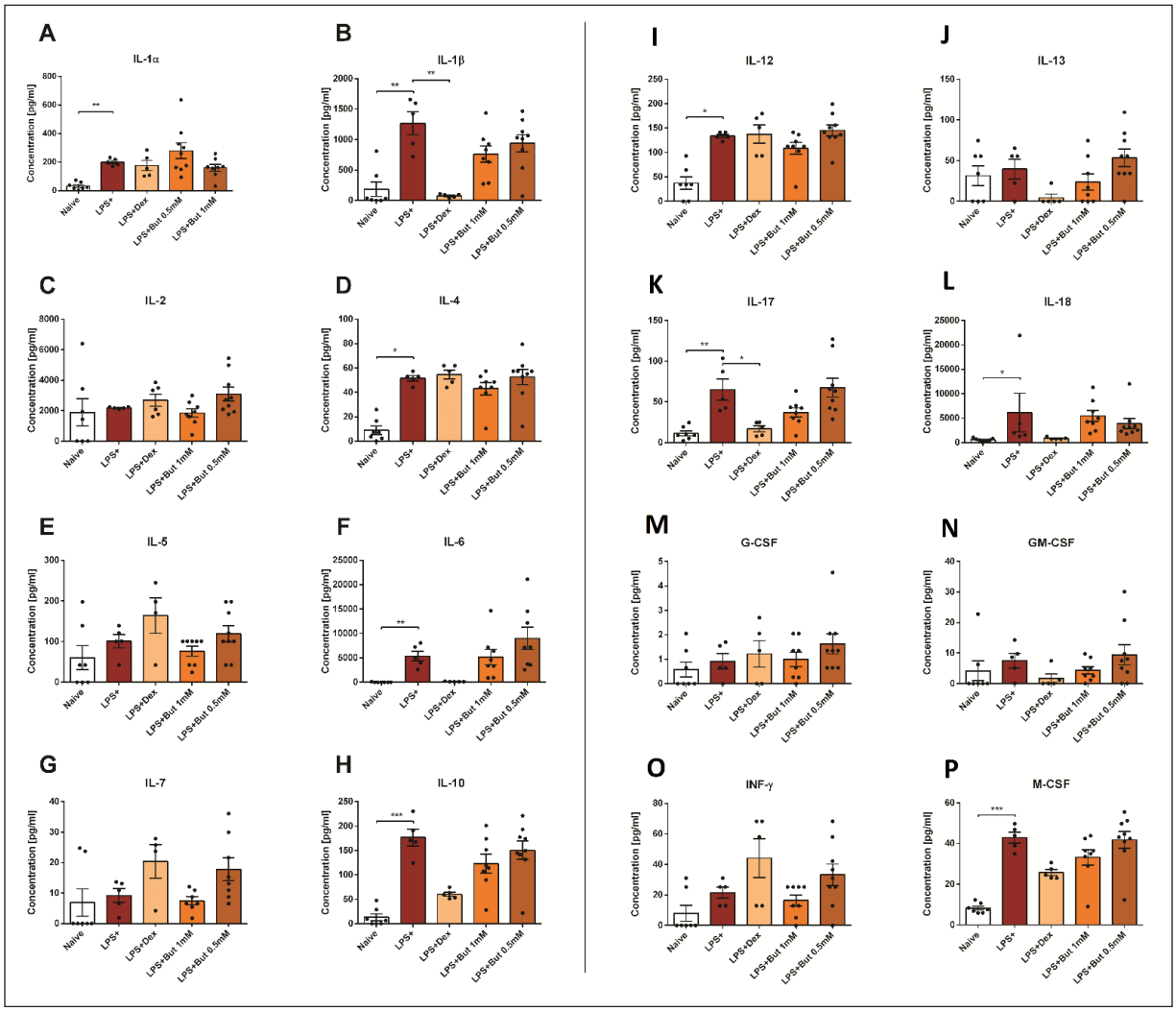

FIGURE 3. The levels of inflammatory factors were determined in the aqueous humor using Luminex. **A.** Interleukin (IL) 1 alpha (IL 1α); **B.** IL-1β; **C.** IL-2; **D.** IL-4; **E.** IL-5; **F.** IL-6; **G.** IL-7; **H.** IL-10; **I.** IL-12; **J.** IL-13; **K.** IL-17; **L.** IL-18; **M.** Granulocyte colony-stimulating factor (G-CSF); **N.** Granulocyte-macrophage colony-stimulating factor (GM-CSF); O. Interferone gamma (IFN-γ); **P.** macrophage colony-stimulating factor (M-CSF). *p < 0.05; **p < 0.01; ***p < 0.001

paring the LPS+ group with the Naive group, only the levels of tumor necrosis factor alpha (TNF-α) and granulocyte colony-stimulating factor (G-CSF) were not significantly elevated. Dexamethasone treatment reduced significantly levels of IL-1β (LPS+: 1268.00 ± 186.40 pg/mL *vs.* LPS+ Dex 78.04 ± 13.36 pg/mL, p < 0.01), and IL-17 (LPS+: 65.15 ± 12.78 pg/mL *vs.* LPS+ Dex: 16.90 ± 3.69 pg/mL, p < 0.05). There were no significant differences between the LPS+ group and the LPS+ But groups (Fig. 3).

Histopathology

Generally, histopathological alterations were subtle with hyperemia of iris/ciliary body or/and choroid as the only microscopic indicator of acute inflammatory reaction, without inflammatory infiltrate. Moreover, edema and stratification of the cornea were observed in some cases. In group 1, mild hyperemia was observed in 2 of 8 cases without corneal alteration. In Group 2, hyperemia was present in all cases. Moreover, corneal alterations were present in 2 of 8 cases. Similarly, in Group 3, hyperemia was present in all cases; corneal alterations were present in 5 of 8 cases. In Group 4, hyperemia was present in 4 of 8 cases; corneal alterations were not present. In Group 5, hyperemia was present in all cases, and corneal alterations were present in 2 of 8 cases. Taken together (hyperemia and corneal stratification), the total score was 3, 8, 15, 17, and 21, in Groups 1, 4, 2, 5, and 3, respectively (Fig. 4).

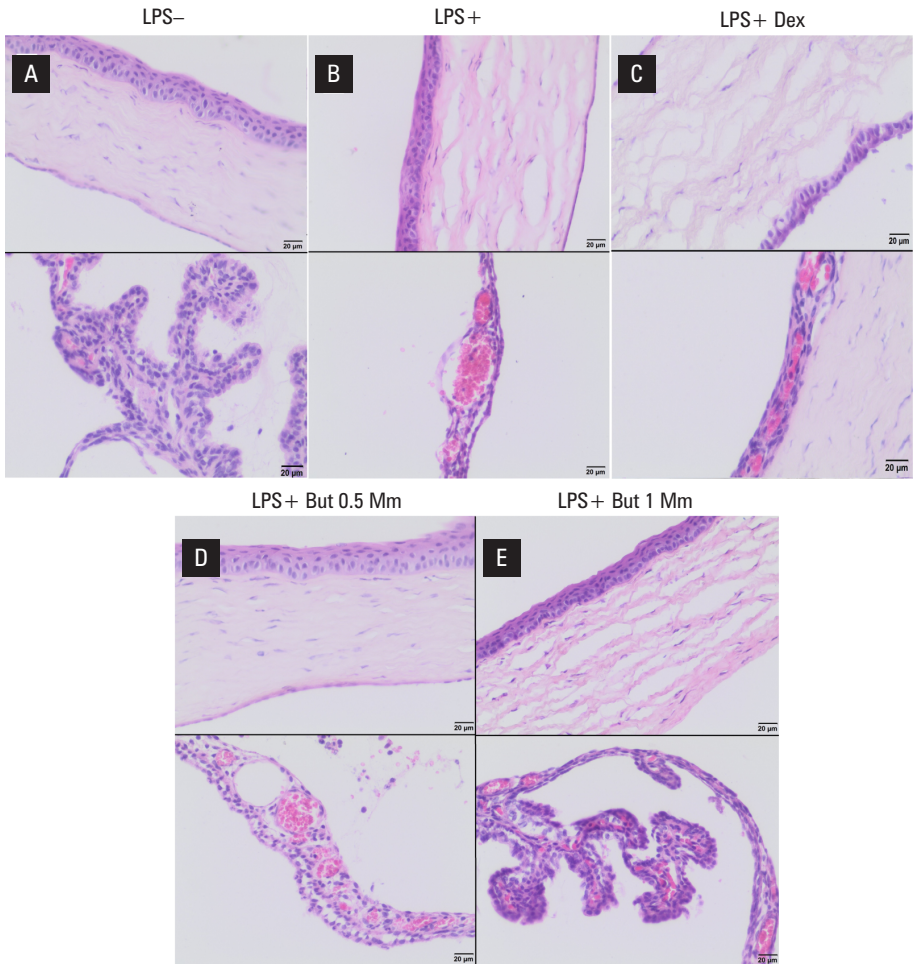

FIGURE 4. Representative histopathological views of cornea (upper figures) and uvea (bottom figures) in particular groups of rats; **A.** Naive group, consisting of rats that received eye drops containing saline (0.9% NaCl) applied topically to the eye — figures present normal structure of cornea and normal structure of anterior uvea; **B.** LPS+ group, consisting of rats that received injections of LPS and eye drops containing saline (0.9% NaCl) applied topically to the eye — figures present stratification of corneal stroma and hyperemia of anterior uvea; **C.** LPS+ Dex group, consisting of rats that received injections of LPS and eye drops containing dexamethasone phosphate (Dexafree 1 mg/ml, Laboratoires Théa, Clermont-Ferrand, France) applied topically to the eye — figures present stratification of corneal stroma and hyperemia of anterior uvea; **D.** LPS+ But 0.5 mM group, consisting of rats that received injections of LPS and eye drops containing sodium butyrate (sodium butyrate solution 0.5 mM) applied topically to the eye — figures present normal structure of cornea and hyperemia of anterior uvea; **E.** LPS+ But 1 mM group, consisting of rats that received injections of LPS and eye drops containing sodium butyrate (sodium butyrate solution 1 mM) applied topically to the eye — figures present stratification of corneal stroma and hyperemia of anterior uvea. Slides stained with hematoxylin and eosin and viewed under 200X magnification (bar 20 μm)

DISCUSSION

Increasing evidence suggests that microbial metabolites may have a protective influence on immune events in the eye [35–41]. Moreover, recent works indicate that butyrate is actively involved in several pathological processes, including autoimmunity, cancer, and neurological disorders [42–44]. To the best of our knowledge, the studies from our lab are the first to investigate the topical effects of BA. In this study, we have demonstrated the anti-inflammatory effect of sodium butyrate administered topically. We also compared the impact of corticosteroid treatment, which has been used frequently and proven effective in uveitis. To investigate the efficacy of topically applied butyrate, we used EIU, a popular model of human acute anterior uveitis that was previously successfully used for the efficacy evaluation of experimental drugs. Our results present a positive tendency in the efficacy of topical application of sodium butyrate aqueous solution in treating EIU in rats. In our study, experimental therapy was initiated two hours before the injection of LPS. We noted a significant decrease in the clinical inflammatory score and a tendency in protection against the development of ocular inflammation in inflammatory cytokines detection

and histopathology examination. Histopathological examination revealed only subtle microscopic changes attributable to acute inflammatory response; among them, only mild hyperemia of uveal structures was observed in some animals. The time from administration of LPS to animal euthanasia was possibly too short for developing a complete set of microscopic changes, including inflammatory infiltrate in our model. Features of hyperemia were observed in all animals from Groups 2, 3, and 5 suggesting an early acute inflammatory response in these cases. In Group 4, hyperemia was also present, but not in every case, suggesting a protective role of BA 0.5 mM, contrary to Group 5 (BA was applied in concentration 1 mM). BA effects could be linked with higher dose toxicity. However, since observed lesions were subtle, they should be treated cautiously. On the other hand, corneal edema and stratification were observed only in Groups 2, 3, and 5, and were not observed in the control group and Group 4 (BA administered at 0.5 mM). Therefore an application of sodium butyrate in lower concentrations can have a protective role in injury produced by the administration of LPS. Additionally, if taken together: hyperemia +corneal stratification, lesions were less common in the group of animals treated with BA in a lower concentration. Butyrate possesses anti-inflammatory properties in part due to inhibition of intereferone gamma (IFN-γ), TNF-α, IL-1β, IL-6, IL-8, IL-17, nuclear factor kappa beta (NF-κB), and upregulation of IL-10 and tumor growth factor beta (TGF-β). Some studies imply that the Th17 cell subtype is critical to the pathogenesis of autoimmune uveitis. Cytokines associated with these cells' differentiation, regulation, and effector functions are IL-6, IL-10, IL-17, IL-22, IL-23, and TNFα [45]. Numerous studies show that treatment with sodium butyrate (NaB) may play a role in attenuating ocular inflammatory response in animals with experimentally induced autoimmune uveitis. A decrease in inflammatory cytokine production may be linked with inhibition of the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase 1 (HO-1)/interleukin-6 receptor pathway [31]. However, the inhibitory changes induced by sodium butyrate in our study were small. Therefore, it is unclear whether sodium butyrate would be clinically effective in controlling these cytokines in topical administration. The possible butyrate mechanism of action seems complex and is not fully understood. The main butyrate action is to induce differentiation of regulatory T-cells by inhibiting histone deacetylase (HDAC) and activating the free fatty acids receptor 2 (FFAR2 receptor). Inhibiting apoptosis of resting and activated CD4+ and CD8+ T cells through HDAC1 inhibition may suppress the inflammation [46]. HDAC may also inhibit activation of NF-κB in various tissues, e.g., in human colonic epithelial cells. Consequently, early immune-inflammatory cytokines, e.g., IL-1b, TNF-α, IL-2, IL-6, IL-8, IL-12, would be suppressed [47]. Importantly, the expression of butyrate receptors (GPR41, GPR43, and GPR109B) and the transporter were detected in the conjunctiva and cornea. Moreover, treating cultured cells originating from ocular tissues with butyrate resulted in the upregulation of TNF-α, IL-1β, and nucleotide-binding domain leucine-rich repeat (NLR) and pyrin domain containing receptor 3 (NLRP3) [48]. Contrary to our expectations, this study did not find any significant differences in the cytokine levels after topical administration of BA. On the other side, some cytokine levels declined, with a clear tendency toward statistical significance [IL-1β, IL-10, IL-17, IL-18, monocyte chemoattractant protein (MCP-1)]. This suggests that a larger study, including more experimental animals, may confirm butyrate's protective effect on cytokine levels. In this study, we tested only two doses of butyrate, and a relatively short observation time was adopted. Over and above, not only rapid changes in cytokine levels play an important role in the inflammatory process, but also a balance between the effects of inflammatory cytokines is thought to determine the disease outcome, whether in the short term or long term [49].

CONCLUSIONS

Our findings suggest that sodium butyrate may alleviate the severity of intraocular inflammation in eyes with uveitis. There were some limitations of the current study. Effects of BA treatment were investigated only in the rat EIU model. Moreover, pharmacokinetic and functional analyses were not evaluated. At this moment, we cannot undoubtedly explain the result that a 0.5 mM dose of butyric acid is more effective than 1 mM; we suggest that further studies are needed to assess butyric acid as a potential treatment of EIU. Therefore, future studies are required to determine the optimal dosing protocol are needed to confirm the therapeutic potential and investigate the anti-inflammatory mechanisms in ocular tissue linked with a topical application of sodium butyrate.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Conception and design of the work: MO, KK-J, MF, MS. Acquisition, analysis, interpretation of the data; KK-J, MO, MS, MF, RS, PG, MR. Drafting the paper: KK-J, MO, MŚ, MF, RS, PG, MR. Funding acquisition: KK-J, MO. All the authors reviewed the manuscript and approved the final version.

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Institutional Review Board Statement

The experiments were performed in accordance with the Directive 2010/63 EU on the protection of animals used for scientific purposes, and guidelines of the Association for Research in Vision and Ophthalmology (ARVO). They were also approved by the II Local Ethics Committee in Warsaw (permission no. 653/2018 and WAW2/051/2020).

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