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Lowering propionic acid levels by regulating gut microbiota with ursodeoxycholic acid appears to regress autism symptoms: an animal study

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ABSTRACT

Aims: Patients with autism have altered gut microbiata, including higher frequency of bacteroidetes and clostridiales that produce of propionic acid (PPA) –a compound that is established as an autism-inducing agent. We hypothesized that lowering the PPA levels by regulating gut microbiata with ursodeoxycholic acid (UDCA) can regress the autism symptoms. The aim of this study is to examine the potential ameliorating effects of UDCA on a PPA-induced rat model of autism.

Methods: Thirty male Wistar albino rats were divided into three groups: controls, PPA-induced (5 days of intraperitoneal 250 mg/kg/day dosage) autism model receiving oral saline, and PPA-induced autism model receiving oral UDCA (100 mg/kg/day). Oral treatments were applied for 15 days. At the end of the 15th day, all rats underwent behavioral tests and MR spectroscopy. At the end of the study, all animals were sacrificed and brain tissue / blood samples were collected for histopathological and biochemical analyses.

Results: Sociability test, open field test and passive avoidance learning tests were impaired, similar to the autism behavioral pattern, in PPA recipients; however, results were closer to normal patterns in the PPA+UDCA group. Biochemically, MDA, TNF-alpha, IL-2, IL-17, NF-kB, lactate, NGF and NRF2 levels in brain tissues showed significant differences between controls and the PPA+Saline group, and between the PPA+Saline group and the PPA+UDCA group ($p< 0.05$, for all). Histopathology showed that PPA injection caused increased glial activity, neural body degeneration, decreased neural count and dysmorphic changes in hippocampal and cerebellar tissues ($p<0.01$, for all). UDCA treatment significantly ameliorated these changes.

Conclusion: UDCA administration has ameliorating effects on PPA-induced autism-like behavioral, biochemical and histopathological changes in rats.

Keywords: Autism, ursodeoxycholic acid, propionic acid, gut microbiota

INTRODUCTION

Autism spectrum disorder is a complex neurobehavioral disorder, with a large phenotypical spectrum, usually including impaired social interaction and communication, restricted and/or repetitive behavioral characteristics, altered cognitive patterns (learning, memory etc.) and sensory perception.[1] The fact that its prevalence has been increasing significantly in recent years has drawn more attention to the potential causes of this disease. While some studies suggest that autism develops on a multigenetic basis[2] many environmental factors, including metabolism-related compounds have been associated with this disorder.[3] Although proteomic analyses are still at the center of determining potential pathways associated with various diseases, advances in recent decades have demonstrated that

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other metabolites including low molecular weight compounds, lipids, and other compounds associated with gut microbiota may contribute to the pathological basis of various diseases.[4-6] Similar relationships have also been demonstrated in autism,[7-10] indicating the need for further research assessing the roles of these compounds in autism.

One such compound is propionic acid (PPA), which is a short-chain fatty acid that enters human metabolism both as an intermediate product of fatty acid metabolism and as a metabolic end product of bacteria in the gut.[11] In gut microbiata, bacteroidetes and clostridiales are largely responsible for the production of PPA, and autistic patients have been shown to have an altered microflora with relatively greater levels of these bacteria.[12] PPA can pass the brain-blood barrier and was shown to alter the release of some neurotransmitters.[1,13] High PPA levels were also shown to induce behavioral, electrophysiological, neuropathological and biochemical effects similar to those observed in autism.[2,3] Autism-like experimental rat models have been established by administration of PPA in various ways, including subcutaneous (500 mg/kg), intragastric gavage (250 mg/kg), intraperitoneal (250 mg/kg), and intracerebroventricular (4 μL, 0.26 M) administration.[14-16]

Although most autistic patients receive pharmacological treatment, there is no accepted medical treatment procedure that can significantly reduce the core symptoms of autism. Based on the potential effects of the gut microbiata on this disease,[2] studies assessing microbiome-mediated therapies have gained importance. One of the most important metabolites of gut microbiota are bile acids. Novel studies provide evidence about the role of bile acids in regulating gut microbiata as well as gastrointestinal functions and intestinal permeability. Furthermore, the pathogenic and therapeutic roles of bile acids and related sterols have been shown in several metabolic,[17,18] and neurodegenerative diseases,[4,19] have been shown. Ursodeoxycholic acid (UDCA) is a secondary bile acid which has therapeutic value in gallstones and inflammatory diseases including primary biliary cholangitis.[20]

Considering studies suggesting that increased PPA production due to alterations in gut microbiata may lead to autism-like symptoms,[21] we hypothesized that lowering PPA levels by regulating gut microbiota could regress symptoms. Therefore, the aim of this study was to examine the potential ameliorating effects of UDCA administration in a PPA-induced rat model of autism.

METHODS

Animals

In this study, thirty 10-12 week old male Wistar albino rats weighing 150-200 g were used. The experiments performed in this study were carried out according to the regulations put forth in the 'Guide for the Care and Use of Laboratory Animals' by the National Institutes of Health (U.S.A). The Animal Ethics Committee's approval for the study procedure was obtained (İstanbul Science University, Date: 2022, Decision No: 31210835). The rats used in the experiment were obtained from Experimental Animal Laboratory of Science University. Rats were fed ad libitum and housed in pairs in steel cages kept in a temperature-controlled environment (22±2°C) with automated 12-hour light/dark cycles.

Experimental Procedures

Thirty male Wistar rats were included in the study. Twenty rats were administered PPA intraperitoneally, at 250 mg/kg/day dosages for 5 days to induce an autism model. PPA-administered rats were randomly divided into 2 groups. Study groups were designed as follows: Group 1: Normal control (orally fed control, n=10); Group 2 (PPA+Saline, n=10): received PPA and were administered saline via oral gavage (1 ml/kg/day%0.9 NaCl), Group 3 (PPA+UDCA, n=10): received PPA and were administered UDCA via oral gavage (100 mg/kg/day; Ursactive capsule 250 mg, Pharmactive). In the literature, UDCA was mostly administered at 100 mg/kg/day, so we used the same dose according to the literature (22, 23). All treatments (saline, UDCA) were administered for 15 days, after which behavioral tests were performed. All behavioral experiments were conducted between 10:00 AM and 3:00 PM. After behavioral tests, animals underwent MR spectroscopy under ketamine anesthesia (50 mg/kg; Ketasol, Richterpharma AG, Austria).

At the end of the study, all animals were sacrificed (cervical dislocation) under high-dose ketamine/xylazine anesthesia (100 mg/kg / 50 mg/kg) (Xylazine Rompun, Bayer, Germany). Targeted brain tissues for histopathology and tissue biochemical analyses were dissected, prepared and stored appropriately. Blood samples for biochemical analyses were collected via cardiac puncture and plasma was obtained via centrifugation.

Behavioral Tests

Three-chamber sociability and social novelty test: Sociability test was performed as previously described with minor modifications (3, 24). Briefly, a Plexiglas cage (40 × 90 × 40 cm) was divided into three equal regions (40 × 30 × 40 cm). On the first day, the rats were allowed to habituate in the test cage for 5 min (pre-test session). Twenty-four hours later, a stranger rat (Stranger 1) was

placed inside a small plastic cage with mesh-like holes in one side chamber and an empty cage in the third chamber. Then, the test rat was placed in the center chamber and the time spent in each region by the test rat was recorded for 10 min. Presence in a chamber was defined when the rats head and two front paws entered the chamber. Time spent with the Stranger rat was calculated and reported as a percentage of total time. All chamber floors were cleaned between each test (70% alcohol) to remove traces of olfactory stimuli (Figure 1).

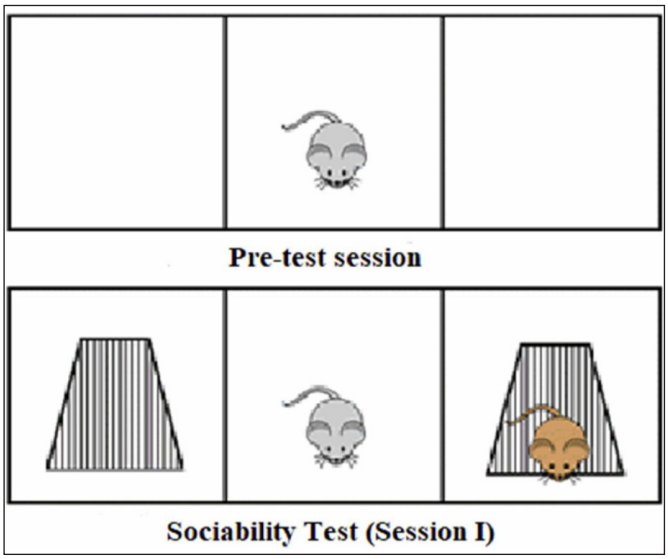

Figure 1. Three- chamber sociability and social novelty test

Open-field: The open-field (OF) paradigm assesses locomotion and exploration. Altered OF behavior is relatively simple to observe; however, concluding the reasons for the observed changes is a complex task. Generally, there are two factors that determine the behavior in this paradigm: The first is the natural exploratory drive of rodents to explore new environments (for food and shelter), while the second concerns precautionary avoidance of open and brightly lit spaces. The OF test is considered useful in determining stereotyped behavior, auto-grooming, and restriction of exploration in autism models.[24] The test was conducted in an open-aired box (50 × 50 × 40 cm). Rats were gently placed in the center of the box and allowed to explore the arena freely for 5 min. Then, each rat was observed for 5 min to evaluate its spontaneous activity level. The total number of ambulation events, defined as the number of floor divisions crossed with four paws, was recorded. The chamber was cleaned between each test (70% alcohol) to remove traces of olfactory stimuli.

Passive avoidance learning (PAL): The passive avoidance learning (PAL) test, as described previously,[26] is comprised of the assessment of fear-motivated avoidance. The healthy rat learns to refrain from stepping through a door leading into an apparently-safe dark chamber (preferred environment) due to prior experience of the fact that the door leads into a chamber in which an electrical shock is delivered. The PAL box was sized 20 × 20 × 20 cm and had dark and lighted chambers. After a 10-second habituation period in the lighted compartment, the guillotine door separating the light and dark chambers was opened. When a rat passed into the dark chamber, the door separating the light and dark compartments was closed. Then a 1.5-mA electric shock was delivered over 3 seconds, and the rat was subsequently removed from the dark chamber and returned to its cage (pre-test experience). Twenty-four hours later, the rats were placed into the lighted chamber of the PAL box again. The duration of time (or latency period) for the rat to travel from the lighted to the dark chamber was recorded, but a shock was not delivered. The latency period was recorded up to a maximum of 300s. The amount of time that the rat refrained from crossing into the dark chamber was recorded as PAL latency.

Magnetic Resonance Imaging Studies

Conventional MRI: All rats were examined using the 3.0-Tesla MRI/MRS scanner (Magnetom, Siemens Healthcare). Conventional MR sequences were as follows: sagittal fast spin-echo T1 weighted imaging for location; axial spin echo T1 weighted imaging [repetition time (TR)/echo time (TE) = 400/11 ms; field of view (FOV) = 60 mm; matrix = 256 × 256; number of excitation pulses = 2; bandwidth = 12.5 kHz; slice thickness = 1 mm; interslice gap = 0.2 mm; total number of scan slices = 16]; fast spin echo T2 weighted imaging (TR/TE = 4000/120 ms; other parameters were identical to the T1 weighted spin echo sequence); and fast fluid attenuation inversion recovery (TR/TE = 4000/120 ms; time of inversion = 2200 ms; matrix = 256 × 192; number of excitation pulses = 1; other parameters were identical to the T1 weighted spin-echo sequence).

MR spectroscopy: An automated multivoxel 2D chemical shift imaging sequence (TR = 1000 ms; TE = 35 ms; phase encoding x = 24; phase encoding y = 24; number of excitation pulses = 1) was used for 1H-MRS. FOV diameter was 60 mm, slice thickness was 4 mm and voxel size of the MRS was 1.87 × 1.87 × 4 mm^3. The volume of interest was chosen within the right striatum. The total duration for 2D 1H-MR spectrum acquisition was 580 seconds. Magnetom software (Siemans Healthcare) was used to process raw data stored in a workstation (Figure 2).

Hippocampus and Cerebellum Histopathology

The Cornu Ammonis (CA) 1 and CA 3 regions of hippocampus and cerebellum were chosen as the target areas to be examined for hippocampus damage. Briefly, following behavioral tests, animals were euthanized

and their brains removed and fixed for 3 days in 10% formaldehyde in 0.1 M phosphate-buffer saline (PBS). Then, they were moved into 30% sucrose and stored at 4°C until infiltration was complete. The brains were cut coronally on a sliding microtome (40 µm) and mounted on gelatinized glass slides. For glial fibrillary acidic protein (GFAP) immunohistochemistry, brain sections were incubated with H_2O_2 (10%) for 30 min to eliminate endogenous peroxidase activity and blocked with 10% normal goat serum (Invitrogen) for 1 h at room temperature. Subsequently, sections were incubated in primary antibodies against GFAP (Abcam, Inc., MA, US; 1/1000) for 24 h at 4°C. Antibody detection was performed with the Histostain-Plus Bulk kit (Invitrogen) against rabbit IgG, and 3,3' diaminobenzidine (DAB) was used to visualize the final product. All sections were washed in PBS and photographed with an Olympus C5050 digital camera mounted on an Olympus BX51 microscope. To calculate the GFAP immunostaining index, GFAP-positive cells were counted at 40X magnification in 3 to 4 randomized sections for each rat. All histopathological examinations were performed by the same investigator who was blinded to the study groups. This procedure was performed with an image analysis system (Image-Pro Express 1.4.5, Media Cybernetics, Inc. USA) in four sections per studied group. Cresyl violet staining to quantify the number of surviving neurons were performed in six sections with the same image analysis system.

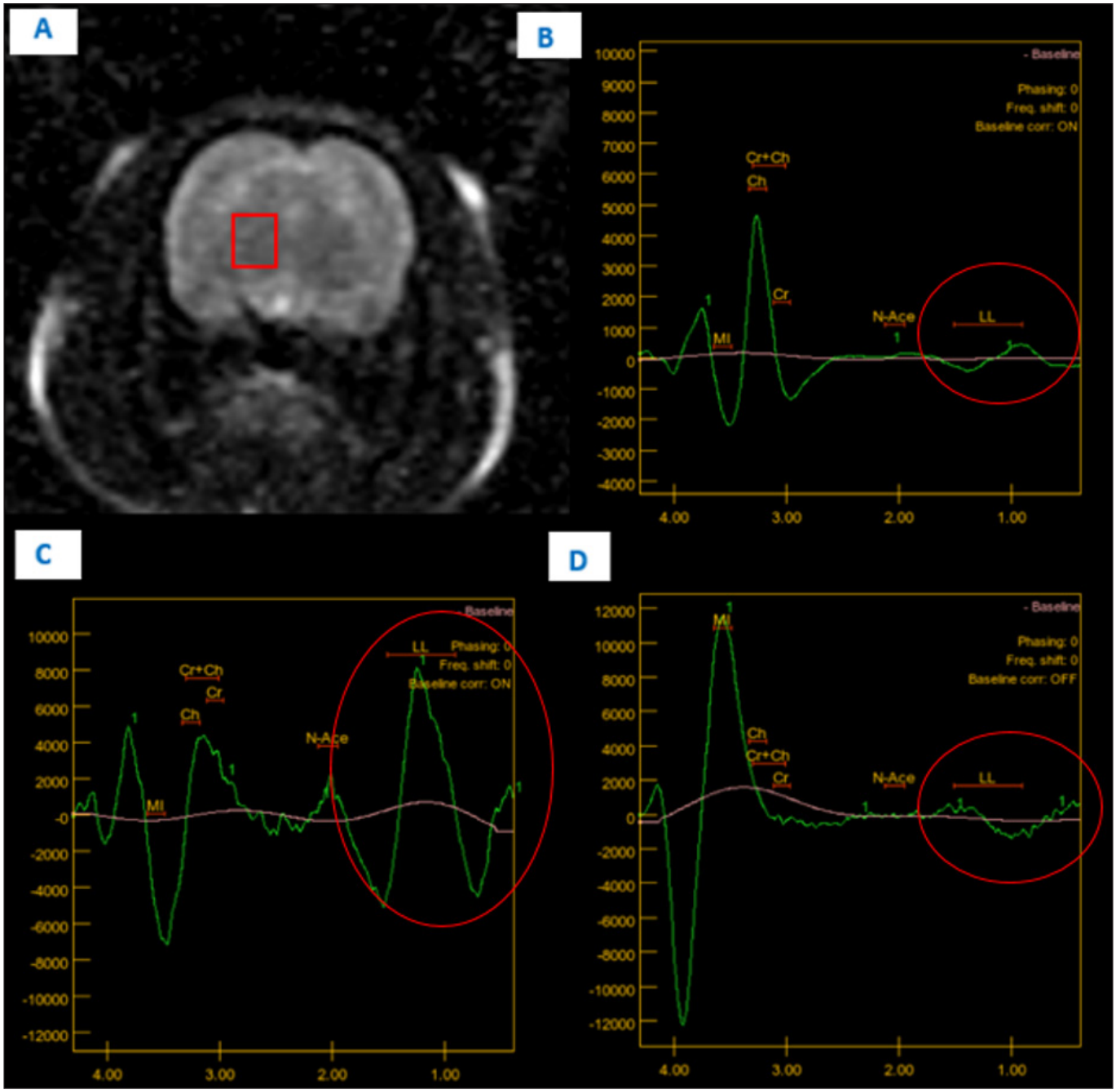

Figure 2: MR spectroscopy image (in the red circles, LL refers to lactate levels). A: MR spectroscopy chosen area (Red box), B: Normal Control Group male Rats, C: PPA and saline group male rats, D:PPA and UDCA group male rats.

Biochemical Analyses

After decapitation, brains were rapidly removed and stored at −20°C until biochemical analysis. For tissue analysis, whole cerebral tissues were homogenized with a glass homogenizer in 5X volumes of phosphate buffered saline (pH=7.4) and centrifuged at 5000×g for 15 min. The supernatant was then collected and total protein concentration in the brain homogenates was determined according to Bradford's method using bovine serum albumin as standard.[27]

The brain levels of TNF-alpha, Nerve Growth Factor (NGF), interleukin (IL)-17, IL-2, NF-kB, NRF2 and lactate in the supernatants were measured using commercially available rat-specific enzyme-linked immunosorbent assay (ELISA) kits. All samples from each animal were measured in duplicate according to manufacturer guidelines. A microplate reader was used for the measurement of absorbances (MultiscanGo, Thermo Fisher Scientific, NH, USA).

Measurement of brain and plasma lipid peroxidation (MDA): Lipid peroxidation was determined in the plasma and brain tissue samples by measuring malondialdehyde (MDA) levels as thiobarbituric acid reactive substances (TBARS). Briefly, trichloroacetic acid and TBARS reagents were added to brain tissue samples, then mixed and incubated at 100 °C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. MDA levels were calculated from the standard calibration curve (prepared using tetraethoxypropane) and were expressed as nmol/gr protein.

Statistical Analysis

Statistical evaluation was performed using SPSS version 15.0 for Windows (IBM, Armonk, NY, USA). The Shapiro-Wilk's W and Levene's tests were used to check normality of distribution and homogeneity of variance, respectively. Comparisons between groups were performed with the one-way ANOVA test. The results are presented as mean±standard error of the mean (SEM) values. p values of <0.05 were accepted to be statistically significant.

RESULTS

Behavioral tests revealed that, in the PPA+Saline group, time spent with stranger rat (%) and PAL latency were significantly shorter, and ambulation events were significantly fewer compared to the control and PPA+UDCA groups (p<0.05, for all) (Table 1).

Compared with the control and PPA+UDCA groups, the levels of brain MDA, plasma MDA, brain TNF-alpha, IL-2, IL17, NF-kB, and lactate were significantly higher, while NGF and NRF2 levels were significantly lower in the PPA+Saline group (p<0.05, for all) (Table 2).

Table 1. Results of behavioral tests

	Control (n=10)	PPA+Saline (n=10)	PPA+UDCA (n=10)
Open field test: number of ambulation	10.2±1.2	6.1±1.08*	8.3±1.3#
Passive avoidance learning (PAL) latency (Sec.)	268.8±20.1	109.1±41.5**	182.5±17.3#

Results were presented as mean±SEM. Statistical analyses were performed by one-way ANOVA. * p< 0.01, ** p<0.001 different from normal groups; # p<0.05, ## p<0.001 different from PPA and saline group.

Table 2. Biochemical parameters obtained from brain tissue and blood samples

Parameters	Control (n=10)	PPA+Saline (n=10)	PPA+UDCA (n=10)
Brain MDA level (nmol/gr protein)	48.8±2.3	195.3±12.9**	92.1±5.9#
Plasma MDA level (nmol/gr protein)	1.63±0.1	4.55±0.4*	3.76±0.2#
Brain TNF-alfa level (pg/mg protein)	14.2±4.4	117.5±9.3**	79.4±7.2#
Brain IL-2 level (pg/g protein)	2.1±0.2	274.1±10.1**	186.1±8.7#
Brain IL-17 level (pg/g protein)	240.9±19.6	598.1±14.5*	405.2±15.6#
Brain NF-KB level (pg/g protein)	19.2±2.1	201.7±14.3**	144.3±17.5#
NGF level (pg/mg protein)	88.1±5.9	39.5±2.3**	66.2±7.03#
NRF2 level (pg/mg protein)	98.2±10.6	45.8±8.4**	88.5±6.08##

Results were presented as mean±SEM. Statistical analyses were performed by one- way ANOVA. * p< 0.01, ** p<0.001 different from normal groups; # p<0.05, ## p<0.001 different from PPA and saline group.

Neuronal counts of CA1, CA3 and Purkinje cells were significantly lower in the PPA+Saline group compared to control and PPA+UDCA groups (p<0.05, for all). GFAP immunostaining index for CA1, CA3 and cerebellum, also MRI spectroscopy lactate values were all increased in the PPA+Saline group, compared to controls. On the other hand, the same indexes decreased for CA1, cerebellum and MRI lactate values (p<0.01, for all) in the PPA+UDCA group compared to PPA+Saline group; whereas CA3 values were similar (Table 3).

Table 3. Cell-count results of hippocampus and cerebellum and MR spectroscopy lactate value

Parameters	Control (n=10)	PPA+Saline (n=10)	PPA+UDCA (n=10)
Neronal Count CA1	87.8±4.4	54.6±2.1 **	66.7±3.3#
Neronal Count CA3	46.5±1.04	28.2±3.2 *	37.5±1.8 #
GFAP immunostaining index (CA1)	31.3±4.5	43.5±2.1 *	35.1±2.5 #
GFAP immunostaining index (CA3)	32.8± 2.1	43.7±2.9 *	38.5±3.3
Purkinje Count Cerebellum	20.8±1.4	10.7±2.8 *	16.5±0.9 #
GFAP immunostaining index (Cerebellum)	16.2±2.5	27.02±1.6*	19.7±0.8#

Results were presented as mean±SEM. Statistical analyses were performed by one- way ANOVA. * p< 0.01, ** p<0.001 different from normal groups; # p<0.05, ## p<0.001 different from PPA and saline group.

Figure 3 shows that PPA+Saline injection caused neural body degeneration and decreased neural count and dysmorphic changes in CA3 and CA1 cells; whereas UDCA treatment increased neural count and improved neural morphology.

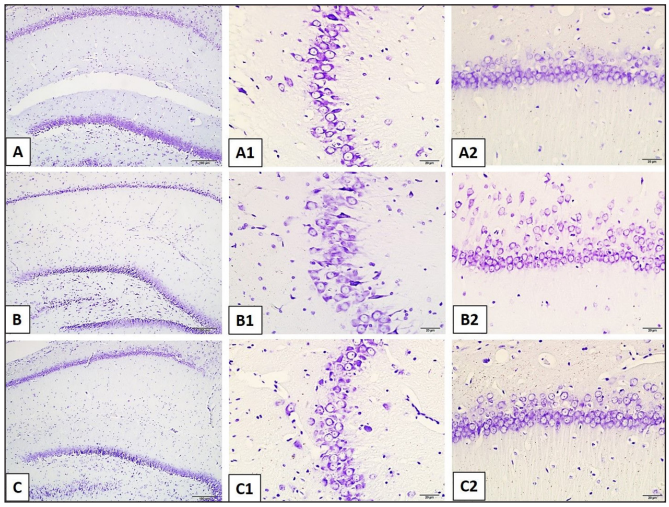

Figure 3: CA3 and CA1 regions of hippocampusCresyl violet stain4 and x 40 magnification. A-A1-A2: Normal Control Group Male Rats CA3 and CA1. Normal pyramidal neuron. B-B1-B2: PPA and saline group male rats have neural body degeneration & decreased neural count and dysmorphological changes CA3 and CA1. C-C1-C2: PPA and UDCA group male rats have increased neural count and improved neural morphology changes CA3 and CA1

Figure 4 shows that PPA+Saline injection caused increased glial activity in hippocampal CA3 and CA1 cells; whereas UDCA treatment reduced this effect of PPA.

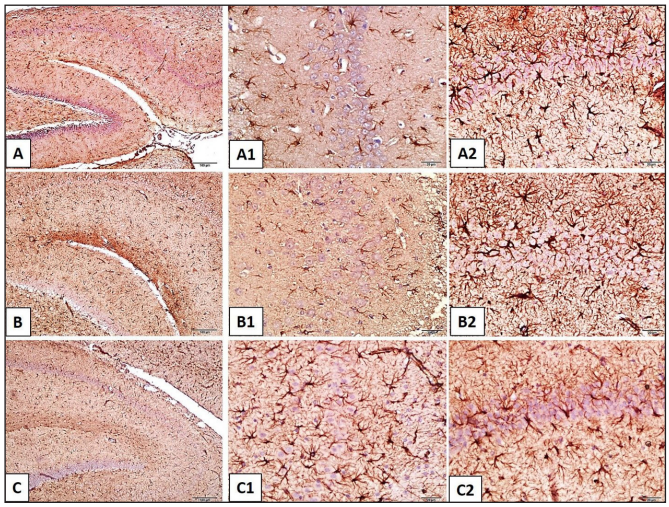

Figure 4: CA3 and CA1 of hippocampus x 40 magnification. Astrogliosis was characterized by GFAP immunostaining(Brown staining). A-A1-A2: Normal Control Group Male Rats CA3 and CA1, B-B1-B2: PPA and saline group male rats have increased glial activity CA3 and CA1.C-C1-C2: PPA and UDCA group male rats have have decreased glial activity CA3and CA1

Figure 5 shows that PPA+Saline injection decreased cell count and caused dysmorphic findings in Purkinje neurons. UDCA treatment increased cell count and improved neural morphological changes in Purkinje neurons.

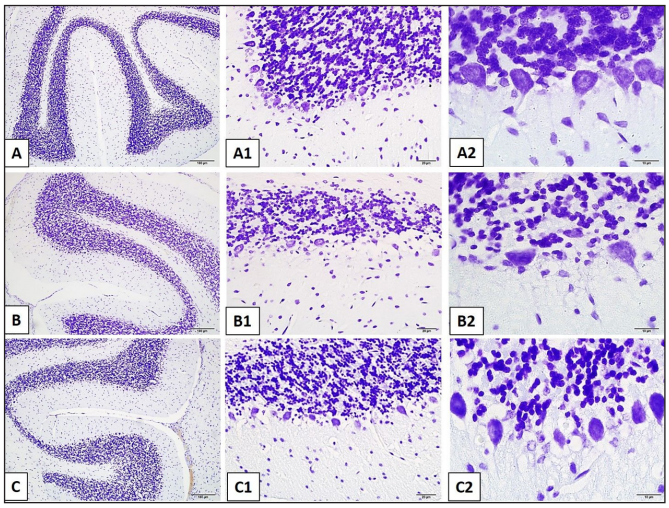

Figure 5: Cerebellum x 4, x 40, x 100 magnification. Cresyl violetstain.A-A2-A3, Normal Control Group Male Rats cerebellum, normal Purkinje Neuron, B-B1-B2: PPA and saline group male rats have decreased count and dysmorphological Purkinje Neuron. C-C1-C2: PPA and UDCA group male rats have increased count and improved neural morphological changes Purkinje Neuron.

Figure 6 shows that the PPA+Saline injected group had increased glial activity in the cerebellum and this activity was found to be decreased in PPA+UDCA recipients.

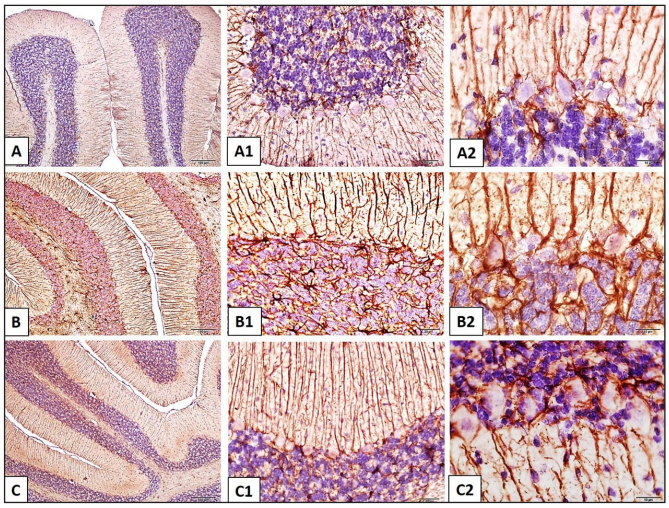

Figure 6: Cerebellum x 4, x 40, x 100 magnification. Astrogliosis was characterized by GFAP immunostaining (Brown staining). A-A1-A2, Normal Control Group Male Rats cerebellum, normal Purkinje Neuron, B-B1-B2: PPA and saline group male rats have increased glial activity cerebellum, C-C1-C2: PPA and UDCA group male rats have decreased glial activity cerebellum.

DISCUSSION

In this study, the results of UDCA treatment were evaluated in a rat model of PPA-induced autism, and it was determined that UDCA yielded ameliorating effects on PPA-induced effects such as disturbances in behavioral tests, biochemical alterations in the blood and brain tissues, and histopathological and MRI findings in brain tissues.

Intraperitoneal or intracerebroventricular injection of PPA (250-500 mg/kg doses) is a widely used

experimental model of autism in rats.[28] In the light of the data obtained in last decades, it has been found that the gut microbiota has significant effects via gut-brain axis on pathophysiology of several neurobehavioral disorders, including autism.[29] These findings are critical in the context of demonstrating the emerging roles of different metabolic compounds in various diseases. Some of these compounds can affect autism pathophysiology and studies support various hypotheses suggesting the functional contributions of lipids and sterol-related compounds in neurodegeneration and autism.[4,8,30] Considering that PPA is a fatty acid and is well-established as a compound that can induce autism-like findings in rats[14,15] the possible roles of altering gut microbiota and influencing the levels of related lipids may be crucial for not only determining treatment targets, but also identifying the pathophysiological contributions of these compounds in autism development.

It is known that most children with autism exhibit gastrointestinal symptoms, such as abdominal pain, vomiting, diarrhea, constipation, intestinal gas problems, and these symptoms are correlated with severity of behavioral or cognitive impairment.[31] When the feces content of children with autism were evaluated, it was seen that microbiota was altered and the levels of clostridiales and bacteroidetes were increased.[32] Some metabolites including PPA produced by these bacterial species can cross the blood-brain barrier and may have neurotoxic effects. Previous studies reported that PPA-treated rats display restricted locomotor activity and attention, impaired cognition, increased repetitive behaviors, and aggressive social behaviors,[15] as well as overexpression of pro-inflammatory cytokines,[33] and astrogliosis in brain tissues.[16] The results were similar in human studies. In autopsy and neuroimaging studies of patients, decrease in cerebellum Purkinje cells, glial activation and cytoplasmic volume change and neuronal cell loss are reported.[32] A novel study revealed that increased plasma levels of proinflammatory cytokines such as interleukins, TNF-alpha and TGF, as well as excessive cellular immune responses were evident in children with autism, and these inflammatory parameters were found to be associated with the severity of autism-related behavioral symptoms.[34] Crawley and colleagues report that the ideal animal model of autism should have at least three diagnostic symptoms unique to people with autism, including deficit in social interaction.[35] We observed that the autism animal model created in this study met this criterion.

The effects of current pharmacological treatment of autism is challenging. The treatment protocol is based on behavioral therapies and rehabilitation. Experimental treatment protocols, on the other hand, focus on different aspects including modulation of gut microbiota by using probiotics, prebiotics, fecal microbiota transplantation, antioxidants, and appropriate diet.[23] The common goal of these supportive treatments is to regulate the gut microbiota, to reduce the permeability of the intestinal barrier to toxins, and to reduce the oxidation and inflammation of brain tissue. Tomova et al.[36] investigated the effects of probiotic treatment on fecal microbiota and assessed plasma hormone and cytokine levels in children with autism. They found that probiotic supplementation altered the composition of gut microbiota and the level of plasma cytokines were decreased after treatment. Similarly, Varesio et al.[37] reported that ketogenic diet therapy had beneficial effects in improving behavioral symptoms in autism due to changes in gut microbiota. To the best of our knowledge, this is the first research assessing the therapeutic effects of UDCA in an experimental autism model. Our results are promising with the therapeutic effects of UDCA on all behavioral, biochemical, and histopathological changes induced by PPA, and support prior studies in terms of the importance of gut microbiota and its alteration which may influence the resultant levels of metabolic compounds. UDCA is a secondary bile acid that can modulate the composition of the gut microbiota via activation of the innate immune system.[38] Tang et al.[39] reported that a 6-month course of UDCA treatment ameliorated gut dysbiosis while not affecting microbial diversity in patients with primary biliary cholangitis.

Behavioral impairment is the most important diagnostic criteria of autism and is also observed in animal models of autism.[40] Correction of behavioral changes may be possible by eliminating the underlying neuroendocrine disorder(s). In this study, the molecular mechanism of the effect of UDCA on neurobehavioral characteristics has not been studied, but the most plausible mechanism is the influence on gut-brain axis through altered microbiota and microbiota-related metabolites. A recent study showed that mice devoid of gut microorganisms exhibited abnormal social behaviors, and restoration of the gut microbiota improved these disturbances.[41]

Increased TNF-alpha, IL-2, IL-17, NF-kB levels in brain tissue are signs of acute inflammation and activation of the innate immune response. Increased MDA and lactate levels and also decreased NGF and NRF2 levels are signs of acidosis and oxidative stress.[42] Today, there is strong evidence concerning the role of brain oxidative stress in the pathophysiology of autism.[43] Accordingly, we can theorize that UDCA treatment may ameliorate oxidative stress in the brain of autistic patients via regulation of gut microbiome. We preferred

the cerebellar and hippocampal regions of the brain to evaluate the histopathological changes, including cell loss, astrogliosis and neurodegeneration. These areas of brain are associated with motor and cognitive functions. Their damage can disturb the functions of connected areas, playing an important role on social behavior, motor, sensory, and memory functions.[44] Therefore, cell loss and neurodegeneration in these areas can explain behavioral changes. As expected, the observed improvement in behavioral disorders may be related with the indirect regenerative effects of UDCA treatment on neuronal tissues.

In some pathological processes, increased concentrations of specific metabolites, such as lactate, may be observed. Lactate is not found in normal brain tissue, and it most commonly arises/increases as a result of anaerobic glycolysis. In cerebral hypoxia, ischemia, seizures and some metabolic diseases, lactate increase can be detected in the brain by MR spectroscopy. Due to the interaction (spin-spin interaction/coupling) between the protons in the methyl and methine groups of lactate, it is observed as a doublet peak in MR spectroscopy and is distinguished from lipid/macromolecules by these two features.[45] Lactate level is thought to be an important biomarker in autism cases. In a study conducted on rats, it was found that while the brain lactate level was quite high in animals with pharmacologically-induced autism, lactate levels decreased in the autism group treated with finasteride.[46] In another study conducted in humans, it was found that lactate doublets increased by 13 times on MR spectroscopy in autistic patients when compared to a healthy control group.[47] In the present study, lactate elevation was also shown in MR spectroscopy in rats with PPA-induced autism, which supports the data in the literature. Consistent with the literature, MR spectroscopy also revealed a decrease in lactate level after UDCA administration in our study.

Limitations of the Study

The most important limitation of our study can be noted as the absence of investigating changes in the composition of gut microbiota with UDCA treatment. Therefore, with current data, the mechanism of UDCA-induced improvements in the autism model cannot be directly associated with PPA levels or changes in PPA levels due to the expected alteration of microbiota via UDCA. It is also possible that UDCA administration caused the observed effects through other mechanisms; however, since UDCA is a bile acid that was administered via oral gavage, overt systemic effects through other mechanisms are unlikely. Nonetheless, further studies are needed to explain the mechanism of action of UDCA on autism-related findings.

CONCLUSION

In Conclusion, we demonstrated that oral UDCA administration had ameliorating effects on PPA-induced autism-like behavioral, biochemical, and histopathological changes in rats. Our results suggest that UDCA administration may ultimately lead to neuroprotective and neuromodulator effects via regulating the gut microbiota. More animal studies examining the relationship between PPA-mediated autism and UDCA treatment could demonstrate the utility of this parameter in clinical practice in humans with autism in more detail.

ETHICAL DECLARATIONS

Ethics Committee Approval: İstanbul Science University Medical Ethics Committee approved all experiments and all procedures and processes in this study. Our study included animal subjects. We considered all ethical, scientific, and legal values. Animals were looked after properly and used in minimum numbers (İstanbul Science University, Date: 2022, Decision No: 31210835).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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