Adverse Effects of Acute Baricitinib Exposure on the JAK/STAT Signaling Pathway and Neural Behavior in Adult Japanese Medaka

Jiao DONG
Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University : Master's Program

Kun CHEN
Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University

Xuchun QIU
Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University

Yanhong SHI
Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University

他

https://doi.org/10.5109/7388809

出版情報：九州大学大学院農学研究院紀要. 70 (2), pp.107-113, 2025. 九州大学大学院農学研究院
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **70** (2), 107–113 (2025)

Adverse Effects of Acute Baricitinib Exposure on the JAK/STAT Signaling Pathway and Neural Behavior in Adult Japanese Medaka

Jiao DONG[1], Kun CHEN[2]*, Xuchun QIU[2], Yanhong SHI[2], Yuki TAKAI[3], Yohei SHIMASAKI[3] and Yuji OSHIMA[3,4]

Laboratory of Marine Environmental Science, Division of Animal & Marine Biosciences, Department of Bioresource Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 819–0395, Japan

(*Received May 5, 2025 and accepted May 16, 2025*)

Baricitinib (BCT) is a Janus kinase (JAK) inhibition medicine, which has been used in the treatment of several self–limiting immune diseases, including the pneumonia caused by COVID–19, rheumatoid arthritis, and alopecia areata *etc*. Researches show that BCT has significant impact on the mammalian immune system. However, the effects of BCT on aquatic organisms remain unclear. This study utilized the Japanese medaka (*Oryzias latipes*) to investigate the effects on the JAK/STAT signaling pathway and the neurobehavioral toxicity alterations induced by a 96–h Baricitinib exposure at the concentrations of 0, 0.05, 0.5, and 5 mg/L. After exposure, behavioral tests were conducted, and tissue samples of spleens and brains were collected and tested to evaluate the alterations of protein levels. The results showed that there were significant alterations in key proteins of the JAK/STAT pathway (JAK1, STAT1, STAT3, SOCS3, and SOCS8) in the spleen of medaka, with distinct sex–specific patterns between male and female individuals. The serotonin turnover rate (5–HIAA/5–HT) in the brain of male medaka was significantly reduced in the high BCT concentration group (5 mg/L) after BCT exposure. However, for female medaka, it did not change significantly in any exposure groups. Furthermore, results of behavioral assessment showed that BCT exposure significantly affected the behavioral activities and exploratory behavior of Japanese medaka. In general, the results suggested that BCT has significant effects the JAK/STAT signaling pathway as it has been to the mammalian animals. The changes of behavioral traits and brain neurotransmitter levels indicated a potential neurobehavioral influence of BCT on fish. People should pay more attention on the disposal of immunomodulatory medicines regarding to their potential adverse impacts on aquatic organisms.

Key words: Baricitinib, Japanese medaka, JAK/STAT signaling pathway, Neural behavior

INTRODUCTION

There is a considerable population suffering from various autoimmune diseases all over the world (approximately 7.6 – 9.4% of the world's population) (Conrad *et al*., 2023). Most autoimmune diseases are incurable and persistent, leading to a substantial demand for long–term therapeutic and pharmaceutical treatment, causing large amount of medicine usage, excretion and disposal. JAK/STAT signaling pathway, as one of the most important regulatory pathways in immunological functions, is a major biologics target in the immunomodulatory drug development pipelines.

Baricitinib (BCT) is a JAK kinase inhibitor that was initially developed as a drug for the treatment of various self–limiting immune system diseases, like rheumatoid arthritis, atopic dermatitis, lupus erythematosus and pneumonia caused by coronavirus (Assadiasl *et al*., 2021; Fetter *et al*., 2020; Mullard, 2018; Zhang *et al*., 2022). During the COVID–19 pandemic, BCT has demonstrated marked therapeutic efficacy in the treatment of moderate to severe pneumonia caused by infection of COVID–19 (Assadiasl *et al*., 2021).

In mammalian, the mechanism of action of Baricitinib is to be a competitive adenosine triphosphate kinase inhibitor that blocks the signaling of certain cytokines by preventing the transfer of phosphate from ATP to JAK, thereby inhibiting activated inflammatory pathways (Kubo *et al*., 2018; Zhang *et al*., 2022). Prior research by Fridman *et al*. (2010) reported that BCT administered at a dose of 10 ml/kg in a rat model of adjuvant arthritis produced a partial inhibitory effect on JAK1 and JAK2 and resulted in apparent anti–inflammatory activity. Research by Makabe *et al*. (2024) demonstrated that Baricitinib inhibits JAK/STAT3 pathway activity and colony stimulating factor 1 (CSF1) expression in neuronal cells, thereby improving inflammatory and neuropathic pain. Furthermore, research by Chen *et al*. (2024) showed that Baricitinib ameliorates moderate to severe atopic dermatitis by inhibiting the JAK/STAT signaling pathway of CD4+ T cells, reducing the activities of downstream pathways, and therefore suppressing the T lymphocyte–mediated inflammation. The aforementioned studies have demonstrated that BCT can regulate the JAK/STAT signaling pathway by inhibiting JAK1 and JAK2, thereby effectively intervening in immune inflammatory responses and disease progression

[1] Master's Program, Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, *Jiangsu University*, Zhenjiang, Jiangsu 212013, China.
[2] Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, *Jiangsu University*, Zhenjiang, Jiangsu 212013, China.
[3] Laboratory of Marine Environmental Science, Faculty of Agriculture, *Kyushu University*, Fukuoka 812–8581, Japan
[4] Institute of Nature and Environmental Technology, *Kanazawa University*, Kanazawa 920–1192, Japan
* Corresponding author (E-mail: chenkun_kunkun@ujs.edu.cn)

in mammalian. The intervention of BCT can significantly reduce the level of inflammation, and improve the balance of the immune system.

With the expanding usage of BCT, the environmental ecotoxicity issues it may pose have also attracted people's attention. The pharmacokinetic and clinical pharmacology test results indicated that the elimination half–life of BCT in the human body is approximately 12–13 hours (Mullard, 2018). According to the 'Safety Data Sheet' of the medicine, Olumiant (Baricitinib), provided by the producer Eli Lilly and Company (LLY), approximately 90% of BCT is excreted in its original chemical structure state through urine (69%) and feces (15%) from the body (LLY). This suggests that BCT will be released into the environment through body excretion and enter the natural water system in its original chemical structure maintaining its bioactivity. In addition, improper disposal of unused medications and the abandon during manufacturing can also lead to water or soil contamination. In consideration of environmental risks, Khan *et al*. (2023) employed quantitative structure–activity relationship (QSAR) models and expert systems to make predictions. The results indicated that BCT is moderately to highly toxic to aquatic species, with a minimum predicted toxicity value of approximately 1 mg/L. Therefore, in the near future, BCT may pose ecotoxicological risks to the aquatic environment.

The Japanese medaka (*Oryzias latipes*) is a suitable and commonly used ecotoxicological model organism to evaluate the toxicological effects of chemical substances (OECD, 2012; Shima and Mitani, 2004). The physiological parameters and behavioral traits of medaka have been well studied, and proved to be appropriate to assess the toxicity of various pollutants (Matsumoto *et al*., 2009). Therefore, in the present experiment, Japanese medaka (*O. latipes*) was employed to investigate the toxicity effects of acute 96–hour exposure to different concentrations of BCT (0, 0.05, 0.5, 5 mg/L) on the JAK/STAT signaling pathway and neural behavior of fish. The study is aiming to provide useful result for assessing the ecological risk of BCT to fish in the aquatic environment.

MATERIALS AND METHODS

Chemicals and reagents

Baricitinib (CAS No. 1187594–09–7) was purchased from Shanghai Send Pharm Co., Ltd. (Shanghai, China). Dimethyl sulfoxide (DMSO) and other reagents were purchased from Sinopharm Chemical Reagent Co., LTD. (Shanghai, China).

Organisms

Six–month–old Japanese medaka (*Oryzias latipes*) were obtained from the broodstock maintained in the Fish Experimental Platform of Jiangsu University (Zhenjiang, Jiangsu Province, China). Male and female Japanese medaka were cultured in rectangular glass aquariums containing dechlorinated tap water (salinity of 1‰; oxygen = 5.2 ± 0.8 mg/L; light : dark cycle=14L : 10D h). The fish were fed with *Artemia nauplii* (< 24 h after hatching) twice per day. The water temperature was maintained at 25 ± 1˚C, and the culturing water was replaced once every three days.

Experimental design and biological sampling

According to the 'Safety Data Sheet' of Olumiant (Baricitinib), the 96–h LC_{50} of BCT for Fathead minnow (*Pimephales promelas*) was greater than 18 mg/L (LLY). We predicted that the 96–h LC_{50} of BCT for Japanese medaka was 25.57 mg/L using Interspecies Correlation Estimation (ICE). Therefore, the maximum exposure concentration of BCT was set to be 1/5 of the LC_{50} values in the present study. The final exposure concentrations of BCT were 0, 0.05, 0.5, and 5 mg/L in the medaka culturing water. The control group (0 mg/L) was assigned with the same concentration of solvent dimethyl sulfoxide (DMSO).

For the exposure experiment, healthy fish were randomly and equitably distributed in 3–L glass cylindrical tanks containing 2.5 L of an exposure solution. Each tank contained 4 fish, and each concentration included 3 tanks as replicates. Medaka of different sex was exposed to BCT separately. This test was conducted for 96–hour under the identical conditions as culturing, except for fasting during the test. Exposure solutions were renewed every day. At the end of the 96–hour exposure, three fish were randomly selected from each glass cylindrical tank for the behavioral test. Subsequently, all the fish were placed into an ice water bath (0 – 4°C, 10 min) for euthanasia, measured for body weight and length, and dissected in order to get the brain, liver, and spleen. Fish tissues were frozen in liquid nitrogen for 5 minutes, and stored at –80°C.

Behavioral assay

After the exposure experiment, three Japanese medaka in each tank (as one group) were randomly selected and positioned in an aquarium (20×9×10 cm) containing 1 L of culturing water. After a 10 minutes acclimatization period, fish locomotor behavior was tracked for 15 minutes using the DanioVision system (Noldus, Wageningen, Netherlands), following the method described by Chen *et al*. (2021). The video data was analyzed using EthoVision XT software (Version 11.5; Noldus). Behavioral traits, the average swimming velocity (ASV), duration of exploratory behavior, and frequency of active mobility (FAM), were used to represent traits of locomotion (Wang *et al*., 2024).

Biochemical assays

For the biochemical assays, an appropriate amount of tissue was weighed, homogenized with physiological saline, and then centrifuged (6000 ×g, 15 min) to obtain the supernatant. ELISA kits (Keshun Biological Technology Co., Ltd., Shanghai, China) were used to measure the levels of 5–HT and 5–HIAA in the brain supernatant, following the manufacturer's instructions, as well as the levels of Janus kinase 1 (JAK1), signal transducer and activator of transcription 1 (STAT1), sig-

nal transducer and activator of transcription 3 (STAT3), suppressor of cytokine signaling 3 (SOCS3), and suppressor of cytokine signaling 8 (SOCS8) in the spleen supernatant. Furthermore, determination of the protein concentration was carried out using a BCA Kit (Beyotime Biotechnology Co., Ltd., Shanghai, China), and the units of each parameter were normalized by the total protein amount (/mgp).

Statistical analysis

The normality of the data and the homogeneity of the variances were determined using Levene's and Shapiro–Wilk's tests, respectively. The generalized linear model (GzLM) was performed to analyze the combined effects of sex and BCT concentration on levels of key proteins in the spleen, as well as on brain neurotransmitter levels and behavioral traits. Subsequently, a simple effects analysis was conducted to ascertain the statistical differences between exposure and control groups across sex. All statistical analyses were performed using SPSS 11.0 J (SPSS Japan, Tokyo, Japan).

RESULTS

During the exposure experiment, all medaka fish survived. The average body lengths (mean ± SE) of male and female Japanese medaka were 1.86 ± 0.22 cm and 2.13 ± 0.34 cm, respectively. The average body weights (mean ± SE) of male and female Japanese medaka were 102.59 ± 0.01 mg and 91.38 ± 0.01 mg, respectively.

Variation in key proteins of the JAK/STAT pathway in the spleen

As shown in Fig. 1, the effects of BCT exposure on the levels of the key proteins in the JAK/STAT signaling pathway were more pronounced in females than in males. In the spleen of female medaka, the levels of all tested proteins in JAK/STAT signaling pathway exhibited significant increases across all concentrations following 96–h acute BCT exposure. On the other hand, for male medaka, 96–h exposure to BCT significantly reduced the levels of JAK1 (0.05 and 5 mg/L, Fig. 1A), STAT1 (5 mg/L, Fig. 1B), and SOCS3 (0.05, 5 mg/L, Fig. 1D) in spleen, meanwhile the level of SOCS8 (0.05 and 0.5 mg/L, Fig. 1E) significantly increased. However, STAT3 level did not significantly affected by the acute exposure of BCT.

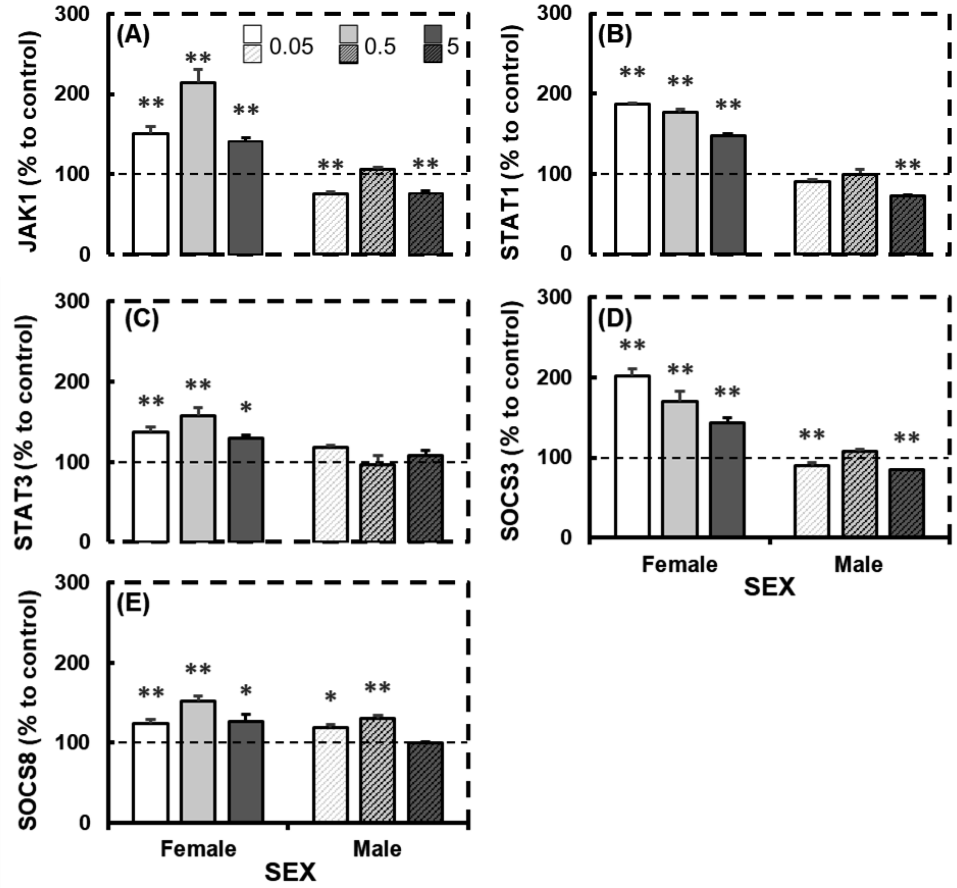

Fig. 1. Levels of key proteins in the JAK/STAT signaling pathway in the spleen of Japanese medaka (*O. latipes*) after 96–hour acute BCT exposure.
Data (mean ± SEM, n = 3) are presented as percentages relative to the control. Asterisks indicate significant differences between the exposure group and the control group (** $p < 0.05$, ** $p < 0.01$). JAK: Janus Kinase; STAT: Signal Transducer and Activator of Transcription; SOCS: Suppressor of Cytokine Signaling.

Variation in behavioral traits and the neurotransmitter levels in brain

In Figure 2, graph A, B and C show the variations of behavioral traits of Japanese medaka after the 96–h BCT exposure in the concentrations of 0.05, 0.5 and 5 mg/L. For female medaka, the frequency of active mobility (FAM) showed significant decrease in 0.5 and 5 mg/L groups, while average swimming velocity (ASV) and duration of exploratory behavior (DEB) were not significantly affected by the exposure to BCT. The DEB of male medaka decreased in the 0.5 mg/L exposure group. And in the concentration groups of 0.05 and 5 mg/L, male ones showed reduced FAM.

Fig. 2C and 2D show the expression changes of serotonin (5–HT), which is a neurotransmitter that regulates mood and sleep, and its primary metabolite, 5– hydroxyindoleacetic acid (5–HIAA). Fig. 2E shows the level changes of serotonin turnover (5–HIAA/5–HT), which reflects the dynamic balance between the synthesis and degradation of serotonin. For female medaka, level of 5–HT in brain increased in the 0.5 mg/L 96–h BCT exposure group, while the level of 5–HIAA increased in 5 mg/L exposure group. However, there was no significant change on serotonin turnover rate in any of the exposure groups. On the other hand, after being exposed to BCT for 96 hours, the levels of 5–HT in all concentration groups and 5–HIAA in both 0.05 and 0.5 mg/L groups showed significant elevation. Meanwhile, there showed a significant decrease of 5–HIAA/5–HT in the 5 mg/L 96–h BCT exposure group.

Combined effect of sex and exposure concentration of BCT

Table 1 shows the results of using GzLM analysis to examining the effects of sex differences, BCT concentration gradients, and their interaction on the expression level of key proteins of JAK/STAT signaling pathway in the spleen, behavioral traits and neurotransmitters in the brain of Japanese medaka.

The expression levels of all protein tested in the JAK/STAT signaling pathway expressed significant influence by sex difference, BCT concentration, and their interactions. Considering the results shown in Fig. 1, levels of the key proteins in the spleen of female medaka were more significantly affected after 96–h BCT exposure.

Results of the GzLM analysis also showed that sex difference had significant effect on the behavioral trait

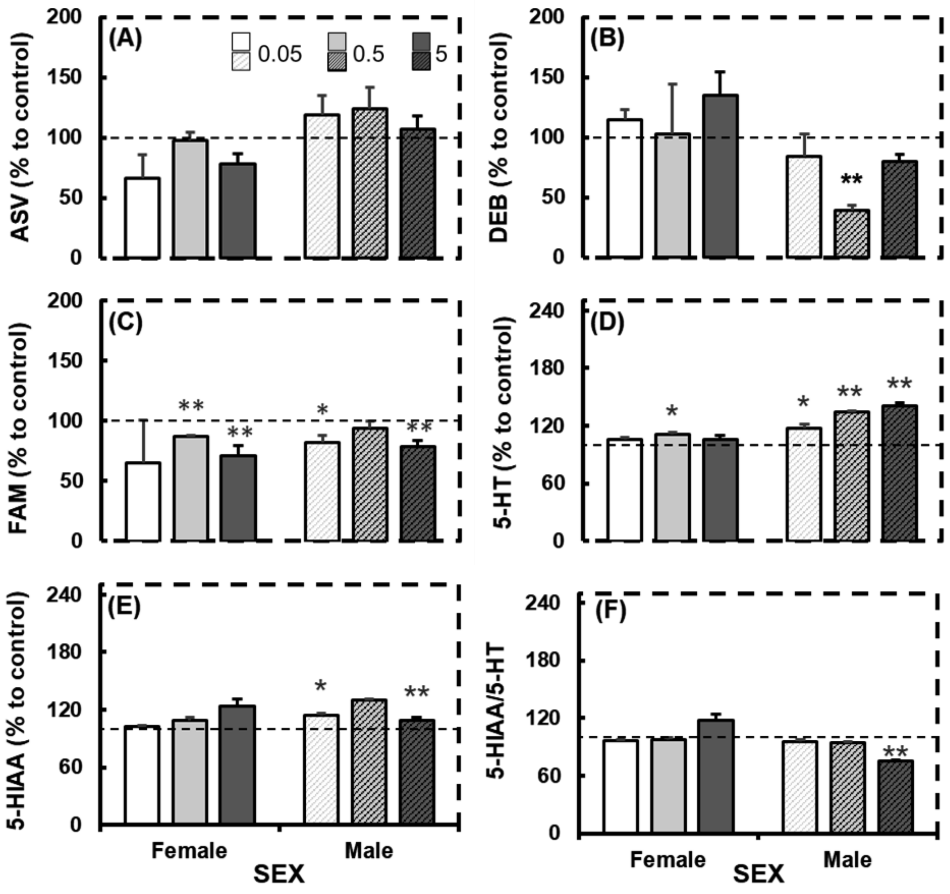

Fig. 2. Changes in behavioral characteristic parameters (A, B, C) and neurotransmitter levels in the brain (D, E, F) of Japanese medaka (*O. latipes*).
Data (mean ± SEM, n = 3) are presented as percentages relative to the control. Asterisks indicate significant differences between the exposure group and the control group (** $p < 0.05$, ** $p < 0.01$). ASV: Average swimming velocity; DEB: Duration of exploratory behavior; FAM: Frequency of active mobility; 5–HT: 5–Hydroxytryptamine; 5–HIAA: 5–Hydroxyindoleacetic Acid.

Table 1. Results of the statistical significance (GzLM) of the effects of different concentrations of Baricitinib, sex-difference, and their interaction on the key protein levels of JAK/STAT signaling pathway, behavioral traits, and neurotransmitters of adult Japanese medaka (*O. latipes*)

Statistical variables (df)	JAK/STAT pathway key proteins					Behavioral traits			Neurotransmitters		
	JAK1	STAT1	STAT3	SOCS3	SOCS8	ASV	DEB	FAM	5-HT	5-HIAA	5-HIAA/5-HT
BCT (df = 3)	42.75**	193.57**	9.87**	72.98**	249.25**	2.24	16.67**	18.90**	48.98**	44.43**	0.63
SEX (df = 1)	109.81**	244.12**	170.36**	28.94**	130.46**	103.89**	3.18	234.90**	23.39**	0.41	6.30*
BCT×SEX (df = 1)	61.16**	186.80**	9.88*	121.59**	35.28**	8.48*	14.20*	14.41**	14.52**	23.36**	25.93**

ASV and FAM, but little effect on the DEB. However, the correlation of sex and BCT concentration could affect the behavior traits of all three parameters.

In the brain of Japanese medaka, level of 5–HT was significantly affected by the BCT concentration, sex, and their interaction. The level of 5–HIAA in the brain was significantly affected by the BCT concentration and its interaction with sex. And the serotonin turnover (5–HIAA/5–HT) was significantly affected by sex and its interaction with BCT concentration.

DISCUSSION

Baricitinib regulates the immune environment of an organism by affecting the activity of the immune system through inhibition of the JAK/STAT pathway. The JAK/STAT signaling pathway is a cornerstone of cellular signaling and can regulate physiological processes such as inflammation and stress (Sarapultsev *et al*., 2023), thereby maintaining immune homeostasis. Extracellular signals induce autophosphorylation and activation of intracellular JAK kinases, which subsequently lead to phosphorylation and translocation of STATs into the nucleus, where they bind to the corresponding regulatory sequence to regulate transcription of target genes (Rawlings *et al*., 2004). Therefore, inhibition of normal JAK function might result in immunosuppression. BCT, as a JAK inhibitor, can inhibit the catalytic activity of JAK kinase, blocking the inflammatory cascade and reducing inflammation (Satarker *et al*., 2021). The uptake of BCT would be likely to suppress the immune system and impact normal immunity of living organisms, and cause side effects (Markham, 2017; Xin *et al*., 2020).

Previous studies have reported the toxicity effects of BCT in mammals. However, the potential toxicity of BCT to aquatic organisms remains unclear. The results of present study indicated that acute BCT exposure affected the expression level of some key proteins in the JAK/STAT signaling pathway in the spleen of Japanese medaka, which might affect the regulation of immune system function. Furthermore, the significant changes in behavioral parameters and neurotransmitters in the brain of medaka indicated that BCT may exhibit neurobehavioral toxicity in Japanese medaka.

JAK1, STAT1, STAT3, SOCS3, and SCOS8 are key proteins in the JAK/STAT signaling pathway. The alteration of their expression level could be critical to immune function. JAK1 is a Janus kinase that is the target site of BCT action and is mainly responsible for immune system development and immune regulation in the JAK family (Liau *et al*., 2019; Xue *et al*., 2023). STAT1 and STAT3 are signaling molecules downstream of JAK and play important roles in the pathogenesis of autoimmune disorders (Xue *et al*., 2023). In the present study, the reducing levels of JAK1 and STAT1 in male medaka and the increasing levels of JAK1, STAT1 and STAT3 in female medaka (Fig. 1A, B and C) after being exposed to BCT exposure indicated that both JAK and STATs expressions were regulated. It has been reported that the abnormal expression of JAKs and STATs would interfere the downstream immune processes in human being (Aota *et al*., 2021; Dang *et al*., 2021; Kubo *et al*., 2018). SOCS3 and SOCS8 are negative feedback regulators that suppress immune responses through JAK/STAT signaling pathway (Shan *et al*., 2023; Xin *et al*., 2020). In mammals, SOCS3 is a direct target of STAT3 signaling, and STAT3 forms a negative feedback regulatory loop by inducing SOCS3 expression (Carow and Rottenberg, 2014; Sims, 2020; Zhang *et al*., 2006). Therefore the abnormal expression changes of STAT3, SOCS3 and SOCS8 may affect the feedback regulation of JAK/STAT signaling pathway in Japanese medaka. Particularly, in female medaka, levels of all tested proteins in JAK/STAT pathway were significant induced in all BCT concentration groups after the 96–h acute exposure. This suggested a stimulation of BCT on JAK/STAT signaling pathway. Considering the result of GzLM analysis and the expression changes of these proteins in both female and male medaka (Fig. 1), sex appears to be an important factor influencing the adverse effects of BCT on the JAK/STAT signaling pathway in Japanese medaka.

The effects of chemicals on fish behavior have direct ecological importance, because behavior is closely related to the overall health and survival of an individual (Brodin *et al*., 2014). Locomotor activity can coordinate fish physiology and improve their metabolism and energy use, which in turn affects their ability to forage and avoid predators (Colwill and Creton, 2011; Dong *et al*., 2022). Exploratory behavior is a response of organism to unfamiliar environments, resources, or objects, which may result to discovering new food sources or reducing competitive pressure (Burns *et al*., 2016). The present results showed that BCT exposure could induce reduced motor activity in Japanese medaka and reduced exploratory behavior in male medaka, as indicated by reduced FAM and DEB scores (Fig. 3). These changes suggested that BCT impairs the behavioral performance of Japanese medaka and might further affect the health status of the fish.

Previous studies showed that BCT could cross the

blood–brain barrier and affect the central nervous system of mammalian (Gavegnano *et al*., 2019; Richardson *et al*., 2020). The serotonin neurotransmitter system is involved in all activities of the central nervous system (De Boer *et al*., 2015; Winberg and Thörnqvist, 2016). The up–regulation of the 5–HT and 5–HIAA levels in the brain of Japanese medaka exposed to different concentrations of BCT for 96–h may cause adverse effect on nervous system (Fig. 2D and E). It is known that the serotonin (5–HT) nervous system in the brain is an important mediator in the modulation of behavioral characteristics in teleost (Winberg and Thörnqvist, 2016). 5–HIAA is the major metabolite of 5–HT, and 5–HIAA/5–HT reflects the relative balance of 5–HT synthesis, release, and metabolism *in vivo*, and the ratio can be used to assess the activity of the 5–HT neurotransmitter system in the brain (Antunes *et al*., 2024; Winberg and Thörnqvist, 2016). In general, increased 5–HT levels are associated with decreased motor activity (Meshalkina *et al*., 2018; Qiu *et al*., 2022; Sallinen *et al*., 2009). Therefore, the presented results of behavioral traits changes of Japanese medaka, and the abnormal expression levels of 5–HT, 5–HIAA and 5–HIAA/5–HT suggested that 96–h BCT exposure affected fish behavior through regulating the expression of neurotransmitters. Besides, because the JAK–STAT pathway is also regulating the reuptake process of 5–HT in the nervous system (Kabiri *et al*., 2020; Kong *et al*., 2015), BCT may affect the 5–HT reuptake process through interfering with the JAK–STAT signaling pathway. However, serotonin turnover was significantly reduced only in the male medaka brain at 5 mg/L BCT exposure (Fig. 2F). Moreover, the effect of BCT on the neural behavior of Japanese medaka may lead to a decrease in fish foraging and viability, thereby affecting population size and ecosystem balance.

The present result revealed sex–specific responses to the 96–h acute BCT exposure, to the extent that the changes or trends in the same parameters were completely opposite in different sex of Japanese medaka. Other research also reported sex–specific toxicity effects of chemicals in teleost. Research by Qiu *et al*. (2023) reported sex differences in monoamine neurotransmitter changes in medaka brains after diazepam exposure. Another research also found that 7–day thiomersal exposure to zebrafish decreased the level of 5–HT in the brains of male fish, but had no significant effect on female ones (Qiu *et al*., 2024). These results suggested that sex–specific variations exert profound influences on toxicological investigation of chemicals.

CONCLUSION

In the present study, adult Japanese medaka (*O. latipes*) was waterborne exposed to a 96–h acute BCT (concentrations of 0, 0.05, 0.5, 5 mg/L) exposure test. Key protein levels in the JAK/STAT signaling pathway in the spleen, neurotransmitter levels in the brain and behavioral traits were detected to explore the adverse effects of BCT on the JAK/STAT signaling pathway and neurobehavioral aspects in adult medaka. Results indicated that BCT have sex–specific effect on the expression levels of key components of JAK/STAT signaling pathway in the spleen of medaka. It may influence the regulation and balance of immune system. Brain neurotransmitters also showed a sex–specific response to BCT exposure, with changes in the 5–HT neurotransmitter system appearing to be more sensitive in the brain of male medaka. Moreover, BCT exposure significantly reduced locomotor activity in both male and female Japanese medaka and reduced exploratory behavior in male medaka. This may be due to changes in neurotransmitter levels in the brain. Therefore, BCT may have neurobehavioral toxicity on fish. In general, the present study suggested adverse effects of Baricitinib on the regulation of immune system and neurobehavioral aspects of Japanese medaka, addressing the deficiency of research on self–limiting immune system medicines in environmental toxicology.

AUTHOR CONTRIBUTIONS

J. Dong did laboratory work, performed the exposure experiment, measured the parameters presented in the experiment, managed the data analysis, and wrote the manuscript. X. Qiu contributed to the design of the experiment, mathematic analysis, and paper wiring. Y. Oshima supervised the design of experiment and contributed to the modification of the manuscript. Y. Shimasaki supervised the experiment process, data analysis, and manuscript modification. Y. Takai supervised the experimental work and modified the manuscript. K. Chen designed the experiment, managed the data analysis, wrote the manuscript, and provided fund for the study.

ACKNOWLEDGEMENTS

This study was funded by the Youth Found of the Natural Science Foundation of Jiangsu Province of China (Grant No. BK20220550).

REFERENCES

Antunes, D. F., P. R. Stettler and B. Taborsky 2024 The role of serotonin in modulating social competence in a cooperatively breeding fish. B*ehav. Brain Res*., **461**: 114819

Aota, K., T. Yamanoi, K. Kani, S. Ono, Y. Momota and M. Azuma 2021 Inhibition of JAK–STAT signaling by Baricitinib reduces interferon–γ–induced CXCL10 production in human salivary gland ductal cells. *Inflammation*, **44**: 206–216

Assadiasl, S., Y. Fatahi, B. Mosharmovahed, B. Mohebbi and M. H. Nicknam 2021 Baricitinib: from rheumatoid arthritis to COVID–19. *J. Clin. Pharmacol*., **61**: 1274–1285

Brodin, T., S. Piovano, J. Fick, J. Klaminder, M. Heynen and M. Jonsson 2014 Ecological effects of pharmaceuticals in aquatic systems—impacts through behavioural alterations. *Philos. Trans. R. Soc. Lond. B. Biol. Sci*., **369**: 20130580

Burns, J. G., A. C. Price, J. D. Thomson, K. A. Hughes and F. H. Rodd 2016 Environmental and genetic effects on exploratory behavior of high–and low–predation guppies (*Poecilia reticulata*). *Behav. Ecol. Sociobiol*., **70**: 1187–1196

Carow, B. and M. E. Rottenberg 2014 SOCS3, a major regulator

of infection and inflammation. *Front. Immunol.*, **5**: 58

Chen, K., M. Wu, C. Chen, H. Xu, X. Wu and X. Qiu 2021 Impacts of chronic exposure to sublethal diazepam on behavioral traits of female and male zebrafish (*Danio rerio*). *Ecotoxicol. Environ. Saf.*, **208**: 111747

Chen, S., C. Li, Z. Tu, T. Cai, X. Zhang, L. Wang, R. Tian, J. Huang, Y. Gong and X. Yang 2024 Off–label use of Baricitinib improves moderate and severe atopic dermatitis in China through inhibiting MAPK and PI3K/Akt/mTOR pathway via targeting JAK–STAT signaling of CD4+ cells. *Front. Pharmacol.*, **15**: 1324892

Colwill, R. M. and R. Creton 2011 Locomotor behaviors in zebrafish (*Danio rerio*) larvae. *Behav. Process.*, **86**: 222–229

Conrad, N., S. Misra, J. Y. Verbakel, G. Verbeke, G. Molenberghs, P. N. Taylor, J. Mason, N. Sattar, J. J. McMurray and I. B. McInnes 2023 Incidence, prevalence, and co–occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population–based cohort study of 22 million individuals in the UK. *Lancet*, **401**: 1878–1890

Dang, C., Y. Lu, X. Chen and Q. Li 2021 Baricitinib ameliorates experimental autoimmune encephalomyelitis by modulating the Janus kinase/signal transducer and activator of transcription signaling pathway. *Front. Immunol.*, **12**: 650708

De Boer, S., B. Olivier, J. Veening and J. Koolhaas 2015 The neurobiology of offensive aggression: Revealing a modular view. *Physiol. Behav.*, **146**: 111–127

Dong, K., L. Li, C. Chen, M. S. Tengbe, K. Chen, Y. Shi, X. Wu and X. Qiu 2022 Impacts of cetylpyridinium chloride on the behavior and brain neurotransmitter levels of juvenile and adult zebrafish (*Danio rerio*). *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **259**: 109393

Fetter, T., P. Smith, T. Guel, C. Braegelmann, T. Bieber and J. Wenzel 2020 Selective janus kinase 1 inhibition is a promising therapeutic approach for lupus erythematosus skin lesions. *Front. Immunol.*, **11**: 344

Fridman, J. S., P. A. Scherle, R. Collins, T. C. Burn, Y. Li, J. Li, M. B. Covington, B. Thomas, P. Collier and M. F. Favata 2010 Selective inhibition of JAK1 and JAK2 is efficacious in rodent models of arthritis: preclinical characterization of INCB028050. *J. Immunol.*, **184**: 5298–5307

Gavegnano, C., W. B. Haile, S. Hurwitz, S. Tao, Y. Jiang, R. F. Schinazi and W. R. Tyor 2019 Baricitinib reverses HIV–associated neurocognitive disorders in a SCID mouse model and reservoir seeding in vitro. *J. Neuroinflammation*, **16**: 1–12

Kabiri, M., A. Hemmatpour, F. Zare, H. Hadinedoushan and A. Karimollah 2020 Paroxetine modulates immune responses by activating a JAK2/STAT3 signaling pathway. *J. Biochem. Mol. Toxicol.*, **34**: e22464

Khan, K., S. Kar and K. Roy 2023 Are we ready to combat the ecotoxicity of COVID–19 pharmaceuticals? An in silico aquatic risk assessment. *Aquat. Toxicol.*, **256**: 106416

Kong, E., S. Sucic, F. J. Monje, S. N. Reisinger, G. Savalli, W. Diao, D. Khan, M. Ronovsky, M. Cabatic and F. Koban 2015 STAT3 controls IL6–dependent regulation of serotonin transporter function and depression–like behavior. *Sci. Rep.*, **5**: 9009

Kubo, S., S. Nakayamada, K. Sakata, Y. Kitanaga, X. Ma, S. Lee, A. Ishii, K. Yamagata, K. Nakano and Y. Tanaka 2018 Janus kinase inhibitor Baricitinib modulates human innate and adaptive immune system. *Front. Immunol.*, **9**: 1510

Liau, N. P., A. Laktyushin, R. Morris, J. J. Sandow, N. A. Nicola, N. J. Kershaw and J. J. Babon 2019 Enzymatic characterization of wild–type and mutant janus kinase 1. *Cancers*, **11**: 1701

Makabe, K., H. Okada, N. Tachibana, H. Ishikura, N. Ito, M. Tanaka, R. Chijimatsu, A. Terashima, F. Yano and M. Asaka 2024 Baricitinib ameliorates inflammatory and neuropathic pain in collagen antibody–induced arthritis mice by modulating the IL–6/JAK/STAT3 pathway and CSF–1 expression in dorsal root ganglion neurons. *Arthritis Res. Ther.*, **26**: 121

Markham, A. 2017 Baricitinib: first global approval. *Drugs*, **77**: 697–704

Matsumoto, Y., H. Oota, Y. Asaoka, H. Nishina, K. Watanabe, J. M. Bujnicki, S. Oda, S. Kawamura and H. Mitani 2009 Medaka: a promising model animal for comparative population genomics. *BMC Res. Notes*, **2**: 1–9

Meshalkina, D. A., E. V. Kysil, K. A. Antonova, K. A. Demin, T. O. Kolesnikova, S. L. Khatsko, R. R. Gainetdinov, P. A. Alekseeva and A. V. Kalueff 2018 The effects of chronic amitriptyline on zebrafish behavior and monoamine neurochemistry. *Neurochem. Res.*, **43**: 1191–1199

Mullard, A. 2018 FDA approves Eli Lilly's Baricitinib. *Nat. Rev. Drug Discov.*, **17**: 460

OECD 2012 305: Bioaccumulation in fish: aqueous and dietary exposure. *OECD Test Guidelines Section*, **3**: 1–72

Qiu, X., S. Wang, M. Zhuo, Y. Shi and K. Chen 2023 Diazepam at environmentally relevant concentrations disturbed social interactions and brain neurotransmitters in adult Japanese medaka (*Oryzias latipes*). *Aquat. Toxicol.*, **265**: 106773

Qiu, X., C. Chen, Y. Shi, K. Chen, M. Li, H. Xu, X. Wu, Y. Takai, Y. Shimasaki and Y. Oshima 2022 Persistent impact of amitriptyline on the behavior, brain neurotransmitter, and transcriptional profile of zebrafish (*Danio rerio*). *Aquat. Toxicol.*, **245**: 106129

Qiu, X., Z. Su, J. Gao, Y. Cui, K. Dong, K. Chen, R.–J. Zhao, S. Wang, T. Wu and Y. Shi 2024 Sex–specific impacts of thimerosal on the behaviors and brain monoaminergic systems in zebrafish (*Danio rerio*). *Aquat. Toxicol.*, **271**: 106921

Rawlings, J. S., K. M. Rosler and D. A. Harrison 2004 The JAK/STAT signaling pathway. *J. Cell Sci.*, **117**: 1281–1283

Richardson, P. J., S. Ottaviani, A. Prelle, J. Stebbing, G. Casalini and M. Corbellino 2020 CNS penetration of potential anti–COVID–19 drugs. *J. Neurol.*, **267**: 1880–1882

Sallinen, V., M. Sundvik, I. Reenilä, N. Peitsaro, D. Khrustalyov, O. Anichtchik, G. Toleikyte, J. Kaslin and P. Panula 2009 Hyperserotonergic phenotype after monoamine oxidase inhibition in larval zebrafish. *J. Neurochem.*, **109**: 403–415

Sarapultsev, A., E. Gusev, M. Komelkova, I. Utepova, S. Luo and D. Hu 2023 JAK–STAT signaling in inflammation and stress–related diseases: implications for therapeutic interventions. *Mol. Biomed.*, **4**: 40

Satarker, S., A. A. Tom, R. A. Shaji, A. Alosious, M. Luvis and M. Nampoothiri 2021 JAK–STAT pathway inhibition and their implications in COVID–19 therapy. *Postgrad. Med.*, **133**: 489–507

Shan, J., G. Wang, H. Li, X. Zhao, W. Ye, L. Su, Q. Zhu, Y. Liu, Y. Cheng and W. Zhang 2023 The immunoregulatory role of fish specific type II SOCS via inhibiting metaflammation in the gut–liver axis. *Water Biol. Secur.*, **2**: 100131

Shima, A. and H. Mitani 2004 Medaka as a research organism: past, present and future. *Mech. Develop.*, **121**: 599–604

Sims, N. A. 2020 The JAK1/STAT3/SOCS3 axis in bone development, physiology, and pathology. *Exp. Mol. Med.*, **52**: 1185–1197

Wang, S., J. Dong, K. Chen, Y. Shi and X. Qiu 2024 Effects of chronic diazepam exposure on the behaviors and oxidative stress homeostasis in the eyes and brains of female Japanese medaka. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **276**: 109812

Winberg, S. and P.–O. Thörnqvist 2016 Role of brain serotonin in modulating fish behavior. *Curr. Zool.*, **62**: 317–323

Xin, P., X. Xu, C. Deng, S. Liu, Y. Wang, X. Zhou, H. Ma, D. Wei and S. Sun 2020 The role of JAK/STAT signaling pathway and its inhibitors in diseases. *Int. Immunopharmacol.*, **80**: 106210

Xue, C., Q. Yao, X. Gu, Q. Shi, X. Yuan, Q. Chu, Z. Bao, J. Lu and L. Li 2023 Evolving cognition of the JAK–STAT signaling pathway: autoimmune disorders and cancer. *Signal Transduct. Target. Ther.*, **8**: 204

Zhang, J., F. Qi, J. Dong, Y. Tan, L. Gao and F. Liu 2022 Application of Baricitinib in dermatology. *J. Inflamm. Res.*, 1935–1941

Zhang, L., D. B. Badgwell, J. J. Bevers, K. Schlessinger, P. J. Murray, D. E. Levy and S. S. Watowich 2006 IL–6 signaling via the STAT3/SOCS3 pathway: functional analysis of the conserved STAT3 N–domain. *Mol. Cell. Biochem.*, **288**: 179–189