Regular Article

Effects of Exogenous Biliverdin Treatment on Neurobehaviors in Mice

Yueyi Li,[a,#] Yan Dong,[b,#] Li Meng,[a] Panpan Yu,[c] Penghui Zhao,[a] Miao Gong,[a] Qiang Gao,[a] Haishui Shi,[a,d,e] Cuili Meng,*[,f] and Yuan Gao*[,a,d,e]

[a] *Neuroscience Research Center, Institute of Medical and Health Science of HeBMU, Hebei Medical University; Shijiazhuang 050017, China:* [b] *Intensive Care Unit of Hebei Hospital of Traditional Chinese Medicine, Hebei University of Chinese Medicine; Shijiazhuang 050200, China:* [c] *Department of State Assets and Laboratory Administrative, Hebei Medical University; Shijiazhuang 050017, China:* [d] *Department of Biochemistry and Molecular Biology, College of Basic Medicine, Hebei Medicinal University; Shijiazhuang 050017, China:* [e] *Hebei Key Laboratory of Neurophysiology, Hebei Medicinal University; Shijiazhuang 050017, China: and* [f] *Department of Biochemistry, School of Basic Medicine, Xingtai Medical College; Xingtai 054000, China.*

Received April 13, 2020; accepted December 17, 2020

The neuroprotective effects of heme oxygenase (HO) have been well investigated. The potential effects of exogenous supplementation of biliverdin (BVD), one of the main products catalyzed by HO, on neurobehaviors are still largely unknown. The present study aimed to investigate the effects of BVD treatment on depression, anxiety, and memory in adult mice. Mice were injected with BVD through tail vein daily for a total 5 d, and depression- and anxiety-like behaviors were conducted by using open field test (OFT), novelty suppressed feeding (NSF), forced swimming test (FST) and tail suspension test (TST) since the third day of BVD administration. Novel object recognition (NOR) paradigm was used for memory formation test. After the final test, serum and hippocampal levels of interleukin-6 (IL-6) and tumor necrosis factor-*α* (TNF-*α*) of mice were analyzed by enzyme-linked immunosorbent assay (ELISA). The results showed that BVD treatment at low dose (2 mg/kg) induced depression-like behaviors, and high dose (8 mg/kg) BVD injection increased anxiety-like behaviors and impaired memory formation in mice. ELISA data showed that BVD treatment significantly increased hippocampal IL-6 and TNF-*α* level while only decreasing serum IL-6 level of mice. The present data suggest that exogenous BVD treatment induced depression- and anxiety-like phenotypes, which may be related to inflammatory factors, providing BVD may be a potential target for the prevention of mental disorders.

Key words biliverdin; depression; anxiety; memory; inflammation

INTRODUCTION

Heme oxygenase (HO)–carbon monoxide (CO) system has attracted more attention since it has anti-inflammatory and anti-oxidative stress activities *in vivo* and *in vitro*.[1,2] Endogenous HO is the limiting step of heme catalytic degradation of pig iron, carbon monoxide, and bilirubin/biliverdin (BVD). HO mainly includes three isoenzymes. Previous evidence showed that HO-1 can be induced by oxidative stress, heat shock, UV irradiation, ischemia reperfusion, heavy metals, bacterial lipopolysaccharides, cytokines, nitric oxide (NO), and its substrate heme, and other stimulants.[3–5] Studies have shown that HO-2 knockout increases the size of cerebral infarction in rats, and primary cortical neurons have been used to demonstrate a similar neuroprotective effect of HO-2.[6] Most endogenous bilirubin is produced in heme oxygenase catalyzed reactions. The biological functions of HO-3 are still largely unknown.[7] Recent studies mainly focus on the biological effects of HO-1 pro-reaction products (BVD, CO and iron).[8] BVD, also known as dehydrobilirubin, is a dark green, lamellar or columnar crystal of bile pigments found mainly in bile and under jaundice within the urine.

The significant neuroprotective effects of HO and its derivatives have been well investigated.[9,10] The experiment concurrently shows that the HO–CO system has the function of relieving anti-anxiety and epileptic seizures.[11,12] The BVD reductase/HO-1 system is also associated with Alzheimer's disease.[4] As the main product of HO *in vivo*, BVD has been reported to affect the levels of inflammatory cytokines in blood samples, to inhibit the expression of adhesion molecules and the aggregation of white blood cells, thereby reducing the production of cytokines and chemokines. Also BVD can inhibit the production of pro-inflammatory proteins and ultimately inhibit the inflammatory response.[13] The HO–CO system has been well known to be associated with many kinds of inflammation-related diseases, such as depression, cerebral ischemia, and traumatic brain injury.[7,14] Notably, the dual prooxidant and antioxidant activities of bilirubin and BVD were reported, suggesting complex effects of HO system *in vivo*.[15] Some studies have shown that the HO–CO system is related to emotional behavior. CO produced by HO enzyme plays an anti-anxiety-like effect by enhancing intracellular 3′-5′-guanosine monophosphate (cGMP).[11,16] HO products, CO and bilirubin, show antioxidant properties in cerebral circulation, so HO–CO system plays a positive role in seizures.[12] The upregulation of HO–CO system and soluble gyanylyl cyclase (sGC)/cGMP pathways play an active role not only in the pathogenesis of hypertension but also in many cardiovascular complications caused by hypertension.[17] Up to now, the potential effects of BVD on neurobehaviors and the underlying mechanism are still poorly understood.

At present, there are still many limitations in the field of

[#] These authors contributed equally to this work.

* To whom correspondence should be addressed. e-mail: mclant@163.com; iaa2002@163.com

antidepressant drugs and strategies and even the pathological mechanism of depression. It is well accepted that excessive inflammatory response and oxidative stress injury are important pathological mechanisms of depression. Recent studies from our and other groups also reported that the antidepressant effects of sulforaphane, arctigenin, methylene blue, *Allium cepa* L. bulb and quercetin, are related to their anti-inflammatory effects.[18–22] To this mind, by using series of behavioral test paradigms, the present study aimed to evaluate the potential effects of exogenous BVD treatment on depression, anxiety, and memory-related behaviors and to investigate the underlying mechanism in mice.

MATERIALS AND METHODS

Animals Six-week old ICR male mice (32–34 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All mice were housed at a constant temperature (23 ± 2 °C) with a period of light/dark of 12 h (lights on from 8:00 p.m. to 8:00 a.m.) and had *ad libitum* access to food and water. The behavioral tests and drug administration were performed in the dark phase. All animal experiments were reviewed and approved by the Local Animal Use Committee of Hebei Medical University, following its guidelines for the use and care of laboratory animals.

Biliverdin Treatment BVD hydrochloride (IX-α with N95% purity, Sigma-Aldrich (Shanghai) Trading Co., Ltd., Shanghai, China) was dissolved in 0.2 N NaOH and adjusted to a final pH of 7.4, and then diluted with normal saline (0.9% NaCl) to a final concentration (8 mg/kg in 0.2 mL, 2 mg/kg in 0.2 mL).The final pH of each BVD solution used in this study was between 7.3 and 7.5. For mice in vehicle group, the saline was obtained by adjusting the pH of 0.2N NaOH with HCl; That volume of the adjusted saline was injected in the same manner.[5]

Behavioral Tests

Open Field Test (OFT)

The apparatus consisted of a 40 × 40 × 35 cm clear plastic arena and the size of the central area is 20 × 20 cm. The test session was videotaped and was analyzed by a video tracking system (SMART 3.0, Panlab, Spain). Mice were placed in the corner of the chamber and allowed free exploration for 5 min individually. Total distance travelled and time spent in central zone were recorded to assess the locomotion and anxiety-like behavior of mice, respectively.[18,23]

Forced Swim Test (FST)

FST was conducted according to our previous studies.[18] Briefly, activity of the animals was tracked for 6 min. Latency of the first float status in the first 2 min, as well as a total float time in the last 4 min was recorded to reflect the depression-like behaviors of mice.

Tail Suspension Test (TST)

TST paradigm was also provided to evaluate depression-like behaviors of mice.[18] Mice were suspended 50 cm above the floor by the tail with duct tape and videotaped for 6 min. Latency to first freezing and total immobility time were evaluated, animals displaying tail climbing behavior were excluded from the analysis.

Novelty-Suppressed Feeding (NSF)

The 5-min NSF test was performed to assess the anxiety-like behaviors of mice based on previous reports.[20] After a 24-h food deprivation, mice were put in the corner of an open field with a small of food in the center. The maximum test time is 5 min. Once the mice began to eat the chow or 5 min later, the mice were put in an individual cage with packing and food for 10 min. Latency to feeding and total food intake in cage was assessed.

New Object Recognition (NOR)

Based on previous report, the NOR paradigm was used to study the effects of exogenous BVD treatment on recognition ability.[24] In short, mice were first given a habituation session to freely explore the open field for 5 min. The training session was conducted 24 h after the habituation period. Mice were placed individually in the experimental apparatus facing the wall, and 2 identical objects (object A1 and A2) were positioned in 2 adjacent corners 5 cm from the walls. Animals freely investigated the objects and open field for 5 min, and long-term memory (LTM) was tested 24 h later. During the test session, mice were allowed to explore freely in the open field for 5 min in the presence of one familiar (object A) and one novel object (object B). Exploration was defined as the mice sniffing a distance of no more than 2 cm with the nose and/or touching an object with the nose or forepaws. Between each individual testing trial, the field and objects were thoroughly wiped with 70% ethanol. Recognition index (%) = TN × 100/(TN + TF) (TN = the time spent exploring the novel object; TF = the time spent exploring the familiar object in the test session).

Timeline of the Experiment Mice were reared in home cages for 5 d for habituation and then used for the experiment. Drugs were administered from day 1 to day 5. OFT and FST were performed 30 min after the administration on day 3, TST was on day 4, and NSF test was on day 5. For NOR, the habituation session was performed on day 6, the training was on day 7, and the LTM was on day 8. After the LTM, tissue samples were collected.

Measurement of Inflammatory Cytokines Serum and hippocampal levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were determined by using commercially available kits (IL-6, ml002293-C; TNF-α, ml002095-C; Shanghai Enzyme Biotechnology Co., Ltd., China) according to the manufacturer's instructions. Briefly, the hippocampus samples were homogenized in RIPA lysis buffer. The supernatants were obtained by centrifugation at 8000 rpm for 10 min. The concentration of IL-6 and TNF-α in the supernatants was measured at 450 nm of wave length with a spectrophotometer.[25]

Statistical Analysis All data was expressed as mean ± standard error of the mean (S.E.M.). One-way ANOVA, followed by Tukey–Kramer test's multiple comparison tests was used to analyze data from enzyme-linked immunosorbent assay (ELISA), depression- and anxiety-like behavioral experiments. Two-way repeated measures ANOVA, followed by Dunnett *t*'s multiple comparison tests was used to analyze data of NOR experiment. The statistical analysis was completed by using the GraphPad Prism software, version 4.0 (GraphPad Software Inc., San Diego, CA, U.S.A.).[25]

RESULTS

Biliverdin Treatment Had No Effects on Body Weight of Mice As shown in Fig. 1A, mice were randomly di-

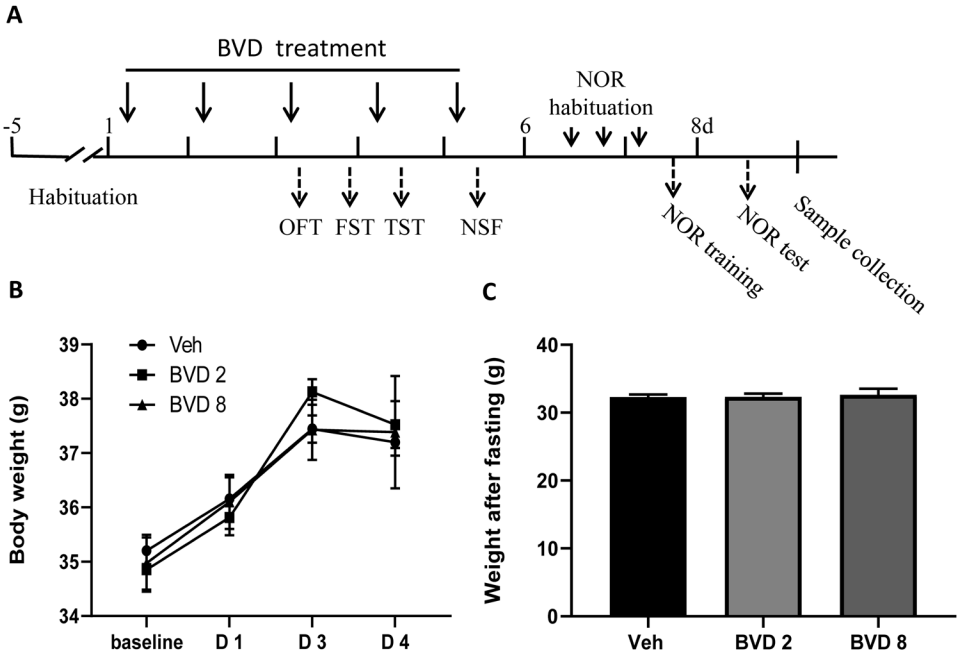

Fig. 1. Biliverdin Treatment Had No Effects on Body Weight of Mice

(A) Experimental procedure. (B) BVD treatment on body weight in mice. (C) Weight after NSF fasting.

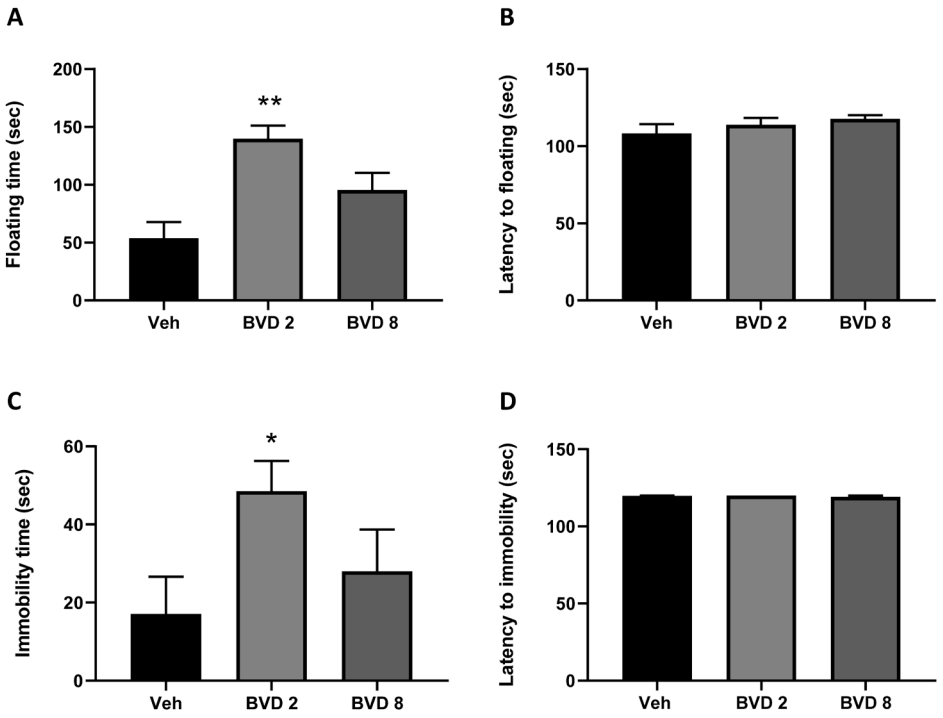

Fig. 2. Biliverdin Treatment Induced Depression-Like Behaviors in Mice

Biliverdin treatment significantly increased floating time (A) without effects on latency to floating (B) in the FST. BVD treatment significantly increased the immobility time (C) without any effects on latency to immobility (D) in the TST. $*p<0.05$, $**p<0.01$ *vs.* Veh group. $n=7$–8 per group.

vided into 3 groups ($n=7$–8 per group): mice were injected with vehicle (Veh group), 2 mg/kg (BVD2 group) or 8 mg/kg BVD (BVD8 group) based on previous reports.[5,26] After a 5-d habituation, exogenous BVD was injected *via* tail vein daily, series of behavioral tests were carried out about 30 min after BVD treatment. Body weight of mice was monitored. The results showed that there was no statistically significant difference in weight gain between the control group and the mice treated with BVD during the experiment [group effect ($F_{2,87}=0.058$, $p=0.943$), time ($F_{3,87}=21.005$, $p=0.001$), interaction ($F_{6,87}=0.379$, $p=0.890$), Fig. 1B]. And there was no significant difference of body weight between the groups after a 24-h fasting before NSF test ($F_{2,19}=0.093$, $p>0.05$, Fig. 1C).

Biliverdin Treatment Induced Depression-Like Behaviors In FST, BVD treatment at 2 mg/kg dose significantly increased the floating time of mice ($F_{2,19}=10.57$, $p<0.01$, Fig. 2A) compared with that in Veh group, without significant effects on latency to floating ($F_{2,19}=1.032$, $p>0.05$, Fig. 2B).

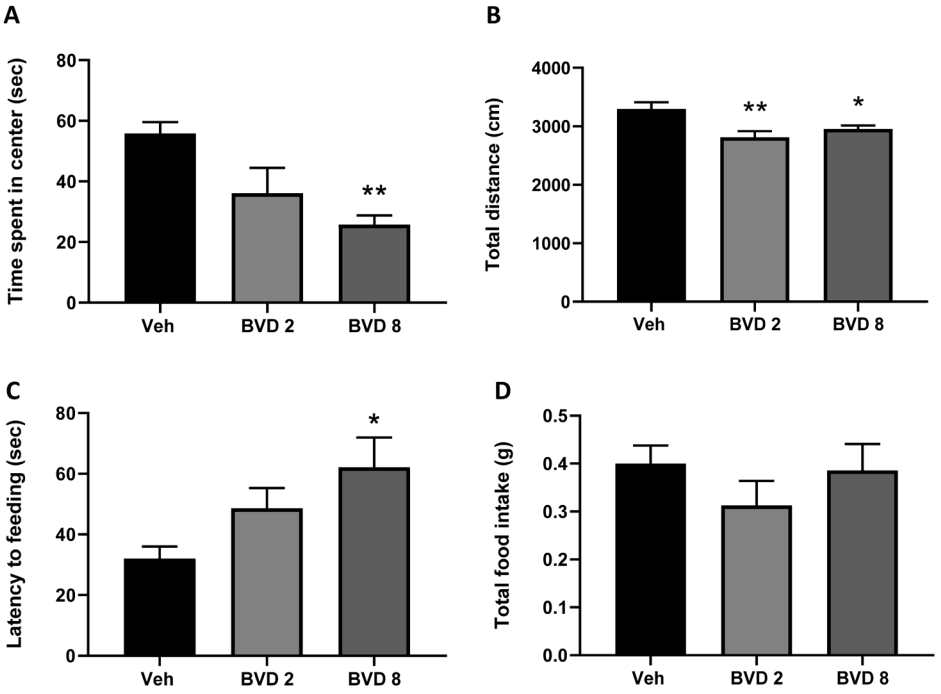

Fig. 3. Biliverdin Treatment Induced Anxiety-Like Behaviors in Mice

BVD treatment significantly decreased time spent in center (A) and locomotion activities (B) of mice in the OFT. BVD treatment significantly increased the latency to feeding (C) without any effects on total food intake (D) of mice in the NSF. $^{*}p<0.05$, $^{**}p<0.01$ *vs.* Veh group. $n=7$–8 per group.

Similar to that of data from FST, low dose BVD treatment increased the immobility time ($F_{2,19}=3.040$, $p<0.05$, Fig. 2C), but had no significant effects on latency to immobility ($F_{2,19}=0.916$, $p>0.05$, Fig. 2D) of mice in TST. And TST data also showed that there was no significant difference in immobility time in high dose group ($p>0.05$, Fig. 2C).

Bliverdin Treatment Increased Anxiety-Like Behaviors OFT and NSF were provided to evaluate anxiety-like behaviors of mice. OFT data showed that BVD treatment showed significantly decreased time spent in center zone ($F_{2,19}=6.319$, $p<0.01$, Fig. 3A) and decreased total distance ($F_{2,19}=6.412$, $p<0.01$, Fig. 3B) in OFT test. Similarly, BVD treatment at 8 mg/kg dose significantly prolonged latency to feeding ($F_{2,19}=4.179$, $p<0.05$, Fig. 3C) without effects on total food intake ($F_{2,19}=0.946$, $p>0.05$, Fig. 3D) in the NSF test. Results from the NSF showed that low dose BVD treatment had no effects on latency to feeding ($p>0.05$, Fig. 3C).

Biliverdin Treatment Impaired NOR Memory Formation The NOR procedure was used to assess the potential effects of BVD treatment on memory formation. Two-way repeated measures ANOVA of data from NOR test revealed that BVD exposure significantly affected recognition of mice [group effect ($F_{2,19}=2.405$, $p=0.117$), time ($F_{1,19}=7.368$, $p=0.014$), interaction ($F_{2,19}=5.223$, $p=0.016$), Fig. 4]. Post-hoc analysis showed that the 8 mg/kg BVD exposure impaired the long-term memory formation ($p=0.037$).

Biliverdin Treatment Increased Hippocampal IL-6 and TNF-α Levels but Decreased Serum IL-6 Levels Further, we conducted a preliminary exploration on the mechanism underlying the effects of BVD on neurobehaviors in mice. We then randomly selected 5–7 from each group for subsequent ELISA tests. The results of Tukey's multiple comparison tests showed that both doses BVD treatment significantly increased the hippocampal IL-6 levels mice ($F_{2,15}=3.834$, $p<0.05$, Fig. 5A), but decreased serum levels of IL-6 ($F_{2,17}=6.174$, $p<0.05$, Fig. 5B). The results of Tukey's multiple comparison tests showed that BVD treatment at 2 mg/kg dose significant increased hippocampal levels of TNF-α ($F_{2,17}=4.617$, $p<0.05$, Fig. 5C) without any effects on serum samples ($F_{2,17}=3.720$, $p>0.05$, Fig. 5D).

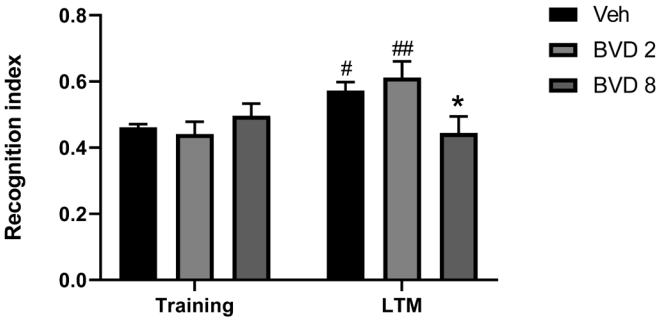

Fig. 4. Biliverdin Treatment Impaired Memory Formation in the NOR Paradigm

The results showed that BVD exposure at high dose impaired the memory formation of NOR. $^{*}p<0.05$ *vs.* Veh group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ within group. $n=7$–8 per group.

DISCUSSION

As an endogenous product of HO, the effects of BVD have been well investigated.[26] However, the potential functions on the central nervous system are still not fully understood. In the present study, we mainly focused on the neurobehavioral effects of exogenous BVD treatment on depression, anxiety, and memory in mice by using series of classic behavioral tests.[27] Contrary to our expectations, the behavioral results showed that exogenous BVD treatment induced significant depression- and anxiety-like behaviors as well as memory im-

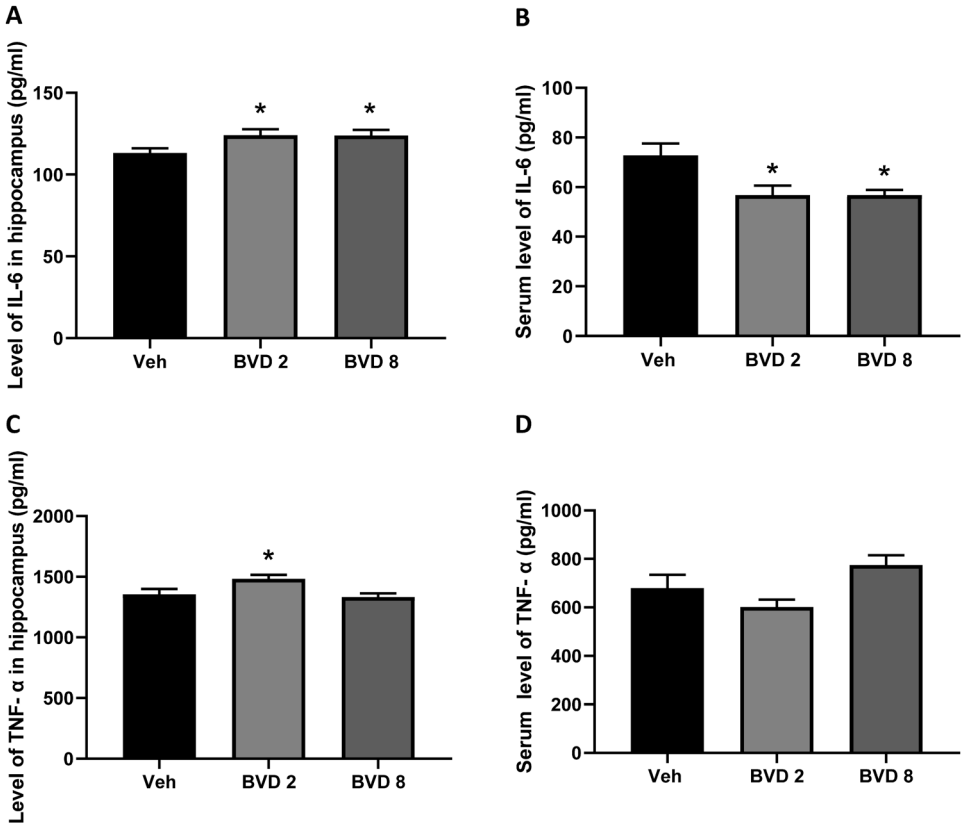

Fig. 5. Biliverdin Treatment Increased Hippocampal Levels of Inflammatory Cytokines While Decreased Serum Levels of IL-6

After all the behavioral tests were completed, serum and hippocampus of mice were collected for ELISA analysis. BVD treatment significantly increased IL-6 levels in hippocampus (A) while decreased that in serum (B) of mice. Low dose BVD treatment significantly increased TNF-α level in hippocampus (C) without any effects on that in serum of mice. $*p < 0.05$ *vs.* Veh group. $n = 5–7$ per group.

pairment of mice under normal condition. ELISA data showed that BVD treatment significantly increased hippocampal levels of IL-6 and TNF-α, while decreased serum levels of IL-6 without any effects on serum TNF-α.

Previous studies showed that extensive inflammation is often accompanied with higher anxiety, learning, and memory impairment in mice, and therapeutic strategies targeting the regulation of the immune system have been paid more attention to.[28] Stress response upregulates the components of the immune system involved in inflammation.[29] The key mediator is proinflammatory cytokines, such as IL-1, IL-6, and TNF-α, which can cause depressive symptoms. Some studies have shown that the level of inflammatory cytokines in the hippocampus can lead to depression-like behavior caused by estrogen deficiency.[30,31] Inflammation is also associated with anxiety-related diseases.[32,33] Studies have shown that hippocampal inflammation is related to depression and anxiety behavior. Considering the regulatory roles of BVD and the relationship between hippocampal inflammation and behavioral dysfunction, we examined several inflammation-related cytokines.[34] Behavioral tests used in the present study, mainly include OFT, NSF, TST and FST which are all widely used for the evaluation of depression- and anxiety-like behaviors and for rapidly screening potential antidepressant- and anxiolytic drugs.[18,24] Our present data showed that mice with exogenous BVD treatment exerted depression- and anxious-like behaviors, accompanied with inflammatory response in both the peripheral and the central nervous system. It is well accepted that memory impairment is also accompanied with depression in both animals and human. As a classic mice memory paradigm, NOR was provided to assess the effects of BVD exposure on memory formation.[24] The results showed that exogenous BVD treatment impaired the memory formation of NOR. All these behavioral data indicated that exogenous BVD treatment induces depression- and anxiety-like and memory impairment effects.

Considering the present data is contrary to expectations, the following possibilities are proposed. First, we make the assumption that BVD plays a dual role, which has been indicated previously.[5,8] It is however worth exploring the different effects of low-dose *versus* high-dose BVD on depression, anxiety, and memory behaviors. The differences may be due to the respective difference in dose. At low doses, BVD can have residual anti-inflammatory, antioxidant, and other neuroprotective effects; while high-dose BVD treatment is associated with inflammation and nerve damage, which can lead to depression and anxious behaviors. No evidence is provided to investigate the effect of tail vein BVD on drug concentration in the target brain area and blood. In combination with the unexpected findings of the present study, we will carry out relevant research in the future. Additionally, it may be due to the injection of high doses of BVD into the tail vein may result in negative feedback regulation of HO-1, which weaken HO-1-mediated anti-inflammatory and antioxidant effects. In addition, we hypothesize that excessive injection of exogenous BVD will produce bilirubin in the body, which has a role in causing central inflammation and oxidative stress damage[35] and passing through the blood–brain barrier,[36–39] this is due

to the conversion between BVD/bilirubin. High serum level of bilirubin has been found to be association with depression.[40] This confirmed that mice in the low concentration group showed depressive behaviors, while mice in the high concentration group showed anxious behaviors and decreased learning and memory ability due to nerve damage. Therefore, we hypothesized that low levels of inflammation would lead to depression in mice, while high levels of inflammation would damage neurons in mice, leading to learning and memory impairment and anxiety-like behaviors. The increase of IL-6 in the hippocampus, the decrease of IL-6 in the serum of mice, and the increase of TNF-α in the hippocampal of the low-dose group, indicate that mice produce the central inflammatory response after administration, but the specific mechanism needs to be further discussed. We presumed that the differences would be attributed to the dual effects of BVD and its metabolic transformation *in vivo* and that tail vein injection of BVD would play the anti-inflammatory role in the peripheral system, while the increase of BVD in the body may promote the metabolism of bilirubin, and the increased bilirubin will pass through the blood–brain barrier,[36,37] thus causing an excessive central inflammatory response.[38,39] Finally, previous studies have shown that anxiety can reduce spontaneous locomotor activity and increase the immobility time in FST.[41,42] Since in this study, there was no significant difference in the latency to floating of the mice in the FST and TST tests, we suspect that the mice in the experimental group had normal activities, and the prolonged floating time and immobility time in the FST and TST tests was not due to the restricted activity of the mice. Therefore, in the case of reduced spontaneous activity in the OFT test, we speculate that the prolonged floating time of FST is due to the depression of the mice.

In conclusion, the preliminary data showed that exogenous BVD supplement could induce depression- and anxious-like effects and memory impairment in mice. We noticed that low dose of BVD treatment increase depression-like behaviors and high dose of BVD increase anxiety-like behaviors. We speculate that there are two possibilities. One is the dose-dependent effect of BVD treatment on different behavioral phenotypes, the other possibility is that the dual effects of BVD treatment on behaviors. Compared with previous research, this experiment used the method of injecting BVD into the tail vein of mice to explore its effect on depression and anxiety. The results of the study were different from those of previous BVD, and the dual effects of BVD on proinflammatory cytokines within the peripheral and central nervous system should be further investigated.

Acknowledgments This study was partly supported by the Natural Science Foundation from Hebei province (H2018206119); Foundation from Hebei Education Department (SLRC2019047); Health Science Foundation from HFPC of Hebei Province (20170471), Foundation for Excellent Undergraduate Students from Hebei Medical University (USIP2018012). We thank Omar Israel Velez de-la-Paz from Hebei Medical University for his help in revising the manuscript.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) El-Achkar GA, Mrad MF, Mouawad CA, Badran B, Jaffa AA, Motterlini R, Hamade E, Habib A. Heme oxygenase-1-dependent anti-inflammatory effects of atorvastatin in zymosan-injected subcutaneous air pouch in mice. *PLoS ONE*, **14**, e0216405 (2019).
2) Fan R, Pan T, Zhu AL, Zhang MH. Anti-inflammatory and anti-arthritic properties of naringenin *via* attenuation of NF-kappaB and activation of the heme oxygenase HO-1/related factor 2 pathway. *Pharmacol. Rep.*, **69**, 1021–1029 (2017).
3) Tang LM, Wang YP, Wang K, Pu LY, Zhang F, Li XC, Kong LB, Sun BC, Li GQ, Wang XH. Exogenous biliverdin ameliorates ischemia-reperfusion injury in small-for-size rat liver grafts. *Transplant. Proc.*, **39**, 1338–1344 (2007).
4) Barone E, Di Domenico F, Mancuso C, Butterfield DA. The Janus face of the heme oxygenase/biliverdin reductase system in Alzheimer disease: it's time for reconciliation. *Neurobiol. Dis.*, **62**, 144–159 (2014).
5) Bai B, Liu Y, You Y, Li Y, Ma L. Intraperitoneally administered biliverdin protects against UVB-induced skin photo-damage in hairless mice. *J. Photochem. Photobiol. B*, **144**, 35–41 (2015).
6) Kim YS, Dore S. Catalytically inactive heme oxygenase-2 mutant is cytoprotective. *Free Radic. Biol. Med.*, **39**, 558–564 (2005).
7) Wu L, Wang R. Carbon monoxide: endogenous production, physiological functions, and pharmacological applications. *Pharmacol. Rev.*, **57**, 585–630 (2005).
8) Chiang SK, Chen SE, Chang LC. A dual role of heme oxygenase-1 in cancer cells. *Int. J. Mol. Sci.*, **20**, 39 (2018).
9) Ayer A, Zarjou A, Agarwal A, Stocker R. Heme oxygenases in cardiovascular health and disease. *Physiol. Rev.*, **96**, 1449–1508 (2016).
10) Park J, Chen Y, Zheng M, Ryu J, Cho GJ, Surh YJ, Sato D, Hamada H, Ryter SW, Kim UH, Joe Y, Chung HT. Pterostilbene 4′-beta-Glucoside Attenuates LPS-Induced Acute Lung Injury *via* Induction of Heme Oxygenase-1. *Oxid. Med. Cell. Longev.*, **2018**, 2747018 (2018).
11) Carvalho-Costa PG, Branco LG, Leite-Panissi CR. Activation of locus coeruleus heme oxygenase–carbon monoxide pathway promoted an anxiolytic-like effect in rats. *Braz. J. Med. Biol. Res.*, **49**, e5135 (2016).
12) Parfenova H, Leffler CW, Basuroy S, Liu J, Fedinec AL. Antioxidant roles of heme oxygenase, carbon monoxide, and bilirubin in cerebral circulation during seizures. *J. Cereb. Blood Flow Metab.*, **32**, 1024–1034 (2012).
13) Wang J, Zhou HC, Pan P, Zhang N, Li WZ. Exogenous biliverdin improves the function of lung grafts from brain dead donors in rats. *Transplant. Proc.*, **42**, 1602–1609 (2010).
14) Chen Z, Xu H, Haimano S, Li X, Li XM. Quetiapine and venlafaxine synergically regulate heme oxygenase-2 protein expression in the hippocampus of stressed rats. *Neurosci. Lett.*, **389**, 173–177 (2005).
15) Sakihama Y, Cohen MF, Grace SC, Yamasaki H. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. *Toxicology*, **177**, 67–80 (2002).
16) Cazuza RA, Pol O, Leite-Panissi CRA. Enhanced expression of heme oxygenase-1 in the locus coeruleus can be associated with anxiolytic-like effects. *Behav. Brain Res.*, **336**, 204–210 (2018).
17) Ndisang JF, Wu L, Zhao W, Wang R. Induction of heme oxygenase-1 and stimulation of cGMP production by hemin in aortic tissues from hypertensive rats. *Blood*, **101**, 3893–3900 (2003).
18) Wu S, Gao Q, Zhao P, Gao Y, Xi Y, Wang X, Liang Y, Shi H, Ma Y. Sulforaphane produces antidepressant- and anxiolytic-like effects in adult mice. *Behav. Brain Res.*, **301**, 55–62 (2016).
19) Yin S, Shao J, Wang X, Yin X, Li W, Gao Y, Velez de-la-Paz OI, Shi H, Li S. Methylene blue exerts rapid neuroprotective effects on lipopolysaccharide-induced behavioral deficits in mice. *Behav.*

Brain Res., **356**, 288–294 (2019).
20) Du Y, Li W, Li Y, Yang J, Wang X, Yin S, Wang X, Velez de-la-Paz OI, Gao Y, Chen H, Yin X, Shi H. Repeated arctigenin treatment produces antidepressant- and anxiolytic-like effects in mice. *Brain Res. Bull.*, **146**, 79–86 (2019).
21) Samad N, Saleem A. Administration of *Allium cepa* L. bulb attenuates stress-produced anxiety and depression and improves memory in male mice. *Metab. Brain Dis.*, **33**, 271–281 (2018).
22) Samad N, Saleem A, Yasmin F, Shehzad MA. Quercetin protects against stress-induced anxiety- and depression-like behavior and improves memory in male mice. *Physiol. Res.*, **67**, 795–808 (2018).
23) Liang M, Du Y, Li W, Yin X, Yang N, Qie A, Lebaron TW, Zhang J, Chen H, Shi H. SuHeXiang essential oil inhalation produces antidepressant- and anxiolytic-like effects in adult mice. *Biol. Pharm. Bull.*, **41**, 1040–1048 (2018).
24) Shi HS, Yin X, Song L, Guo QJ, Luo XH. Neuropeptide Trefoil factor 3 improves learning and retention of novel object recognition memory in mice. *Behav. Brain Res.*, **227**, 265–269 (2012).
25) Bisht K, Tampe J, Shing C, Bakrania B, Winearls J, Fraser J, Wagner KH, Bulmer AC. Endogenous tetrapyrroles influence leukocyte responses to lipopolysaccharide in human blood: pre-clinical evidence demonstrating the anti-inflammatory potential of biliverdin. *J. Clin. Cell. Immunol.*, **5**, 1000218 (2014).
26) Bellner L, Wolstein J, Patil KA, Dunn MW, Laniado-Schwartzman M. Biliverdin rescues the HO-2 null mouse phenotype of unresolved chronic inflammation following corneal epithelial injury. *Invest. Ophthalmol. Vis. Sci.*, **52**, 3246–3253 (2011).
27) Gong M, Huai Z, Song H, Cui L, Guo Q, Shao J, Gao Y, Shi H. Effects of maternal exposure to bisphenol AF on emotional behaviors in adolescent mice offspring. *Chemosphere*, **187**, 140–146 (2017).
28) Su WJ, Zhang T, Jiang CL, Wang W. Clemastine alleviates depressive-like behavior through reversing the imbalance of microglia-related pro-inflammatory state in mouse hippocampus. *Front. Cell. Neurosci.*, **12**, 412 (2018).
29) Gao X, Cao Q, Cheng Y, Zhao D, Wang Z, Yang H, Wu Q, You L, Wang Y, Lin Y, Li X, Wang Y, Bian JS, Sun D, Kong L, Birnbaumer L, Yang Y. Chronic stress promotes colitis by disturbing the gut microbiota and triggering immune system response. *Proc. Natl. Acad. Sci. U.S.A.*, **115**, E2960–E2969 (2018).
30) Xu Y, Sheng H, Bao Q, Wang Y, Lu J, Ni X. NLRP3 inflammasome activation mediates estrogen deficiency-induced depression- and anxiety-like behavior and hippocampal inflammation in mice. *Brain Behav. Immun.*, **56**, 175–186 (2016).
31) Slavich GM, Irwin MR. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychol. Bull.*, **140**, 774–815 (2014).
32) Felger JC. Imaging the role of inflammation in mood and anxiety-related disorders. *Curr. Neuropharmacol.*, **16**, 533–558 (2018).
33) Dempsey E, Abautret-Daly Á, Docherty NG, Medina C, Harkin A. Persistent central inflammation and region specific cellular activation accompany depression- and anxiety-like behaviours during the resolution phase of experimental colitis. *Brain Behav. Immun.*, **80**, 616–632 (2019).
34) Li N, Wang Q, Wang Y, Sun A, Lin Y, Jin Y, Li X. Fecal microbiota transplantation from chronic unpredictable mild stress mice donors affects anxiety-like and depression-like behavior in recipient mice *via* the gut microbiota-inflammation-brain axis. *Stress*, **22**, 592–602 (2019).
35) Qaisiya M, Brischetto C, Jasprova J, Vitek L, Tiribelli C, Bellarosa C. Bilirubin-induced ER stress contributes to the inflammatory response and apoptosis in neuronal cells. *Arch. Toxicol.*, **91**, 1847–1858 (2017).
36) Ghersi-Egea JF, Gazzin S, Strazielle N. Blood-brain interfaces and bilirubin-induced neurological diseases. *Curr. Pharm. Des.*, **15**, 2893–2907 (2009).
37) Gazzin S, Strazielle N, Tiribelli C, Ghersi-Egea JF. Transport and metabolism at blood–brain interfaces and in neural cells: relevance to bilirubin-induced encephalopathy. *Front. Pharmacol.*, **3**, 89 (2012).
38) Feng J, Li M, Wei Q, Li S, Song S, Hua Z. Unconjugated bilirubin induces pyroptosis in cultured rat cortical astrocytes. *J. Neuroinflammation*, **15**, 23 (2018).
39) Schiavon E, Smalley JL, Newton S, Greig NH, Forsythe ID. Neuroinflammation and ER-stress are key mechanisms of acute bilirubin toxicity and hearing loss in a mouse model. *PLOS ONE*, **13**, e0201022 (2018).
40) Tang WK, Liang H, Chu WC, Mok V, Ungvari GS, Wong KS. Association between high serum total bilirubin and post-stroke depression. *Psychiatry Clin. Neurosci.*, **67**, 259–264 (2013).
41) Zhou X, Gan T, Fang G, Wang S, Mao Y, Ying C. Zeaxanthin improved diabetes-induced anxiety and depression through inhibiting inflammation in hippocampus. *Metab. Brain Dis.*, **33**, 705–711 (2018).
42) Rajabi M, Mohaddes G, Farajdokht F, Nayebi Rad S, Mesgari M, Babri S. Impact of loganin on pro-inflammatory cytokines and depression- and anxiety-like behaviors in male diabetic rats. *Physiol. Int.*, **105**, 116–126 (2018).