Study of the serum levels of polyunsaturated fatty acids and the expression of related liver metabolic enzymes in a rat valproate-induced autism model

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ABSTRACT

To investigate whether the decreased level of serum polyunsaturated fatty acids (PUFAs) in patients with autism is associated with the expression of related liver metabolic enzymes, we selected rats that were exposed to valproic acid (VPA) on embryonic day 12.5 (E12.5) as a model of autism. We observed the serum levels of PUFAs and the expression of related liver metabolic enzymes, including Δ5-desaturase, Δ6-desaturase and elongase (Elov12), in VPA-exposed and control rats on postnatal day 35 (PND35) and conducted sex dimorphic analysis. We found that the levels of serum PUFAs and related liver metabolic enzymes in the VPA rats were significantly reduced, in association with autism-like behavioral changes, the abnormal expression of apoptosis-related proteins and hippocampal neuronal injury, compared to the control rats and showed sex difference in VPA group. This finding indicated that rats exposed to VPA at the embryonic stage may exhibit reduced synthesis of serum PUFAs due to the down-regulation of liver metabolic enzymes, thereby inducing nervous system injury and behavioral changes, which is affected by sex in the meantime.

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1. Introduction

Autism is a neurodevelopmental disorder. Its hallmark symptoms are abnormalities in social interaction and communication, repetitive behaviors, and narrowed interests (Association, 2013), and these symptoms are accompanied by various neurobehavioral disorders, such as learning disability, hyperactivity and anxiety (O’Brien and Pearson, 2004; De Giacomo et al., 2014; Towbin et al., 2005). Recently, data from the US indicated that the incidence of autism spectrum disorder (ASD) was 14.7‰, with a male to female ratio of 4.1 (CDC, 2014). The causes of autism are complex, involving genetic, epigenetic and environmental factors (Currenti, 2010).

VPA is a common anti-epileptic drug that displays teratogenic effects. From day 20 to day 24 of pregnancy, before closure of the nerve canal, if pregnant women use teratogenic drugs such as VPA, the probability that their offspring develop a neurodevelopmental disorder increases (Rice and Barone, 2000). The intraperitoneal injection of VPA into female rats during early pregnancy results in autism-like changes in the offspring, and the brain structures and the biomarker levels in these offspring are similar to those in autism patients (Kim et al., 2013a; Schneider et al., 2008). Thus, this study adopted this animal model for subsequent research. There has been increasing evidence that the change of metabolic pathway of PUFAs could affect the normal function of nervous system which is related to pathogenesis of some disease such as autism (Brown et al., 2014; Gumpricht and Rockway, 2014). PUFAs are crucial for proper functioning of cell membranes, particularly in the brain (Caramia, 2008; Extier et al., 2009). As precursors of n-3 and n-6 PUFAs, α-linolenic acid (ALA) and linoleic acid (LA) must be absorbed from food because they cannot be synthesized in the human body. These precursors are synthesized into long-chain PUFAs such as docosahexaenoic acid (DHA) and arachidonic
acid (AA) by metabolic enzymes after entering the human body. Δ5-desaturase, Δ6-desaturase and Elov12 are important metabolic enzymes in the synthesis of long-chain PUFAs, which primarily express in the liver (Rapoport et al., 2007), and their functional status directly affects the synthesis of long-chain PUFAs (Nakamura and Nara, 2004). Studies have revealed that autistic patients may exhibit abnormal PUFAs metabolism, which manifests as varying decreases in the levels of PUFAs in samples such as serum and plasma (Sun et al., 2013; El-Ansary et al., 2011a,b; Mostafa et al., 2015a,b; Mostafa and Al-Ayadhi, 2015). Additionally, reduced DHA uptake via food and a decreased serum level of DHA have been observed in autistic children (Al-Farsi et al., 2013).

To explore the possible explanation for the decline in the levels of PUFAs and nervous behavioral changes in autism, we utilized a relevant well-developed animal model of autism, the exposure of rats to VPA on day E12.5, to evaluate the serum levels of PUFAs, the expression of liver metabolic enzymes, the survival rate of hippocampal CA1 region neurons and the protein expression of caspase-3, Bax and Bcl-2 in the hippocampus and used the sex dimorphic analysis.

2. Materials and methods

2.1. Ethical approval

All experimental procedures were conducted in strict accordance with the guidelines established by the Ministry of Health of China and were approved by the Animal Care Committee of Harbin Medical University.

2.2. Experimental animals

Adult Wistar rats (YiSi, China) were housed overnight in one cage (male:female = 1:1). The next day, the detection of a vaginal plug in the female rats marked the success of copulation and was recorded as E1. The gestational female rats were randomly divided into two groups. The first group, referred to as the model group, received a single intraperitoneal injection of 600 mg/kg VPA (Sigma, USA) dissolved in physiological saline at a concentration of 250 mg/ml on E12.5. The other group, referred to as the saline group, was injected with the same volume of physiological saline. The offspring born from the rats in the model and saline groups were included in the VPA and control groups, respectively (Schneider and Przewlocki, 2005). On PND21, the offspring were weaned and divided into different cages according to sex. The rats were freely provided with the same food and water. The laboratory temperature was 21 ± 1 °C, with a humidity of 55% and a 12-h photoperiod. The experiment was conducted from 9am to 3pm.

2.3. Behavioral tests

2.3.1. Morris water maze test

The Morris water maze test (on PND21) (WMT-100, TME, China) was used to evaluate the learning and memory ability of the rats (Morris, 1984). An acrylic acid platform (with a diameter of 8 cm) was placed at the center of the third quadrant of the water maze and was hidden 1 cm below the water surface. From day 1 to day 3, the rats were trained to locate the hidden platform within 60 s. From day 4 to day 7, the escape latency of the rats to climb on the platform was recorded. On day 8, the hidden platform was removed, and the number of times that the rats crossed the former platform position within 60 s was recorded.

2.3.2. Open field test

This test evaluated the locomotor activity of the rats on PND28. The rats were habituated to the test box for 5 min before the test (Kim et al., 2013a). An auto-tracking camera system (YH-OF, YiHong, China) was used to record the total distance traveled and the duration of movement in the test box within 30 min.

2.3.3. Sociability and social preference tests

The sociability test evaluated the social interaction of the rats on PND31 (Moy et al., 2004). We used three interconnected compartments in this test. The compartments on both sides contained an empty cage. First, the experimental rat was placed in the central compartment for adaptation for 5 min. During this period, the rat was free to enter each compartment. Then, the experimental rat was removed, and a cage containing a conspecific stranger rat to the tested rat was placed in the left compartment, labeled as stranger rat no. 1, whereas an empty cage was placed in the right compartment. Then, the tested rat was placed in the empty central compartment again to begin the 10-min sociability test. Afterwards, the social preference test was immediately performed. A new stranger rat no. 2 was added to the empty cage from the right compartment, which was then swapped with the left cage. The experimental rat was placed in the empty central compartment again according to the same method as described above. The same camera system as that used for the open field test was used to record the duration spent and the distance traveled in the three compartments by the experimental rats.

2.4. Fatty acid analysis

The rats were anesthetized on PND35 after 12-h fasting. Blood was then collected from their abdominal aorta and was immediately centrifuged at 3000 × g for 10 min at room temperature. Then, the serum was separated from the blood, and 0.2 ml of serum, 0.2 ml of internal standard solution (200 μg/ml, C17:0) and 2 ml of 10% sulfuric acid–methanol solution were added to a 10-ml test tube (Liu et al., 2010). Then, the tube was suspended for 1 min. The plug was sealed tightly and the tube was incubated in a 62 °C water bath for 2h. Then the sample was cooled. A certain amount of anhydrous sodium sulfate and 2 ml of hexane were added to the tube, followed by incubation for 1 min. The tube was centrifuged at 4000 × g for 5 min, and the supernatant was pipetted into a 2-ml test tube and dried using nitrogen gas. Finally, 0.1 ml of n-hexane was used to dissolve the sample, and 1 μl of each sample was employed for analysis using a TRACE gas chromatograph with a Polaris Q mass spectrometer (Thermo Finnigan, Austin, TX, USA). All fatty acid standards were purchased from Sigma Company (USA, >99% purity) and were classified into two groups. The n-3 PUFAs included ALA (C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3) and DHA (C22:6n-3), whereas the n-6 PUFAs included LA (C18:2n-6), γ-linolenic acid (GLA, γ-C18:3n-6), AA (C20:4n-6), and docosatetraenoic acid (DTA, C22:4n-6).

2.5. Western blot analysis

On PND35, the rats were narcotized and then killed. Hippocampus and liver were rapidly removed on ice. An aliquot of each collected sample was mixed with RIPA lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 1% (w/v) Nonidet P-40, 5 mM EDTA and 1 mM phenylmethylsulfonyl fluoride) at a ratio of 1:5 (w/v). This mixture was centrifuged at 13,200 × g at 4 °C for 20 min, and the supernatant was used for protein concentration estimation using a BCA protein assay kit (Beyotime, China). Then, 30-μg protein samples were subjected to sodium dodecyl sulfate-polycrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Millipore, MA). The membrane was blocked with 5% skim milk for 1 h and incubated in the following antibodies at 4 °C overnight for immunodetection: rabbit anti-FADS1 (1:200, Santa Cruz, USA), goat anti-FADS2 (1:200, Santa Cruz, USA), goat anti-
Evol2 (1:200, Santa Cruz, USA), rabbit anti-caspase-3 (1:300, Santa Cruz, USA), rabbit anti-Bax (1:200, Santa Cruz, USA), rabbit anti-Bcl-2 (1:200, Santa Cruz, USA) and mouse anti-GAPDH (1:2000, Kang Chen, China). Following washing, the membranes were incubated for 2 h in an HRP-labeled goat anti-rabbit (1:5000, Santa Cruz, USA), or anti-mouse antibody (1:5000, Santa Cruz, USA). The enhanced chemiluminescence kit (Pierce, CA) was used for immunodetection.

The membrane images were digitized using a scanner, and the protein band gray values were analyzed using Quantity One software (BioRad, USA).

2.6. Nissl staining

On PND35, the rats from each group were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) (4 °C). The brains were then removed, and frozen coronal sections (5 μm) were generated on slides. The slides were immersed in 0.1% cresyl violet for 10 min at room temperature. The sections were then dehydrated in a graded alcohol series and cleared using dimethylbenzene, and the slides were sealed using neutral resin. The slides were observed under the microscope and photographed. To evaluate neuronal survival in the hippocampal CA1 subfield, neurons with round and palely stained nuclei represented surviving cells, whereas shrunken neurons with pyknotic nuclei represented damaged cells. Hippocampal neuronal damage in the CA1 region was determined by counting the surviving neurons under a light microscope by blinded analysis. The data were presented as the number of surviving neurons/fiel.

2.7. Biochemical analysis

The rats were anesthetized on PND35 after fasting for 12 h. Blood was then collected from their abdominal aorta and was immediately centrifuged at 3000 × g for 10 min at room temperature. Biochemical analyses were performed by using kits of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Nanjing JianCheng, China).

2.8. Statistics

All data were analyzed using SPSS version 13.0 (Beijing Stats Data Mining Co., Ltd.). The results were statistically assessed by parametric analysis of variance (ANOVA) with two levels of prenatal status (VPA vs. control) and sex (males vs. females) as between subject factors. If significant effects were found in any one of the factors, post hoc comparisons were conducted using Bonferroni’s post-tests. The differences in the escape latency on the Morris water maze test were analyzed via repeated measures ANOVA. p < 0.05 indicated a significant difference, and all data were presented as the means ± S.E.M.

3. Results

3.1. Morris water maze test

Escape latency (Fig. 1A): Results of repeated measures ANOVA showed significant effects of group (F(1,36) = 84.14, p < 0.001), time (F(3,108) = 159.48, p < 0.001), and group × time (F(3,108) = 31.89, p < 0.001). Post hoc comparisons revealed that, compared to control, escape latency of VPA rats was significantly longer. In the process of training, the shortening escape latency both in VPA and control rats indicated that the rats in both group had the learning and memory ability to some extent. But the shortening degree of the escape latency in VPA rats was smaller than control rats. There was no sex difference in both groups.

The number of times crossed the center of 3rd quadrant (Fig. 1B): Results of two-way ANOVA revealed a significant group effect (F(1,36) = 60.70, p < 0.001). VPA group was less than that of the control group, but not by sex difference and interaction.

3.2. Open field test

The distance spontaneously traveled (Fig. 1C): Results of two-way ANOVA showed significant effects of group (F(1,36) = 69.38, p < 0.001), sex (F(1,36) = 7.60, p = 0.009), and group × sex (F(1,36) = 5.88, p = 0.021). Post hoc comparisons revealed that, for the VPA group, the distance spontaneously traveled was significantly longer, than that by the control group. VPA males traveled longer than VPA females.

The spontaneous movement duration (Fig. 1D): Results of two-way ANOVA indicated significant effects of group (F(1,36) = 97.50, p < 0.001), sex (F(1,36) = 7.13, p = 0.011), and group × sex (F(1,36) = 6.29, p = 0.017). Post hoc comparisons showed that, for the VPA group, the spontaneous movement duration was significantly longer, than that by the control group. The duration of VPA males was longer than that of VPA females.

3.3. Sociability and social preference tests

3.3.1. Sociability test

The time spent exploring stranger rat no. 1 and the empty cage (Fig. 1E): Results of two-way ANOVA revealed a significant group effect (F(1,36) = 94.30, p < 0.001; F(1,36) = 64.46, p < 0.001). The time spent exploring stranger rat no. 1 indicated significant effects of sex (F(1,36) = 4.74, p = 0.036) and group × sex interaction (F(1,36) = 8.63, p = 0.006). Post hoc comparisons showed that, for the VPA group, the time spent exploring stranger rat no. 1 was significantly less and the time spent exploring the empty cage was significantly longer, than that in control group. VPA males spent less time in the stranger rat no. 1 side than VPA females.

The frequency exploring stranger rat no. 1 and the empty cage (Fig. 1F): Results of two-way ANOVA indicated a significant group effect (F(1,36) = 10.17, p = 0.003; F(1,36) = 25.52, p < 0.001). The frequency exploring the empty cage revealed significant effects of sex (F(1,36) = 9.38, p = 0.004) and group × sex interaction (F(1,36) = 17.09, p < 0.001). Post hoc comparisons found that, for the VPA group, the frequency exploring stranger rat no. 1 was significantly lower, and the frequency exploring the empty cage was significantly higher, than that in control group. The frequency of exploring the empty cage in VPA males was significantly higher than that in VPA females.

3.3.2. Social preference test

The time spent exploring stranger rat no. 1 and the stranger rat no. 2 (Fig. 1G): Results of two-way ANOVA showed a significant group effect (F(1,36) = 57.18, p < 0.001; F(1,36) = 108.45, p < 0.001), but not by sex difference. The time spent exploring stranger rat no. 2 revealed a significant effect of group × sex interaction (F(1,36) = 14.89, p < 0.001). Post hoc comparisons indicated that, for the VPA group, the time spent exploring stranger rat no. 1 was significantly longer and the time spent exploring the stranger rat no. 2 was significantly less, than that in control group. VPA males spent less time in the stranger rat no. 2 side than VPA females.

The frequency exploring stranger rat no. 1 and the stranger rat no. 2 (Fig. 1H): Results of two-way ANOVA revealed a significant group effect (F(1,36) = 47.14, p < 0.001; F(1,36) = 49.81, p < 0.001). The frequency exploring the stranger rat no. 1 showed significant effects of sex (F(1,36) = 6.97, p = 0.012) and group × sex interaction (F(1,36) = 6.52, p = 0.015). Post hoc comparisons indicated that, for the VPA group, the frequency exploring stranger rat no. 1 was significantly higher and the frequency exploring the stranger rat no. 2 was significantly lower.
Fig. 1. Behavioral tests. (A and B) Morris water maze test. (C and D) Open field test. (E and F) Sociability test. (G and H) Social preference test. Data expressed as mean ± S.E.M., n = 10. Statistical significance (p < 0.05). * compared to control males; † compared to VPA males; ‡ compared to control females; ¶ compared to VPA females.
was significantly lower than that in control group. The frequency of exploring the stranger rat no. 1 by the VPA males was significantly higher than that in the VPA females.

3.4. Fatty acid analysis

The levels of the eight examined types of PUFAs in serum samples from the VPA males were significantly lower than those from the control males \(p < 0.05\). The levels of n-3 PUFAs, n-6 PUFAs and total fatty acids (total of the eight examined types of PUFAs) in the VPA males were lower than those in the control males \(p < 0.05\). Therefore, the levels of EPA, DPA, DHA and total fatty acids in serum samples from the VPA females were significantly lower than those from the control females \(p < 0.05\). Only the levels of GLA in serum samples from the control females were lower than those from the control males \(p < 0.05\) (Table 1).

<table>
<thead>
<tr>
<th>PUFAs (μg/ml)</th>
<th>Control Male (n = 12)</th>
<th>Female (n = 18)</th>
<th>VPA Male (n = 14)</th>
<th>Female (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>448.35 ± 29.89</td>
<td>440.33 ± 29.97</td>
<td>323.30 ± 27.67a</td>
<td>381.04 ± 40.21</td>
</tr>
<tr>
<td>GLA</td>
<td>19.28 ± 1.73</td>
<td>13.68 ± 1.47†</td>
<td>13.86 ± 1.60†</td>
<td>16.81 ± 1.97</td>
</tr>
<tr>
<td>ALA</td>
<td>193.76 ± 25.94</td>
<td>202.98 ± 24.78</td>
<td>107.85 ± 24.01a</td>
<td>132.93 ± 33.24</td>
</tr>
<tr>
<td>AA</td>
<td>245.70 ± 11.55</td>
<td>232.01 ± 11.21</td>
<td>198.08 ± 10.69a</td>
<td>225.05 ± 15.04</td>
</tr>
<tr>
<td>EPA</td>
<td>6.80 ± 0.95</td>
<td>9.88 ± 0.87</td>
<td>6.04 ± 0.88b</td>
<td>6.94 ± 1.10†</td>
</tr>
<tr>
<td>DTA</td>
<td>63.87 ± 5.42</td>
<td>57.85 ± 4.62</td>
<td>42.65 ± 5.02b</td>
<td>49.79 ± 6.20</td>
</tr>
<tr>
<td>DPA</td>
<td>12.98 ± 1.29</td>
<td>11.68 ± 1.37</td>
<td>4.75 ± 1.19a</td>
<td>6.53 ± 1.83†</td>
</tr>
<tr>
<td>DHA</td>
<td>63.84 ± 3.87</td>
<td>60.03 ± 3.99</td>
<td>38.04 ± 3.58a</td>
<td>45.20 ± 5.35†</td>
</tr>
<tr>
<td>n-3 PUFAs</td>
<td>281.38 ± 29.48</td>
<td>284.57 ± 28.48</td>
<td>156.67 ± 27.29a</td>
<td>191.59 ± 38.21</td>
</tr>
<tr>
<td>n-6 PUFAs</td>
<td>777.20 ± 38.07</td>
<td>743.87 ± 40.71</td>
<td>577.88 ± 35.24a</td>
<td>672.69 ± 54.62</td>
</tr>
<tr>
<td>Total</td>
<td>1058.57 ± 82.81</td>
<td>1028.43 ± 67.61</td>
<td>734.55 ± 76.66a</td>
<td>864.28 ± 90.71</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.E.M., \(n = 10\), Statistical significance \(p < 0.05\).

* Compared to control males.

† Compared to control females.

![Fig. 2](image-url) Western blot analysis. (A) Immunoblot of Δ5-desaturase, Δ6-desaturase and Elovl2. (B) Immunoblot of caspase-3, Bcl-2 and Bax. (C) Normalization of the densitometry for Δ5-desaturase, Δ6-desaturase and Elovl2 with GAPDH. (D) Normalization of the densitometry for caspase-3, Bcl-2 and Bax with GAPDH. Data expressed as mean ± S.E.M., \(n = 10\), Statistical significance \(p < 0.05\). a compared to control males; b compared to VPA males; c compared to control females; d compared to VPA females.
3.5. Western blot analysis

The expression of Δ5-desaturase (Fig. 2C): Results of two-way ANOVA revealed significant effects of group \(F(1,36) = 42.40, p < 0.001\) and sex \(F(1,36) = 26.61, p < 0.001\). Post hoc comparisons showed that VPA group was significantly down-regulated compared to the control group. Females had higher expression than males in both VPA and control groups.

The expression of Δ6-desaturase (Fig. 2C): Results of two-way ANOVA indicated significant effects of group \(F(1,36) = 118.58, p < 0.001\), sex \(F(1,36) = 11.04, p = 0.002\). Post hoc comparisons indicated that VPA group was significantly down-regulated compared to the control group. Females had higher expression than males in both VPA and control groups.

The expression of Elovl2 (Fig. 2C): Results of two-way ANOVA revealed significant effects of group \(F(1,36) = 42.19, p < 0.001\), sex \(F(1,36) = 10.41, p = 0.003\). Post hoc comparisons showed that VPA group was significantly down-regulated compared to the control group. Females had higher expression than males in both VPA and control groups.

The expression of caspase-3 (Fig. 2D): Results of two-way ANOVA showed significant effects of group \(F(1,36) = 68.81, p < 0.001\), sex \(F(1,36) = 8.09, p = 0.007\), and group × sex interaction \(F(1,36) = 7.78, p = 0.008\). Post hoc comparisons revealed that VPA group was significantly up-regulated compared with the control group. Males had higher expression than females in VPA group.

The expression of Bcl-2 (Fig. 2D): Results of two-way ANOVA indicated significant effects of group \(F(1,36) = 34.42, p < 0.001\), VPA group was significantly down-regulated compared with the control group. There were no sex difference and interaction.

The expression of Bax (Fig. 2D): Results of two-way ANOVA revealed a significant group effect \(F(1,36) = 254.36, p < 0.001\), VPA group was significantly up-regulated compared to the control group, but there were no sex difference and interaction.

3.6. Nissl staining

No pathological tissue abnormalities were observed in the hippocampal CA1 region of the control group: the neurons displayed a round morphology. The nuclear coloration was light, and a complete Nissl body was detected. However, many injured neurons were found in the hippocampal CA1 region of the VPA group; the number of Nissl bodies was markedly decreased, and the neurons exhibited a shrunken morphology (Fig. 3A).

Number of surviving neurons in the hippocampal CA1 region (Fig. 3B): Results of two-way ANOVA revealed a significant effect of group \(F(1,36) = 197.22, p < 0.001\). No sex difference and interaction were observed. Post hoc comparisons showed that the number of viable neurons of the VPA group was significantly lower than that of the control group. Female has more surviving neurons than male in VPA group.

3.7. Biochemical analysis

Liver cytotoxicity was investigated by the serum activity of the hepatic enzyme markers AST and ALT. Prenatal exposure to VPA did not modify the activity of the enzymes in blood as compared to control group (Table 2).

4. Discussion

The results of the present experiments demonstrated that prenatal exposure to VPA on E12.5 had long-term and sex-specific effects on postnatal behaviors, the levels of serum PUFAs, the expression of related liver metabolic enzymes of PUFAs, survival rate of hippocampal neurons and the expression of apoptosis-related proteins in VPA rats.

Consistent with previous studies (Bambini-Junior et al., 2011; Kim et al., 2013b), we found that the VPA male rats showed impaired social interaction and hyperactivity, compared to the control group. Additionally, same behavioral changes were observed in VPA females, compared with control females. Meanwhile, there was a significant sex difference between male and female in VPA group. But in learning and memory, our results differed from Edalatmanesh et al. (2013) study. The impaired social interaction was regarded as the core symptom of autism. Our study revealed that prenatal exposure to VPA may cause impaired social interaction in rats. Generally, once VPA rats learned certain task normally, when challenged to try a new strategy, they showed a lack of flexibility and spent more trials on the first reward arm, which we observed in social interaction test. It could be related to behavioral rigidity (South et al., 2007), which is very typical in autism. The hyperactivity might be partially explained by elevated

<table>
<thead>
<tr>
<th>Table 2: Activity of hepatic enzyme markers in serum.</th>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>AST(U/L)</td>
</tr>
<tr>
<td>ALT(U/L)</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.E.M. Statistical significance (p < 0.05).
anxiety (Schneider et al., 2008), as fear and anxiety have a profound influence on those behaviors in rats. It is well known that autism is often accompanied by learning disorder (O’Brien and Pearson, 2004). In this study, VPA rats showed spatial learning impairment, which is related to the hippocampal neuronal injury. The hippocampal region is involved in social behavior and memory, and regulates behavioral and cognitive functions. The hippocampal neuronal injury will cause series of behavioral and cognitive changes. In this study, the survival rate of neurons in hippocampus CA1 in VPA group was decreased compared to control group, while less effect was observed in VPA females. This indicated that the prenatal exposure to VPA on E12.5 may have a long-term effect on neuronal injury in rats and be sex specific. This could be one of the factors that contributed to the sex difference in behaviors. The next detection of expression of apoptosis-related proteins in hippocampus revealed that the expression of caspase-3 and Bak in VPA rats was up-regulated while the expression of Bcl-2 was down-regulated, compared with control group. Besides, there was a sex difference in the expression of caspase-3 in VPA group, i.e., male was higher than female. The study suggested that male and female rodents in early development could be associated with the different response to teratogenic and toxic substances (Geller et al., 2001). The abnormal expression of apoptosis-related proteins observed in this study was consistent with that observed in the subjects with autism (Sheikh et al., 2010; Malik et al., 2011).

Our data also showed that, under the same dietary conditions, the levels of the eight examined types of PUFAs, n-3 PUFAs, n-6 PUFAs and total fatty acids in serum samples from the VPA males were significantly lower than those from the control males. The serum levels of EPA, DPA, DHA and total fatty acids in VPA females were significantly lower than control females. In our study, the group difference in the serum levels of PUFAs was similar to that observed in subject with autism (Meguid et al., 2008). But there have been no reports of sex difference in study of subject with autism. Only the levels of GLA in serum samples from the control females were lower than those from the control males. There may be many factors affecting the serum levels of PUFAs, in which related metabolic enzymes of PUFAs play an important role. Our study revealed that the expression of related liver metabolic enzymes of PUFAs in VPA group was significantly lower than those in control group and had sex difference in both groups, i.e., female was higher than male. This result may partly explain why the serum levels of PUFAs in VPA group were lower than those in control group. Although a sex difference in the expression of related metabolic enzymes existed, there was no a significant sex difference in the serum levels of PUFAs. The study revealed that the up-regulation of Δ5- and Δ6-desaturase expression in the liver of sexually mature females contributed to their greater capacity for synthesizing DHA from dietary ALA (Childs et al., 2008). Like human subjects, female rats have higher plasma DHA concentrations than males. This could be associated with hormonal regulation of PUFAs metabolism. The concentrations of n-3 PUFAs in rat plasma and tissue are positively associated with circulating concentrations of oestradiol and progesterone and negatively associated with circulating concentrations of testosterone. These findings suggested that sex hormones may act to modify plasma and tissue n-3 PUFAs content, possibly by altering the expression of desaturase and elongase enzymes in the liver (Extier et al., 2010). While in this study, the same diet in both groups aimed to reduce the effect of diet factor. We evaluated the enzyme markers of liver cytotoxicity AST and ALT, as a way of investigating if impairments in VPA rats could be correlated to alterations in hepatic metabolism. However, these parameters were not changed between the VPA and control rats, indicating absence of hepatic damage.

PUFAs are important components of cell membrane phospholipids that promote nervous system development and repair following injury. DHA is an important molecule that participates in cellular signal transduction and cell proliferation (Salem et al., 2001). DHA also promotes the survival of hippocampal neurons, down-regulates the activity of caspase-3, inhibits the apoptosis of neurocytes, and greatly affects nervous system development (Kim et al., 2000; Calderon and Kim, 2007). Studies have revealed that neuroprotectin D1 (NPD1), which is formed from DHA, counteracts H2O2/TNF-α-induced apoptosis via the up-regulation of the anti-apoptotic proteins Bcl-2 and Bcl-XL and the down-regulation of the pro-apoptotic proteins Bax and Bad. In addition, NPD1 inhibits oxidative stress-induced caspase-3 activation and apoptosis (Mukherjee et al., 2004; Lukiw et al., 2005). Therefore, a decrease in the serum levels of PUFAs may be a causal factor of neuronal injury and abnormal expression of apoptosis-related proteins in the hippocampal region in VPA rats.

The sex difference in the PUFAs metabolic enzymes was observed in normal rats. However, the specific mechanism underlying which exposure to VPA on E12.5 leads to the down-regulation of the expression of related liver metabolic enzymes of PUFAs remains unclear. The decreased levels of serum PUFAs cannot be explained only by the down-regulated metabolic enzymes expression. The exact mechanism needs to be investigated in the future studies. In conclusion, this was the first study on the utilization of rats exposed to VPA on E12.5 to describe the sex differences in the serum levels of PUFAs and the expression of liver metabolic enzymes. The results not only demonstrated that the levels of serum PUFAs and related liver metabolic enzymes in the VPA rats were significantly reduced, in association with autism-like behavioral changes, the abnormal expression of apoptosis-related proteins and hippocampal neuronal injury, compared to the control rats, but also showed sex dimorphism characteristics. For the disorders with sex differences, such as autism, systematic investigations of the sex differences in animal models of developmental disorders will lead to a better understanding of the pathogenesis of these diseases.

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References


