Serum Dopamine Level in Acute Murine Toxoplasmosis

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Abstract

Toxoplasmosis is a globally parasitic zoonotic disease transmitted by *Toxoplasma gondii* protozoa. This infection in its chronic form can cause a change in its host's specific behavior and is also associated with developing neuropsychological symptoms in humans. Changes in neurotransmitters' levels, especially dopamine, have been identified as a behavior change factor in the infected host. This study aimed to evaluate serum dopamine levels in acute murine toxoplasmosis. In this study, 50 mice infected with *Toxoplasma* were studied in 5 separate groups, and ten healthy mice were considered a control group. For five consecutive days after parasite injection, blood sampling and serum isolation were performed daily from one of the groups. Serum dopamine levels were measured by HPLC method. Statistical studies showed that serum dopamine on the first to the fourth day after parasite inoculation was the same as the control group, but the fifth day began to increase. The present study results indicate that dopamine production in mice infected with *Toxoplasma gondii* increases from day five after infection. This result suggests that in acute toxoplasmosis, dopamine production is low, and the trend of chronic disease increases dopamine production.

Introduction

Toxoplasmosis is a common parasitic disease among humans and animals. About one-third of the world's population is chronically infected with this protozoal infection (Robert-Gangneux and Dardé 2012; Saadatnia and Golkar 2012). Felidae are the final host, containing sexual parasite stages and product infective step (Oocyst) in their small intestine enterocytes(Innes 2010). Birds and a wide variety of mammals, including humans, are intermediate hosts and carriers of parasitic tissue cysts in their brains and muscles (Dubey 2020). Humans become infected by eating contaminated meat with tissue cysts, water, or vegetables contaminated with the Oocyst and congenitally (Asgari et al. 2011; Dubey 2020; Innes 2010). Infection is often asymptomatic but is especially important in immunosuppressed individuals and congenital forms (Omidian et al. 2020; Robert-Gangneux and Dardé 2012; Shiadeh et al. 2020). Chronic Toxoplasmosis was initially thought to have no clinical significance, but in recent decades the potential role of *Toxoplasma* brain cysts in causing mental disorders in humans has become controversial (Flegr 2013a).

Since the 1950s, researchers have drawn attention to the relationship between Toxoplasma and mental disorders (Torrey and Yolken 2003). The high prevalence of Toxoplasmosis, the high affinity of *Toxoplasma* parasites to the brain in the chronic phase, and the significant concurrency between anti-*Toxoplasma* antibodies and mental disorders have raised researcher's doubts about the causal relationship between parasites and mental diseases (Del Grande et al. 2017; Fekadu et al. 2010; Pearce et al. 2012; Xiao et al. 2018). *Toxoplasma* can manipulate the host brain cells and consequently behavioral changes in their host (Boillat et al. 2020). It seems that the parasite's ability to induce behavioral changes in the intermediate host leads to its predation by the final and other intermediate host and facilitates parasite transmission (Boillat et al. 2020; Hammoudi and Soldati-Favre 2017).

The main suspect for behavioral change in latent Toxoplasmosis in intermediate hosts is dopamine (Flegr 2013a; b). Dopamine is made in mammals by the adrenal glands as well as dopaminergic cells in the brain. This catecholamine is made by removing a carboxyl group from its precursor levodopa (L-dopa). Also, L-dopa is synthesized by the enzyme tyrosine hydroxylase from L- Tyrosine (Berke 2018; Iversen and Iversen 2007). Two aromatic amino acid hydroxylases)AAH1 and AAH2(enzymes have been discovered in the *Toxoplasma*. These enzymes are responsible for catalyzing phenylalanine conversion to tyrosine and tyrosine to L-dopa (Gaskell et al. 2009). The parasite synthesizes L-dopa to build its Oocyst wall (Wang et al. 2017).

Several studies have been performed on the *Toxoplasma* cyst stage's role in altering neurotransmitters' levels, especially dopamine, and subsequently induce behavioral changes (Johnson and Johnson 2020). *Toxoplasma* has been shown to change testosterone levels in addition to neurotransmitters (Bahreini et al. 2020; Kaňková et al. 2011; Lim et al. 2013). Outside the central nervous system, dopamine functions are not clear (Eisenhofer et al. 2004). various roles have been proposed for dopamine, Including local paracrine messenger, vasodilator function (in average concentrations), Help to excretion of sodium and urine, reduces insulin production, reduces gastrointestinal motility to protect the intestinal mucosa, reduces the activity of lymphocytes (Bucolo et al. 2019; Carey 2001; Eisenhofer et al. 2004; R Buttarelli et al. 2011; Sarkar et al. 2010).

In 2009, Gaskell et *al*. Showed that encoding genes)AAH1 and AAH2(enzymes are activated in the chronic phase when tachyzoites converted to bradyzoite (Gaskell et al. 2009). On the other hand, Carruthers et *al*. demonstrated that the *Toxoplasma* parasite could effectively develop schizophrenia, even in the acute phase (Carruthers and Suzuki 2007). Strobl et *al*. observed an increase of *Toxoplasma* tachyzoites proliferation in human fibroblast cell culture media after adding dopamine volumes (Strobl et al. 2012). with these explanations, the parasite's role in changing dopamine levels in the acute phase and its possible effects remains unclear. In our previous study, tyrosine as a dopamine precursor was measured in consecutive days post-*Toxoplasma*-infection in mice models (Asgari et al. 2020). In this regard, this work aimed to measure the serum dopamine levels in acute murine Toxoplasmosis.

Materials And Methods

Ethics approval

This work was aimed to evaluate the serum dopamine levels in acute murine Toxoplasmosis in 2018 in Shiraz, Iran. The present study is based on guidelines for the care and use of laboratory animals (Council 2011). The Ethics Committee of Animal Experiments of the Shiraz University of Medical Sciences approved this research project (permit number IR.SUMS.REC.1398.023).

Parasite's preparation

Toxoplasma gondii parasite RH strain was injected intraperitoneally into BALB/c mice. After 72 hours, the mice were euthanized according to ethical standards. The peritoneal area was flushed with physiological

saline via a 5 cc syringe, and the parasites were collected from the peritoneal site and then washed with PBS. The parasites were mechanically isolated from the host cells bypassing the aspirated fluid through high gauge needles. The solution was then centrifuged at 200 g for 10 minutes to remove cell debris. The supernatant was separated and centrifuged at 800 g for 10 minutes. The sediment was washed three times with Phosphate-buffered saline (PBS) at a pH of 7.2 and prepared as a pure tachyzoite.

Animals

Sixty BALB/c mice aged 6 weeks and weighing 30-35 g were obtained from the Comparative Medical Institute of Shiraz University of Medical Sciences. All animals were kept in standard conditions: temperature of 22±2 °C, the humidity of 60-40%, dark-light cycles of 12 hours, proper ventilation, and access to adequate water and food. Sixty mice were divided into 6 groups of 10. Each group was kept in separate cages. The parasites were subcutaneously injected into groups 1 to 5 (10^5 tachyzoites per mice). The sixth group was considered as the control group (only PBS was injected). After 24 hours from parasite injection, the sampling began so that a group of 10 mice was sampled daily after anesthesia for five consecutive days. Then the samples were taken to the Medical School of the Shiraz University of Medical Science. The serum was isolated and kept at 70 °C until the test.

HPLC

Chromatography conditions

HPLC was used to determine serum tyrosine levels (Waters, USA). Chromatographed Samples on an inverted phase column (Spherisorb C1; Waters) with a C18 column in isocratic mode. A 5% water-soluble acetonitrile at a 1 ml/min flow rate was used as the mobile phase. The absorption of diluted serum and control samples was reading at 225 nm in a UV detector (LC 95; Perkin-Elmer, U Berlin, Germany).

Standard curve preparation

The HPLC apparatus was set up to draw a Standard curve by 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 µg/ml concentration of standard dopamine solution. An amount of 0.01 mg of dopamine was dissolved in 1 cc of 5% perchloric acid to obtain a Homogeneous solution, increased the volume solution to 10 cc, and passing through the syringe filter. Followed by Serial dilution was performed using 5% perchloric acid to obtain the desired concentrations. Finally, Determined concentrations of dopamine solutions were made by absolute ethanol solvent. Each density was run three times in the HPLC device, and the standard curve was drawn using software (SQS 98; Perkin-Elmer) by different concentrations (Fig. 1).

Sample examination

The sera were taken out of the freezer, and after thawing at room temperature, 50 µl of each serum was mixed with an equal amount of 5% (v/v) perchloric acid solution. This step was performed for all 60 samples, injected 50 µl of each sera sample into the device by an HPLC needle. Finally, by applying a

retention time of 3.5 minutes, each sample's curve was drawn at a wavelength of 236 nm. Dopamine peaks of sera samples were determined by comparing the retention times of standard dopamine. (Fig. 2)

Statistical analysis

ANOVA and Post Hoc test evaluated statistical data in software SPSS version 22 (Chicago, IL, USA). P-value≤ 0.05 was considered as statistical differences.

Results

Dopamine concentration in serum samples was calculated based on the standard curve. After preparing standard dopamine solutions in different concentrations and injecting them into the device, the relevant curves were plotted. The standard equation was obtained based on the area below the curve. Then, based on the area below the calculated curve for each unknown sample and the equation's slope, the samples' dopamine concentration was calculated. The mean serum dopamine level on the first, second, third, and fourth days after injection of the parasite was similar and unmeasurable with its level in the control group. The mean serum dopamine level in the fifth group was raised, and significant difference from that in the control group(P=0.042). (Fig. 3).

Discussion

The present study was conducted to evaluate the *Toxoplasma* parasite's effect on changes in blood dopamine levels in acute murine toxoplasmosis. For this purpose, serum dopamine levels of 50 mice as a case group and 10 mice as a control group were measured on 5 consecutive days post-infection. Dopamine levels in the first to fourth days were not different in the case and control groups, but on the Late acute phase (fifth day of infection), dopamine levels increased. Our previous study, which focuses on tyrosine production (one of the dopamine precursors) in acute murine toxoplasmosis, showed the highest and lowest tyrosine levels in the second and fifth-day post-infection, respectively (Asgari et al. 2020). While in the current study, dopamine production began on day 5 after infection. It considered that, decrease the tyrosine levels and increase the dopamine may be correlated in acute toxoplasmosis. It has been shown that the *Toxoplasma* parasite has a tyrosine hydroxylase enzyme that catalyzes L-dopa from L-tyrosine (Gaskell et al. 2009). Decreased tyrosine levels and increased dopamine levels in the late acute phase of mouse toxoplasmosis (day 5) may be related to these two genes' function and activation. And the parasite probably consumption the tyrosine to produce dopamine using these enzymes.

Stiibs et *al*. Showed a similar dopamine production pattern in the control and case group in acute murine toxoplasmosis. But dopamine production in chronic Toxoplasmosis was 14% higher in the case group than in the control group (Stibbs 1985). Mirzaei et *al*. have examined tyrosine levels and dopamine in chronic toxoplasmosis in mice models; They showed low levels of tyrosine and high dopamine levels in the serum (Mirzaeipour et al. 2020). In the present study, we used the parasite's RH strain (lethal strain) to

observe dopamine changes in the acute phase only. This strain is killing mice due to its high pathogenicity (Asgari et al. 2013).

Several studies have been performed to understand the potential of Toxoplasma's induction of behavioral disorders in the intermediate host (Fekadu et al. 2010). Major studies agree on the parasite's ability in its chronic form to alter the levels of neurotransmitters (Flegr 2013a; b). It has been concluded that the parasite in the chronic stage causes mood and physiological changes by manipulating the nervous system function. For example, mice with Toxoplasmosis do not respond to cat urine and its presence; consequently, they are easier to hunt (Berdoy et al. 2000). According to the manipulation hypothesis, *Toxoplasma* parasite has evolved to alter the intermediate host's nervous function system. In doing so, the parasite increases its chances of transmission to the next host and its survival (Flegr 2013b). Unlike the acute phase, Many studies have been conducted to highlight the relation between chronic toxoplasmosis and dopamine brain levels in animal models and humans because of dopamine's importance in behavioral changes (Babaie et al. 2017; Berenreiterová et al. 2011; Parlog et al. 2015; Xiao et al. 2014; Xiao et al. 2018). So, the present study was conducted in BALB/c mice to survey the dopamine level in acute toxoplasmosis. Our result suggests that dopamine production is negligible in the acute phase of infection and begins in part as we approach this phase's end.

Conclusion

Measurement of dopamine levels in the serum of Toxoplasma mice infected five consecutive days after parasite inoculation showed that dopamine production did not change on days one to four compared to the control group. Only on day 5 in some mice dopamine production increased. This result indicates that dopamine production is deficient in Toxoplasmosis's acute period and that dopamine production increases chronically over time.

Declarations

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Conflict of interest

The authors declare that there is no conflict of interest.

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Figures

Figures 1-3 are not provided in this version