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Original Article

Motor defects, dopamine concentration and brain-derived neurotrophic factor in a rat model of Parkinson's disease can be affected by pre-infection with *Toxoplasma gondii*

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Abstract

Introduction: Parkinson's disease (PD) is a neurodegenerative disorder with progressive degeneration of dopaminergic neurons in the nigrostriatal system. *Toxoplasma gondii* (TG) is a parasite that has gene for tyrosine hydroxylase and can produce dopamine. It is not clear whether TG infection has an effect on PD and its motor defect or not. The aim of the present study was to investigate the effects of 6-hydroxydopamine (6-OHDA) model of PD on motor defects, striatal dopamine and brain-derived neurotrophic factor (BDNF) levels in pre-infected TG rats.

Methods: Fifty Sprague-Dawley adult male rats weighing 200-300g were used in five groups. Induction of PD were done by unilateral intra-striatal injection of 6-OHDA, and to prove PD induction the animals were tested for drug-free elevated body swing behavior and bar test. Dopamine and BDNF concentration in striatum were measured by ELISA kits. Giemsa staining of brain smears confirmed TG infection.

Results: The results showed that TG infection prior to PD induction attenuated the elevated body swing bias and the latency in movement on the bar compared to PD rats without infection. The levels of striatal dopamine and the BDNF in TG infected PD rats was significantly higher than the PD rats without infection.

Conclusion: Motor defects in experimental 6-OHDA- induced PD rats can be improved by pre-infection with TG through the increased levels of striatal dopamine and BDNF.

Keywords:
Parkinson's disease;
Toxoplasma gondii;
BDNF;
Dopamine

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Introduction

Parkinson's disease (PD) is the second most commonly diagnosed neurodegenerative disease that affects about 1.5% of people over the age of 65 (Garbayo et al., 2013). Parkinson is produced by the progressive and slow destruction of dopaminergic neurons in the substantia nigra and is characterized by certain clinical symptoms including bradykinesia, akinesia, catalepsy and imbalance in the motion (Dickson, 2012). Nerve inflammation mechanisms are also likely to participate in the cascade of events that lead to nerve damage (Miman et al., 2010).

Toxoplasma gondii (TG) is one of the protozoan parasites that about 30% of the world's population and a quarter of the US population are infected with this parasite (Prandovszky et al., 2011; Beste et al., 2014). Cat's digestive system is exclusively used by TG under a reproductive cycle and ultimately creates oocysts that can infect through the stool. In all other hosts, after the arrival of oocyst into the body, a rapid growth phase called tachyzoite begins. The adaptive responses of the tachyzoite to the host immune system make them distinct to bradyzoite, that it is a part of the life cycle of the parasite; a semi-silent phase in which the parasite remains in the form of intracellular cysts in the muscle and brain tissues (Wang et al., 2015). Research has shown that TG can increase dopamine production levels. It also has the ability to produce antioxidant and anti-inflammatory substance, as well as a brain-derived neurotrophic factor (BDNF) (Ödberg-Ferragut et al., 2000; Xiao et al., 2014). Few investigations can be found on the effect of TG infection on PD in human and experimental animals. Given that humans are greatly exposed to TG and seroprevalence of this parasite is high, its study in humans is not valuable and reliable (Miman et al., 2010). On the other hand, there is an overlap between the cellular pathways of TG and the key genes involved in pathways of PD (Carter, 2013).

With regard to dopaminergic neurons destruction in PD and TG ability to produce dopamine, the present study was aimed to investigate the possible effects of pre-infection with TG on motor defects, intra-striatal dopamine and BDNF levels of PD induced by 6-hydroxydopamine (6-OHDA) in rats.

Materials and methods

All the procedures involving animal subjects reviewed and approved by the Institutional Research Ethics Committee of the School of Veterinary Medicine of Shiraz University. In this study, fifty Sprague-Dawley male rats weighing 200-300g were used. Animals were kept under standard conditions of 22±2°C and 12 hours of light-dark cycle with free access to water and food. Rats were randomly divided into five groups (n=10): 1- control group, intact rats; 2- sham group, in which animals received intra-striatal injection of artificial cerebrospinal fluid; 3- positive control group 1, induction of PD, without TG infection;

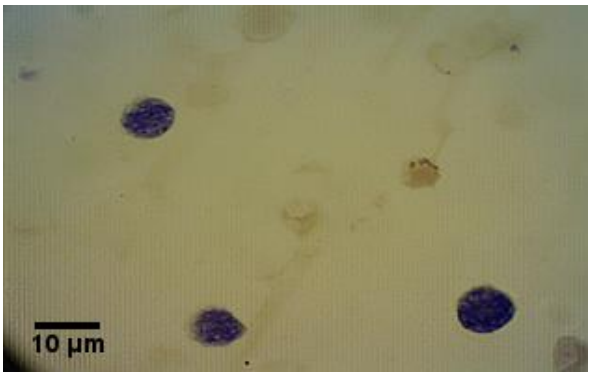

Fig.1. Microphotograph represent the TG cysts in the cerebral hemisphere of infected rats (Giemsa stained, 100 ×10).

Fig.2. Unilateral ink injection site of striatum that represented the injection site of 6OHDA according to atlas of Paxinos and Watson (Paxinos and Watson, 2004).

4- positive control group 2, infection by TG without PD induction and 5- experimental group in which, 8 weeks after TG infection PD induction was done.

For induction of TG infection, tachyzoite of RH strain of Razi Vaccine and Serum Institute was prepared and used. Prior to infection, tachyzoites were counted by a hemocytometer and 10,000 tachyzoites were injected intraperitoneally in each rat. To confirm induction of TG infection, after killing the rats, brain smears were prepared and the presence of tissue cysts in the brain was confirmed by Giemsa staining. Figure 1 confirms the formation of cysts in the brain.

Stereotaxic surgery: ketamine (100mg/kg) and xylazine (8mg/kg) were used as anesthetic. For PD induction, microinjection of 20µg of 6-OHDA (in 4µl of saline containing 0.2mg/ml ascorbic acid) unilaterally in the striatum (coordinates 7.0mm A-P, 3mm ML and 5mm vertically) was performed through stereotaxic surgery. Brain was cut into 300µm thick sections to determine the injection site of striatum according to the atlas of Paxinos and Watson (Fig. 2).

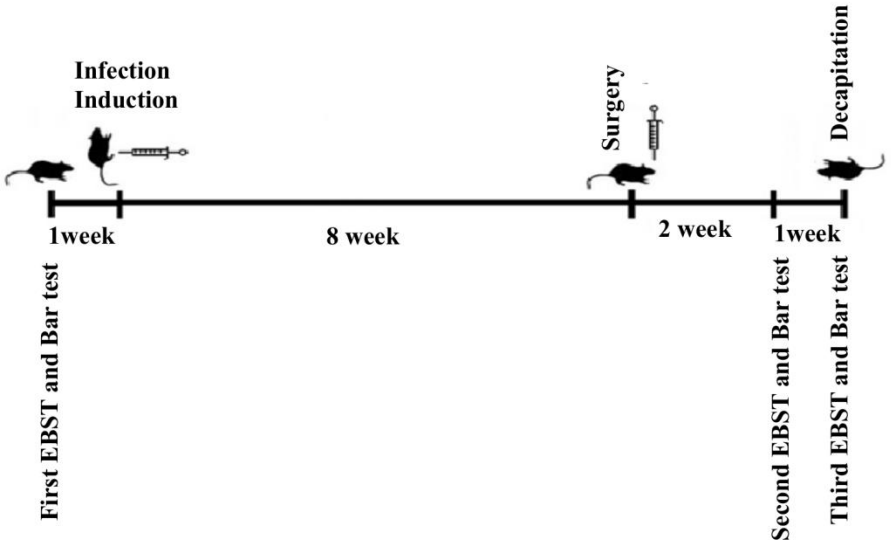

Fig.3. The overall design of the study and scheduling of behavioral tests.

Behavioral tests

To confirm of PD induction, motor defects were evaluated using two methods: 1- elevated body swing test (EBST) and 2- bar test. EBST test was performed according to the method described by Roghani et al. (2002). This test were perform to represent asymmetrical behavior. Bar test behavior was perform to catalepsy measurement in PD rats. The bar test device consists of a bar 0.9cm in diameter at a height of 9cm from the base and the animal was placed slowly on the bar so that both the front paws of the animal are placed on the bar and the time is recorded (Beyer, 2016). Once the animal removes one or both of its hands from the rod, the time is recorded. Both EBST and bar tests were performed 3 times: one week before TG infection, the second and third weeks after stereotaxic surgery (Fig. 2). After behavioral tests, rats were killed to remove the brain. Then the one brain hemisphere was used for confirmation of TG cyst formation and the other brain hemisphere was used to dopamine and BDNF levels measurement.

Dopamine measurement

The dopamine level was measured in striatum tissue using RE59161, BL kit (Hamburg, Germany) at 405nm wavelength.

BDNF assay

The BDNF level in striatum tissue was measured by ELISA kit MBS824814, My Bio Source, Rat BDNF ELISA kit according to manufacturer's instructions.

Statistical analysis

Data were analyzed by SPSS software version 21. Normality of behavioral data was confirmed by the Kolmogorov test. One-way ANOVA and Tukey's post hoc test were used to analyze the data. Data were represented as mean±SEM. The significance level was considered $P<0.05$.

Results

As shown in Figure 4, there was no significant difference between groups during the week before TG induction infection (EBST 1). However, in the second week after induction of PD (EBST 2), significant increase in one-sided oscillation was observed in the positive control 1 group compared to other groups (P<0.001) and the experimental group showed significant increase in one-sided oscillation compared to control, sham groups (P<0.01) and positive control 1 (P<0.0001). In the third week after PD induction (EBST3), positive control 1 group showed a significant increase in the number of one-sided oscillations compared to control ($P<0.001$), sham and positive control 2 groups ($P<0.05$) and experimental group ($P<0.05$). The experimental group showed significant ($P<0.05$) increase in one-sided oscillation compared to control and sham groups.

As can be seen in Figure 5, one week before induction of TG, there was no significant difference in

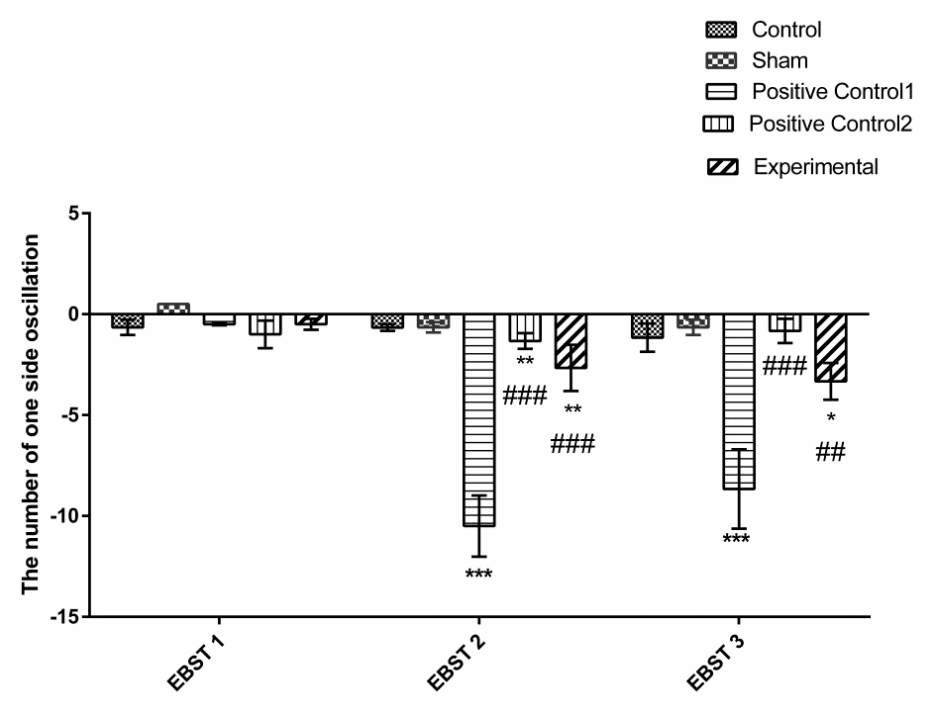

Fig.4. The number of one-sided oscillations in three different time intervals. The abbreviation of EBST 1, EBST 2 and EBST 3 respectively indicate, one week before the TG infection, two weeks after the surgery and three weeks after surgery. *P<0.05 significant difference compared to control and sham groups; **P<0.01 significant difference compared to control and sham groups; ***P<0.001 significant difference compared to control and sham groups; $^{\#\#}$P<0.01 significant difference compared to positive control 1; $^{\#\#\#}$P<0.001 significant difference compared to positive control 1. Negative numbers indicate a turn to the opposite side of the injury. Data are displayed as mean±SEM. EBST: elevated body swing test.

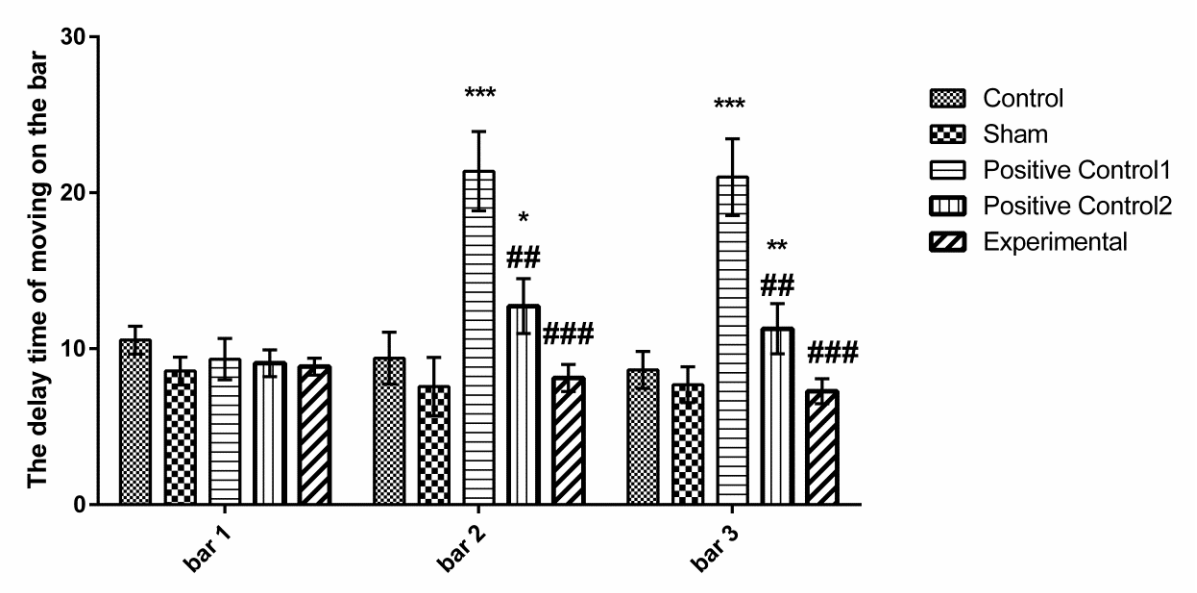

Fig.5. The delay time of moving on the bar, in three different time intervals. The abbreviation of Bar 1, Bar 2 and Bar 3 respectively, one week before the TG infection, two weeks after the surgery and the three weeks after surgery. *P<0.05 significant difference compared to control and sham groups; **P<0.01 significant difference compared to control and sham groups; ***P<0.001 significant difference compared to control and sham groups; $^{\#\#}$P<0.01 significant difference compared to positive control1; $^{\#\#\#}$P<0.001 significant difference compared to positive control1. Data are represented as mean±SEM.

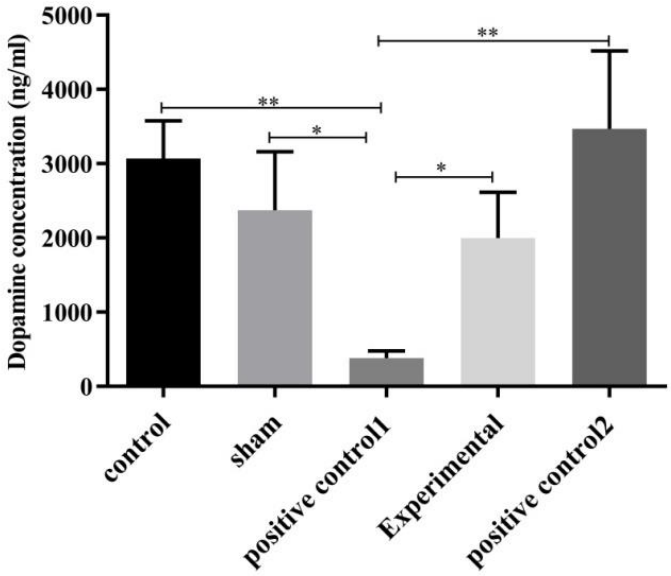

Fig.6. The effect of PD induction 8 weeks following TG infection on the dopamine concentration in the striatum. *P<0.05 significant difference; **P<0.01 significant difference.

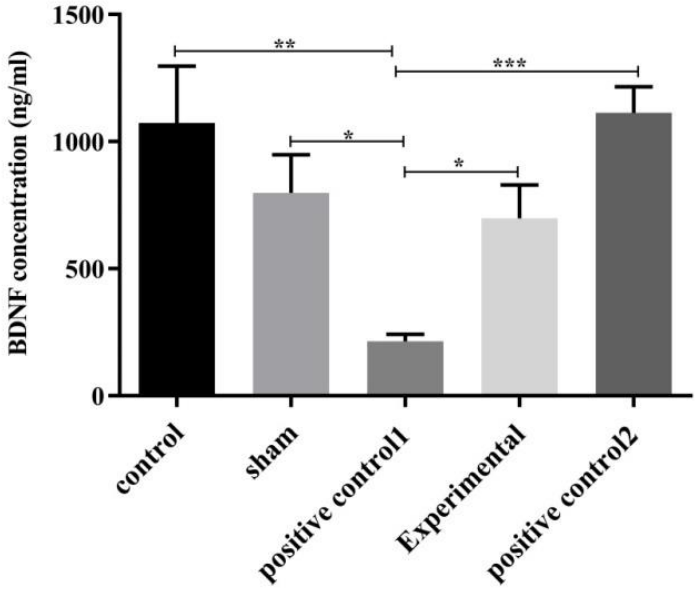

Fig.7. The effect of PD induction 8 weeks following TG infection on the BDNF concentration in the striatum. *P<0.05 significant difference; **P<0.01 significant difference; ***P<0.001 significant difference.

the delay time of moving on the bar between the groups. However, two weeks after induction of PD in rats, there was a significant increase in the delay time of moving on the bar in the rats in the positive control 1 group compared to the control, sham (*P*<0.001), positive control 2 (*P*<0.01) and experimental group (*P*<0.001). While, in experimental rats, there was no significant increase in the delay time of moving on the bar compared to the control and sham groups. Three weeks after PD induction, positive control 1 group showed significant increase in delay time of moving on the bar compared to control, sham (P<0.001), positive control 2 (P<0.01) and experimental groups (P<0.001). The experimental group did not show a significant difference in the delay time of moving on the bar compared to the control and sham groups.

According to Figure 6, induction of PD caused a significant reduction in the concentration of dopamine in the rats in the positive control 1 group compared to the control, positive control 2 (P<0.01), experimental

and sham (P<0.05). In the experimental group, the level of dopamine did not show significant difference compared to the control and sham groups.

Figure 7 shows the striatal concentration of BDNF in rats. In the positive control 1 group, the concentration of BDNF was significantly lower compared to the control (P<0.01), sham, experimental (P<0.05) and positive control 2 groups (P<0.001). However, in the experimental group, the level of BDNF in the striatum did not differ significantly with control and sham groups.

Discussion

One of the neurodegenerative disorders is PD, which is caused by the destruction of dopaminergic neurons in the midbrain and subsequent reduction of the level of dopamine in striatum. Any factor that increases the synthesis of dopamine and protects neurons against dopaminergic degeneration seems to play a useful role in the treatment of PD (Yurek and Fletcher-Turner, 2001; Tieu, 2011). The TG parasite is interest to researchers because of its ability to cross the blood-brain barrier and infect brain without causing neuronal apoptosis, as well as it has extensive effects on host brain biochemistry and behavior (Carter, 2013).

The effect of TG on neurodegenerative diseases such as PD is little known. Therefore, the present study examined the interactions between pre TG infection and PD. The injection of 6-OHDA in the striatum causes damage to the dopaminergic neurons of the nigrostriatal pathway, which is characterized by motor defects, such as, catalepsy and asymmetry.

The results of the present study were shown that positive control 1 group had a high degree of one-sided oscillation compared to other groups, two and three weeks after PD induction, which was consistent with previous studies (Roghani et al., 2002). However, TG infection before induction of PD led to reduction of one-sided oscillation compared to animals in the positive control 1 group. No significant difference was found between the experimental, control and sham groups. The results of this study showed that the delay in movement on bar in the positive control 1 group was significantly higher than that in other groups. However, pre-infection by TG in experimental rats was improved delay time of movement on bar relative to the positive control 1. Therefore, it seems that TG infection before PD induction can improve asymmetrical and bar test behavior in the PD. There are no reports on the effect of TG on the asymmetrical behavior of PD rats.

Two tyrosine hydroxylase-encoding genes exist in TG parasite genome. Tyrosine hydroxylase is a rate-limiting enzyme for the synthesis of dopamine that can be expressed in the brain tissue cysts (Nayebi et al., 2010; Xiao et al., 2013). As a result, the synthesis and release of dopamine increases with infection of dopaminergic neurons with TG. A study has shown that the activity of dopamine synthesis in rat brain after TG parasite has increased (Gatkowska et al., 2013). The results of the present study were shown that TG infection increased the level of dopamine. Improvement of PD behavioral deficits in TG-infected animals is probably due to its ability to increase the striatal dopamine. Nayebi et al. (2010) reported that an increase in the activity of dopaminergic neurons could reduce the 6-OHDA induced catalepsy.

One of the most important neurotrophic factors of the brain is BDNF, which has been identified in different parts of the brain and plays a crucial role in neural plasticity, neuronal survival and neurogenesis in the mammalian nervous system (Gaskell et al., 2009). The expression of BDNF in the PD has been shown to decrease, so increased levels of this neurotrophic factor in the striatum can help improve the disease (Shulman et al., 2011). The results of the present study have shown that PD induces significant reductions in BDNF levels in striatum compared with other groups. However, BDNF levels in the rats of the experimental group have no significant difference relative to the control and sham groups, but in this group BDNF level was significantly higher than that in the positive control 1 group. Xiao et al. (2014) showed that infection with TG increases the expression of BDNF gene in the striatum of rat. On the other hand, the protective effects of BDNF on dopaminergic neurons versus 6-OHDA have been proven (Shults et al., 1995). Therefore, it appears that the TG infection by increasing BDNF gene expression in striatum, protects dopaminergic neurons against the neurotoxic effects of 6-OHDA.

Conclusion

The results of the present study indicate that the TG

infection, due to the presence of tyrosine hydroxylase encoding genes, increases the level of dopamine in the PD rat's striatum. This infection also increases the level of BDNF in this region of the brain. In addition, through its protective effects, the BDNF prevents the damaging effects of neurotoxin 6-OHDA on the dopaminergic neurons in the rat striatum and ultimately prevents the induction of motor defect caused by PD.

According to the present study, it can be found a new solution for PD. Given that the number of people over the age of 65 are increasing in the world, if a vaccine is prepared that can improve the level of dopamine and other factors involved in PG without any side effect of the TG, it will be of great help to human in all around the world.

Acknowledgments

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Conflict of interest

All the authors confirm that, there is no financial or other relationship, which could cause a conflict of interest.

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