THE EFFECTS OF TWO ANTIPSYCHOTIC DRUGS AND PROLACTIN ON *TOXOPLASMA GONDII* TACHYZOITES IN VIVO

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Abstract

Chronic toxoplasmosis is associated with psychotic disorders. Multiple in vitro studies showed that many antipsychotic medications have anti-*Toxoplasma* activity. Meanwhile, there is very limited data regarding the inhibitory effect of them on *Toxoplasma* in vivo. This study evaluated the activity of Chlorpromazine®, Clozapine®, and prolactin (a pituitary gland hormone) on toxoplasmosis infected rats.

Clean laboratory breed rats were chronically infected with non-virulent *T. gondii* strain and classified into 8 groups: negative, positive control groups, chlorpromazine, clozapine, and metoclopramide drug control groups, and 3 infected treated groups. Rats' sera were screened with ELISA test to evaluate level of dopamine, interferon gamma, and prolactin. All drugs were effective against *T. gondii* in infected rats. Dopamine level and INF-γ level were significantly decreased in chlorpromazine infected treated rats. There was significant decrease of dopamine level and increase of prolactin level in clozapine infected treated rats. In metoclopramide infected treated rats prolactin level significantly increased, but INF-γ level significantly decreased.

Key words: Rats, *Toxoplasma gondii,* Antipsychotic drugs, ELISA,

Introduction

Toxoplasma gondii is a zoonotic intracellular protozoan parasite of worldwide distribution infected many species of warm-blooded animals, including birds (Dubey and Beattie, 1988). *T. gondii* effects were not due to manipulation in an evolutionary sense but, to its neuropathological or neuroimmunological effects (Fond *et al*, 2013). Although the medical profession treated these latent cases as asymptomatic and clinically unimportant, results of animal studies and recent studies of personality profiles, behavior, and psychomotor performance led to reconsider this assumption (Flegr, 2013). Epidemiological and neurobiological studies indicated that schizophrenic toxoplasmosis was the second strongest associated after autism (Flegr, 2020). Also, rodents chronic infection with *T. gondii* increased dopamine and metabolite (Ihara *et al*, 2016), a neurotransmitter involved in human schizophrenia (Mahmoud *et al*, 2017).

There were complex mechanisms including immune, humoral, neurotransmitter, epigenetic, genetic, and structural effects (Sorlozano-Puerto, 2016). Psychiatric disorders were mostly mediated by brain immune reaction to *T. gondii* infection affected glutamatergic systems, and *T. gondii* biochemical activity affected dopaminergic systems (Flegr, 2015).

The first antipsychotic inhibition of *T. gondii* was an *in-vitro* study using phenothiazine trifluoperazine (Stelazine), which have membrane-active detergent-like effects on *T. gondii* (Pezzella *et al*, 1997). Other in vitro studies of different antipsychotics and mood stabilizer drugs compared their inhibitory effect on *T. gondii* against trimethoprim effectiveness, the standard toxoplasmosis treatment (Jones-Brando *et al*, 2003; Goodwin *et al*, 2008; Fond *et al*, 2014). Many antipsychotics inhibited cultured *Toxoplasma* (Goodwin *et al,* 2011), but with few limited data on *T. gondii* in vivo (Saraei *et al*, 2016).

Hyperprolactinemia is a common side effect of antipsychotics, and prolactin is one of the most important human hormones in immuno-regulation (López-meza *et*

al, 2010). Prolactin inhibited the multiplication of *Toxoplasma* in murine microglial cell cultures (Benedetto *et al*, 2001), and significantly restricted *Toxoplasma* intracellular growth in mice and human cell lines (Dzitko *et al*, 2010; 2012). Women with hyperprolactinemia had few *T. gondii* prevalence rate (Dzitko *et al*, 2008). Also, *T. gondii*-induced intermediate host behavior alterations and parasite transmission and with postulated relationship between its sero-prevalence and infection in certain disorders such as human schizophrenia (Gatkowska *et al,* 2013).

The present study aimed to evaluate the possible role of antipsychotic drugs and hyperprolactinemia in controlling toxoplasmosis in experimental animal models.

Materials and Methods

This experimental study was conducted from January 2018 to December 2019 according to the ethical rules of dealing with experimental animals.

Drugs: Chlorpromazine (CHZ) (Neurazine, coated tablet manufactured by Misr Pharmaceutical Co., Cairo) as the typical antipsychotics at dose of 30mg/kg/day (Tse *et al*, 2018). Clozapine (CLZ) (Clozaril, coated tablet supplied by Novartis Co., Cairo) as another typical antipsychotics at dose of 5mg/kg/day (Sun and Lau, 2000). Metoclopramide (MTP) (Primpran, coated tablet supplied by Sanofi Aventis Co., Cairo) a prokinetic drug used in nausea, vomiting, diabetic gastric stasis and gastro-esophageal reflux, is a potent stimulator of prolactin release at dose of 5mg/kg/day (Uchida *et al*, 2005). Drugs were separately grinded, dissolved in Ringer`s solution and given orally to Wistar Rats.

Experimental animals: Clean laboratory bred Wister Rats 40days old & about 130 gm each maintained in animal house, Faculty of Medicine was used. The Rh strain of *Toxoplasma gondii* tachyzoites as peritoneal exudate of mice used was kindly obtained from Department of Zoonotic Diseases, National Research Center, Dokki

Rats were intra-peritoneally infected with 5×10^6 tachyzoites. All experiments were done between 6 & 8 weeks' post-infection (Dubey *et al*, 2016). Rats were divided into eight groups of 6 rats each and housed in separate metallic cage with free access to food & water. GI: Negative control, neither infected nor treated. GII: Positive control only infected. GIII: Chlorpromazine, GIV: Clozapine & GV: Metoclopramide only treated positive drug controls. GVI: Infected & chlorpromazine treated with an oral dose of CHZ daily for 3 weeks on the 8th week post-infection (PI). GVII: Infected & clozapine treated with an oral dose of CLZ daily for 3 weeks on the 8th week PI. GVIII: Infected & metoclopramide treated with MTP daily for 3 weeks on the 8th week PI.

Blood was obtained from rats' orbital sinus 60days P.I. and sera were tested for *T. gondii*- IgG antibodies by modified agglutination test (MAT) to ensure infectivity (Mainar-Jaime and Barberán, 2007).

Histopathological examination: After 3rd week post-treatment, rats were intraperitoneally injected with xylazine/ketamine anesthesia (Nelissen *et al,* 2019), and sacrificed by cervical dislocation to prevent pre- and postsynaptic effects of anesthesia, and brains were quickly removed. Portions of brain were fixed in buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin & eosin and examined under low, high and oil immersions.

Neurotransmitter and biochemical markers: Levels of serum dopamine (DA), serum interferon gamma INF-γ & serum prolactin PRL of all rats were done by ELISA with antibody pairs of Sino-Gene-Clon-Biotech Co., Ltd (Hangzhou, China Catalog No: SG-20553, No: SG-20041& No: SG20835). Technique was processed according to the manufacturer given protocol.

Statistical analysis: Data were collected, tabulated, and analyzed by using SPSS version 24. ANOVA & post-hoc tested variance analysis & significance. P value less

than 0.05 were considered significant.

Results

Infectivity: Sera of all infected rats tested for *T. gondii*-specific IgG antibodies by modified agglutination test (MAT) before and after treatment. All showed positive results before receiving drugs. While all sera showed negative result after therapy.

Microscopic examination of negative and drugs controls showed brain parenchyma with unremarkable pathological findings. Positive controls showed infected cerebral parenchyma by tachyzoites were few, vacuolar degeneration was marked in infected cells and sparse inflammatory cells were detected. Infected chlorpromazine treated (CHZ) showed brain parenchyma with unremarkable pathological findings. Infected clozapine treated and metoclopramide treated ones showed brain parenchyma with vacuolar degeneration and multiple inflammatory cell infiltration including eosinophilic cells.

Biochemistry data: Dopamine (DA) level was significantly increased in positive control as compared to negative ones. All drug control groups showed significantly increased DA level as compared to negative control in chlorpromazine (CHZ) and clozapine (CLZ) infected treated groups DA level significantly decreased compared to positive control.

Details were given in tables (1, 2 & 3) and figures (1, 2, 3 & 4).

Table 1: Dopamine level (ng/L) difference between groups.

Groups		Mean ± SD	Median (IQR)	P value
Control	Negative control	10.05 ± 0.7	10 (1)	
	Positive control	26.45 ± 9.4	24 (18)	
Control Treated	CHZ treated	22.20 ± 0.6	22.5 (1)	
	CLZ treated	16.42 ± 0.7	17 (1)	< 0.001
	MTP treated	16.75 ± 0.7	17 (1)	
Infected Treated	CHZ treated	18.27 ± 0.6	18 (1)	
	CLZ treated	19.37 ± 5.9	18.5 (11)	
	MTP treated	20.77 ± 8.9	17.5 (6)	
P-value	GI vs. GII < 0.001	GI vs. GVI = 0.008	GII vs. GVII=0.018	GIV vs. G VII=0.336
	GI vs. GIII < 0.001	GI vs. GVII = 0.003	GII vs. GVIII=0.059	
	GI vs. GIV = 0.035	GI vs. GVIII =0.001	GIII vs. GVI=0.186	
	GI vs. GV = 0.027	GII vs. GVI = 0.008	GV vs. GVIII= 0.177	

There was a significant increase in INF-γ level in positive control as compared to negative one. CHZ & MTB positive controls showed significant decrease of INF-γ level as compared to their negative controls, while CLZ rats showed significant increase. But, in chlorpromazine (CHZ) and (MTB) infected treated INF-γ level significantly decreased compared to their positive controls.

Table 2: INF-Y level (ng/L) difference between groups.

Groups		Mean ± SD	Median (IQR)	P value
Control	Negative control	12.10 ± 2.1	10 (1.5)	
	Positive control	20.47 ± 4.9	20 (8.5)	
Treated Control	CHZ treated	5.63 ± 0.6	6 (1)	
	CLZ treated	38.48 ± 1.1	39 (2.5)	< 0.001
	MTP treated	2.38 ± 0.5	2.5 (1)	
Treated Infected	CHZ treated	11.42 ± 1.2	13.5 (6.5)	
	CLZ treated	20.00 ± 2.6	21 (8.5)	
	MTP treated	13.57 ± 2.3	15 (6.5)	
P-value	GI vs. GII = 0.005	GI vs. GVI = 0.811	GII vs. GVII = 0.870	GIV vs. GVII < 0.001
	GI vs. GIII = 0.028	GI vs. G VII = 0.008	GII vs. G VIII=0.020	
	GI vs. GIV < 0.001	GI vs. GVIII = 0.608	GIII vs. GVI = 0.048	
	GI vs. GV = 0.001	GII vs. GVI = 0.003	GV vs. GVIII < 0.001	

There was a significant decrease in PRL level in sera of positive control as compared to negative one. All drug positive controls showed significant increase in PRL level as compared to negative control All infected treated rats showed significant increase in PRL level compared to each positive control rats.

Table 3: Prolactin level (ng/mL) difference between groups

Groups		Mean ± SD	Median (IQR)	P value
Control	Negative control	16.07 ± 1.1	16 (1.5)	
	Positive control	12.08 ± 1.6	12 (3)	
Control Treated	CHZ treated	26.55 ± 2.6	26.5 (2)	
	CLZ treated	50.43 ± 5.3	50.5 (2.5)	< 0.001
	MTP treated	56.60 ± 4.2	56.5 (2)	
Infected Treated	CHZ treated	17.22 ± 1.6	17.5 (3)	
	CLZ treated	25.52 ± 4.3	26 (8.5)	
	MTP treated	33.98 ± 2.2	33.5 (3.5)	
P-value	GI vs. GII = 0.002	GI vs. GVI = 0.339	GII vs. GVII< 0.001	GIV vs. GVII < 0.001
	GI vs. GIII < 0.001	GI vs. GVII < 0.001	GII vs. GVIII<0.001	
	GI vs. GIV < 0.001	GI vs. GVIII< 0.001	GIII vs. GVI < 0.001	
	GI vs. GV < 0.001	GII vs. GVI < 0.001	GV vs. GVIII < 0.001	

Discussion

No doubt, RH strain, passed for 50+yr as tachyzoites lost not only oocysts capacity formation, but also showed a reduction or absence of tissue cyst (bradyzoites) formation (Dubey *et al,* 1999)

In the present study, chlorpromazine, clozapine, and metoclopramide were effective against *T. gondii*, with the antipsychotic CHZ gave the best impact even with small used dose that caused small prolactin elevation. CHZ is a dopamine D2 receptor antagonist with significant anticholinergic/alpha-adrenergic blocking activity (Kapur, 2003). Association between toxoplasmosis and increased level of dopamine (DA) in brains of animal model contained two genes for enzyme tyrosine hydroxylase (Flegr *et al*, 2003), the key enzyme for the DA synthesis from its precursor and could release it into surrounding tissue of infected animal and also man (Skallová *et al*, 2005; Wang *et al*, 2015)**.**

In the CHZ infected treated group**,** DA level significantly decreased compared to infected control group**. T**his might be related to the feedback regulation effect of dopamine level which means improvement of symptoms caused by toxoplasmosis.

In the present study, INF-γ level significantly decreased in CHZ infected treated rats compared to corresponded control, but significantly increased compared to the CHZ drug control. CHZ independently down-regulated production of various T cell-derived lymphokines (IL-2, IFN-γ, IL-4, TNF, & GM-CSF) and up-regulated the secretion of IL-10 (Tarazona *et al*, 1995). However, *T. gondii* infection led to host immune response, production of pro-inflammatory cytokines, activates Th-cells that secrete IFN-γ; a major cytokine mediates resistance against it (Suzuki *et al*, 1988). So, the present result showed a direct effect of the drug itself on the INF-γ level. But, the most convincing conception that CHZ inhibited replication of *T. gondii* tachyzoites, without significant difference between the INF-γ levels in infected CHZ treated rats and corresponded negative control. Besides, PRL level significantly increased in CHZ infected treated compared to infected control. PRL is inversely proportional with *Toxoplasma* human positivity that increased excretion correlated with decreased toxoplasmosis prevalence and vice versa (Dzitko *et al*, 2008).

No doubt, CHZ is the most dependable effective drug control experimentally induced toxoplasmosis is not just neurotransmitter results but also MAT, detects of *T. gondii* IgG antibodies, converted negative in sera of all infected rats after therapy. Besides, unremarkable pathological findings were revealed in rats' brain parenchyma, rather than other drug tested.

The inhibitory effect of CHZ drug on *Toxoplasma* infection in the present study agreed with similar previous findings in different in vitro studies. It was found that the valproate (mood-stabilizing agent) and haloperidol may have respectively a higher in vitro anti-toxoplasmic activity com-

pared to trimethoprim. While chlorpromazine and fluphenazine showed some activity but less than trimethoprim (Jones-Brando *et al,* 2003). This result was further replicated in animal models that showed improvement in the suicidal attempts in rats (Webster *et al*, 2006). A partial replication of this study was carried out using some different methods and assays. In this study, haloperidol and valproic acid had no measurable effect, but fluphenazine, thioridazine, and trifluoperazine did (Goodwin *et al*, 2008; 2011). Also, Fond *et al.* (2014) showed that fluphenazine and zuclopenthixol possessed a good inhibition of *T. gondii,* whereas haloperidol, levomepromazine, and loxapine had a fair inhibition. However, the result was conflicted with in-vivo study of Saraei *et al.* (2016), who reported that thioridazine and fluphenazine could not significantly inhibited brain cystogenesis of *T. gondii* in mice. Only, each of them reduced insignificantly the percent of cysts at higher dose compared to lower one.

CHZ acted by directly minimizing *T. gondii* replication and invasion of host brain cells in vitro (Jones-Brando *et al*, 2003), anti-*T. gondii* activity was related to drug calcium inhibitory properties (Ballejo *et al*, 1986).

T. gondii tachyzoites require calcium to invade host cells, and this invasion is inhibited by calcium channel blockers and calmodulin antagonists, including trifluoperazine, another phenothiazine antipsychotic (Pezzella *et al*, 1997; Carruthers *et al*, 1999). Transient rises in intracellular calcium within tachyzoites serve as a trigger for parasite egress from host cells and participate in the cell gliding motility and host-cell invasion processes (Nagamune *et al*, 2008; Lourido *et al*, 2010). Calmodulin may apply its role in invasion by mediating calcium-dependent conoid extrusion and through calcium-stimulated lytic enzymes secretion at the attachment site on the host cell. In these ways, calmodulin

inhibition would suppress tachyzoite invasion and protect host cell monolayers (Pezzella *et al*, 1997).

One could propose that CHZ's superior therapeutic impact here may be through a combination of both its ability to inhibit replication of *T. gondii* and to decrease, directly and indirectly, dopamine levels.

In the present study, clozapine gave moderate anti-*Toxoplasma* activity as biochemical findings showed a significant decrease of DA level and increase of prolactin level in comparing CLZ infected treated rats with the infected control ones. Moreover, MAT became negative after three weeks' therapy and brain histopathology revealed inflammatory cells and vacuolar degeneration without tachyzoite cyst.

CLZ is the prototype agent in Second-generation (atypical) antipsychotic drugs. These drugs target the dopamine D2 receptor, as well as the serotonin receptor, and, as a class, bind less tightly to the dopamine receptor than typical antipsychotics (Miyamoto *et al*, 2005; Seeman, 2002). Also, it is adrenergic ($\alpha 1$), histaminergic (H1), and muscarinic acetylcholine receptor antagonist (Kapur and Remington, 2001). Consequently, it also increases PRL secretion (Kuroki *et al,* 2008).

The present study showed that CLZ high significantly increased INF-γ level compared to CLZ drug control with negative control rats. Also, it significantly decreased CLZ infected treated rats compared to its drug control. But, the INF-γ level showed an insignificant result in comparing CLZ infected treated rats to the positive control ones. Song *et al.* (2000) in vitro showed that with a different concentration of CLZ had a different effect on cytokine production by human whole blood. CLZ 10^{-6} & 10^{-8} M (microliter) significantly increased the unstimulated and stimulated production of IL-1RA (receptor antagonist). The CLZ 10^{-6} M significantly increased the IFN-γ production, which led to intracellular degradation of tryptophan

by the host cell and restricted the *T. gondii* growth in two ways (Vogel *et al*, 2014). Tryptophan metabolites were toxic to the parasite in a manner that was reversible by tryptophan. Degradation tryptophan reduced its concentration in host cell and the intracellular parasites would lack essential amino acid (Goodwin *et al*, 2011). This agreed with in vitro study of Jones-Brando *et al.* (2003) who found that risperidone, clozapine, & quetiapine (mood-stabilizing agent) had moderate anti-toxoplasmosis activity less than trimethoprim. A partial replication of this study was done using different methods and assays. In this study, clozapine had no measurable effect (Goodwin *et al*, 2008; 2011). But, in another study, amisulpride, risperidone and valproic acid showed poor *Toxoplasma* inhibition (Fond *et al*, 2014). The present study disagreed with Saraei *et al.* (2015), who reported that aripiprazole had no inhibitory effect on brain cystogenesis of *T. gondii* ME49 strain explained that by second generation antipsychotics were not calmodulin antagonists, and even some cellular actions of clozapine showed dependence upon calmodulin, a prototypical calcium sensor (Chin and Means, 2000) activated kinase activity (Shin *et al*, 2006).

In the present study, no reason was to exclude the idea that actions of atypical antipsychotics drugs on ion channels also contribute to inhibition of tachyzoite propagation in host cells. Multiple mechanisms may be at work here. CLZ exhibits anti-inflammatory activity through the inhibition of calcium/calmodulin-dependent Akt, alpha serine/threonine-protein kinase, activation in microglia (Jeon *et al*, 2018), which results in the inhibition of cell cycle progression (Shin *et al*, 2006). Clozapine increase host cell cytosolic Ca^{2+} and thus significantly decreased tachyzoite invasion (Pezzella *et al*, 1997). *In-vivo*, the intracellular Ca^{2+} increased concentration via up-regulation of neuronal Ca sensors, such as visinin-like protein 1 (VILIP-1) &

neurocalcin δ (NCALD) (Kedracka-Krok *et al*, 2015).

Metoclopramide MTP is a potent dopamine receptor antagonist but does not affect the central DA. It abolishes the inhibitory effect of dopamine on the PRL secretion that led to PRL level increase (Dzitko *et al*, 2008).

In the present study, MTB gave a moderate inhibitory effect in *T. gondii* by significant increased prolactin level, and decreased INF-γ level, with insignificant decreased dopamine. But, MAT became negative after three weeks where brain showed inflammatory cells and vacuolar degeneration without tachyzoites. MTP increased the PRL level and stimulated Th1 cytokine production, especially IFN-γ excretion (Matalka, 2003). MTP acted as an immunomodulating agent with release cytokines by macrophage as IL-1 & IL-6 (Abd Ellah *et al*, 2019).

Based on present study data, higher levels of the prolactin in patients' sera may restrict the *T. gondii* tachyzoites growth. But, this disagreed with Mavoungou (2006) who found that prolactin concentration did not differ according to *P. falciparum* status, despite its increase during pregnancy, regardless of parity. Also, Dzitko *et al.* (2008) reported increased prevalence of latent toxoplasmosis in women with an aberrant level of prolactin. However in favor of the present study, the pre-incubation of *Toxoplasma* tachyzoites with the recombinant human prolactin (rhPRL) in vitro caused a significant reduction (up to 36.15%) in its replication abilities due to a limited parasites capacity to penetrate human cells (Hs27 y HeLa) as noticed by reduced number of infected cells (Dzitko *et al*, 2010). Besides, Dzitko *et al.* (2012) found a significant increase in serum PRL level during pregnancy limited *T. gondii* spreading, and exogenous human recombinant prolactin (rhPRL) & autologous endogenous prolactin (sPRL) from inactivated sera significantly restrict-

ed *Toxoplasma* intracellular growth in peripheral blood cultures. *T. gondii* replication was reduced in murine L929 cells prolactin treated with (Dzitko *et al*, 2013). Protective immune response of man and animal infected with intracellular pathogens as *T. gondii* was based mainly on IFN-γ synthesis (Denkers, 2003). PRL may play a role similar to IFN-γ, because, alone or together with TNF-α, it stimulates the intracellular killing of the parasite (Benedetto *et al*, 1995). Also, PRL at a normal physiological concentration enhanced IFN-γ production in NK cells (Matera *et al*, 1999). It stimulated Th1 cytokine production, especially IFN-γ excretion, by mononuclear cells, due to activation of interferon regulator factor-1 (Peeva *et al*, 2004). But, PRL induced the secretion of cytokines such as IL-6 & IL-10, a cytokine with a broad immunoregulatory activity that controls an inflammatory response during acute toxoplasmosis. Also, cytokine is a unique product of Th$_2$ lymphocytes, antagonizes many effects of IFN-γ (Lang *et al*, 2007). So, possible PRL action could be bidirectional, namely, PRL limited *Toxoplasma* proliferation via surface host cell receptors (Dzitko *et al*, 2013) led to release of protective type-1 cytokines (Matalka, 2003), and inhibited penetration ability (Dzitko *et al*, 2010).

Conclusion

Antipsychotic chlorpromazine drug has good anti-*Toxoplasma* activity in rat. Psychotropic drugs for anti-*Toxoplasma* activity in schizophrenic patients might improve prognosis and treatment efficacy. Exact mechanism of interaction between hormone, host cell, and parasite is still unknown. Chlorpromazine may affect *T. gondii.*

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Explanation of figures

Fig.1. Brain (stained with H & E, x1000) represents A. negative-control (GI). B. positive-control (GII) arrow pointed to a tachyzoite. C. chlorpromazine infected treated (GVI). D. clozapine infected treated (GVII) circle surround eosinophilic cell. E. metoclopramide infected treated (GVIII).

Fig. 2: Mean dopamine level among groups.
Fig. 3: Mean INF-Y Level among groups.
Fig. 4: Mean prolactin level among groups.