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SHORT COMMUNICATION

Comparative analysis of anti-toxoplasmic activity of antipsychotic drugs and valproate

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Abstract Recent studies have shown a strong link between *Toxoplasma gondii* infection and psychiatric disorders, especially schizophrenia and bipolar disorders (odd ratio ≈ 2.7 for each disorder). Antipsychotic drugs and mood stabilizers may have anti-toxoplasmic activity that potentially may be associated with better effectiveness in these disorders, but previous results have been few in number and conflicting. We therefore sought to determine which daily prescribed antipsychotics and mood stabilizer have the best anti-toxoplasmic activity during the development phase of the parasite. In the present study, we examined the effects of commonly used antipsychotic drugs (amisulpride, cyamemazine, fluphenazine, haloperidol, levomepromazine, loxapine, olanzapine, risperidone and tiapride) and one mood-stabilizing agent (valproate) on toxoplasmic activity. We replicated that fluphenazine has a high anti-toxoplasmic activity, but it does not seem to be a phenothiazine-specific class effect: indeed, we found that another first-generation antipsychotic, zuclopenthixol, has a high anti-toxoplasmic activity. Valproate, tiapride and amisulpride have no anti-toxoplasmic activity on parasite growth, and the other antipsychotic drugs showed low or intermediate anti-toxoplasmic activity. As it is not possible to know the intracellular concentrations of antipsychotics in the brain, further clinical studies are warranted to determine whether these in vitro findings have potential implications in treatment of toxo-positive patients with schizophrenia. These findings may be potentially relevant for the choice of the first-line antipsychotic drug or mood stabilizer in previously infected patients.

Keywords *Toxoplasma gondii* · Antipsychotic · Mood stabilizer · Schizophrenia · Calmodulin · Phenothiazine

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Background

The implication of infectious events in the development of major psychosis has recently gained increasing attention (see for review [2, 3, 7]). Rubella, herpes simplex virus (HSV), cytomegalovirus (CMV), *Toxoplasma gondii* and other infections have been shown to be potent disrupters of fetal neurodevelopment, leading to abnormalities of brain and behavior, including psychiatric disorders. In this context, one of the most studied link between a pathogen and psychiatric disorders concerns the association between *T. gondii* and schizophrenia [1, 5, 11, 17, 18, 22, 23, 28, 29] but also recently in bipolar disorders [9].

Toxoplasmosis is a major public health concern: it is the most common protozoan parasite infection in the western

developed countries, and it concerns one third of the global human population [14]. All vertebrates can be infected as intermediate hosts. Two types of infections have been described in humans: congenital infection (transmission from mother to child during pregnancy) and acquired infection, the subject contaminates itself orally (by undercooked meat from infected animals or parasite-contaminated vegetables) or through contact with infected cats, cats being the definitive host of the parasite [6]. The parasite, after crossing the intestinal barrier, infects all types of nucleated cells by forming an intracellular vacuole. This intracellular parasite alters the expression of host cell genes (including brain cells) and persists in the form of cysts, which can reactivate and release parasites by neo-spread throughout the host, depending on its immune status. Therefore, the parasite exists in two forms in the human body: tachyzoites are found in the acute phase of infection, and bradyzoites that are present in the cysts during chronic infection. Medicine currently considers clinically asymptomatic latent infection as unimportant in immunocompetent individuals [27].

Several studies have shown, however, behavioral changes induced by infection in animals: Toxoplasma is an obligate intracellular parasite capable of infecting 30 % of microglial cells and 10 % of neurons and astrocytes among rats [4]. The tachyzoites manipulate the cells: they alter the expression of genes coding for apoptosis, anti-microbial functions and immune cell maturation. Again in mice, chronic infection with *T. gondii* causes an increase in 14 % of brain concentrations of dopamine [21], a neurotransmitter involved in schizophrenia in humans [20]. It was further demonstrated that treatment with a dopamine reuptake inhibitor suppressed exploratory behavior in infected male mice, whereas it had the opposite effect in controls, suggesting lasting changes in response to dopaminergic stimulation in infected rodents [20]. To our knowledge, the mechanism behind this difference has not been elucidated yet in the literature.

The contextual link between Toxoplasma infection in humans and psychiatric disorders (in particular schizophrenia) can be summarized as follows: (1) proven *T. gondii*'s neurotropism and its impact on dopamine pathway [19], (2) shared epidemiological characteristics between Toxoplasma exposure and schizophrenia such as urban living, (3) parallel increase in *T. gondii* infection and incidence of psychosis (especially schizophrenia) in various populations (USA, Turkey and Iran) [2, 24]), (4) a significantly high levels of antibodies to *T. gondii* in maternal sera whose offspring(s) subsequently develop schizophrenia later in life [26] and (5) a 2.73-fold increase in overall odds of *T. gondii* seropositivity for schizophrenia measured in a meta-analysis [22]; the same result has been recently found in bipolar disorders [9]. It has been recently hypothesized that *T. gondii* may reactivate human endogenous retroviruses (HERV) that may lead to brain immunoinflammation and then mental disorders [10, 16]. It may then be advantageous to choose an antipsychotic and/or mood-stabilizing molecule having anti-toxoplasmic properties in patients that have been previously infected by Toxoplasma.

However, conflicting results have been reported on anti-toxoplasmic activity of psychotropic drugs. In particular, Holfels et al. [12] studied the in vitro effects of the calmodulin inhibitor trifluoperazine hydrochloride, as well as other antimicrobial agents on *T. gondii* tachyzoites. Trifluoperazine hydrochloride was not inhibitory at lower concentrations, but higher concentrations were toxic to cell cultures in vitro, and therefore, their assay could not be used to assess its effects. Nine years later, Jones-Brando and collaborators [13] found that haloperidol had the highest therapeutic index (18.4), followed by 9-OH-risperidone, the principal metabolite of risperidone (6.7), and fluphenazine (5.1). The other antipsychotics such as chlorpromazine (TI = 2.3) had modest therapeutic indices.

The aim of the present study was thus to determine which antipsychotics or mood stabilizers prescribed in daily practice have the best anti-toxoplasmic activity during the development phase of the parasite. The present study is innovative because it tests one mood-stabilizing drug on the development phase of the parasite and 5 antipsychotics never tested.

Materials and methods

Cells and parasites

Parasites were maintained by serial passage in human foreskin fibroblasts grown in Dulbecco's Modified Eagle Medium (DMEM) (GibcoBRL, http://www.invitrogen.com) supplemented with 10 % fetal calf serum (FCS) and 2 mM glutamine. Tachyzoites of the RH strain of *T. gondii* were used throughout the study. Intracellular tachyzoite multiplication was measured on HFFs plated on 12-mm coverslips in 24-well plates and fixed 16 h after infection by equal numbers of freshly released tachyzoites. Infection was performed by incubating a parasite suspension in normal culture medium for 4 h on the monolayers; then, the wells were washed with medium, and a dilution in culture medium of the drug to be tested was added to the well for the duration of the experiment. Coverslips were then fixed with methanol and stained with eosine–methylene blue (RAL 555) and then mounted permanently (Pertex; Microm Microtech, France). In all cases, the mean number of parasites per vacuole in the control was in the 4–8 ranges.

Drugs and range testes

All antipsychotics and mood stabilizers used in daily practice in France were tested, that is, amisulpride, cyamemazine, fluphenazine, haloperidol, levomepromazine, loxapine, olanzapine, risperidone, tiapride, valproate and zuclopenthixol. In all cases, the commercial soluble formulation was used, and diluted in the culture medium. Growth inhibition was measured as the ratio between parasite number par vacuole in the drug treated versus untreated cultures. In an initial screening (Table 1), fields were randomly selected, and the numbers of parasites per vacuole were counted using a 40× objective in ten fields per coverslip, with three coverslips per assay.

In a second phase, we chose the antipsychotic which showed the highest anti-toxoplasmic activity in the screening phase, to determine precisely its IC 50 using 3 independent experiments. Data were analyzed using GraphPad/Prism4, to produce a nonlinear regression curve reporting the ratio of intracellular growth versus control to the drug concentration. The IC 50 was calculated from the three replicates with a confidence interval of 95 %. We also chose fluphenazine as a positive control, and haloperidol for which results in the literature are contradictory.

Results

An initial single screening involving all drugs diluted in the range 1–50 μM was performed and the results are reported in Table 1. We differentiated the specific anti-toxoplasmic activity from the general toxic effect, which entirely destroys the cell monolayer ("toxic") or causes obvious damages (i.e., pycnotic or loosely attached cells) due to drug exposure ("subtoxic").

Table 1 Preliminary screening of antipsychotic drugs anti-toxoplasmic activity

Concentrations (μM)	10	50
Amisulpride	1	1.09
Cyamemazine	0.54	Subtoxic
Fluphenazine	0.19	Toxic
Haloperidol	0.73	0.24
Levopromazine	0.68	Subtoxic
Loxapine	0.69	0.35
Olanzapine	1	0.56
Risperidone	0.93	0.82
Tiapride	1	1
Valproic acid	1	1
Zuclopenthixol	0.37	Toxic

The values reported correspond to the ratio between the mean numbers of parasites par vacuole in treated versus non-treated samples fixed 16 h after infection. We differentiated the specific anti-toxoplasmic activity from the general toxic activity, which destroy the cell monolayer ("toxic") or cause obvious damages due to drug exposure ("subtoxic")

Table 2 Anti-toxoplasmic dose-effect of zuclopenthixol, haloperidol, and fluphenazine (3 independent experiments per antipsychotic, except for fluphenazine)

Concentrations (μM)	Zuclopenthixol	Haloperidol	Fluphenazine
2	84.03 ± 4.67	97.89 ± 4.77	73.61 ± 2.78
5	71.19 ± 7.76	nd	40.81 ± 2.54
10	43.18 ± 6.19	82.33 ± 9.26	26.55 ± 1.78
20	27.07 ± 5.76	65.39 ± 6.17	nd

Data are reported as mean growth ratio ± SD

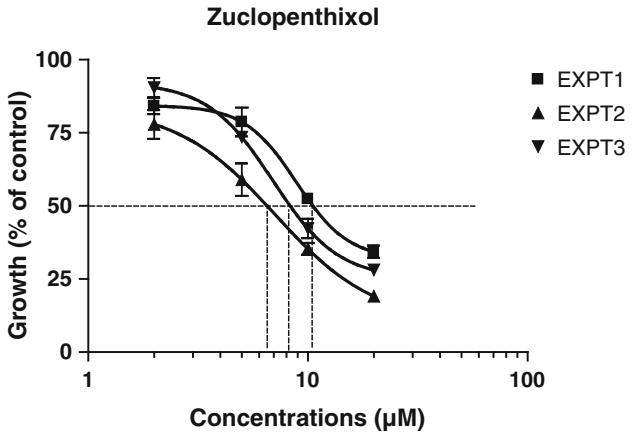

Fig. 1 Dose-effect inhibitory activity of zuclopenthixol on Toxoplasma tachyzoites' growth. The nonlinear regression curves correspond to the 3 independent experiments, the IC 50 was then calculated as a mean with a 95 % CI from the 3 curves

Amisulpride, tiapride and valproate did not have inhibitory activity in the assay at 10 or 50 μM. Among the others, zuclopenthixol appearing to be the most efficient after fluphenazine was further explored. Haloperidol having been reported before to produce contradictory results was also analyzed. The fluphenazine assay was performed once, as a control; since the result was similar to published data (IC 50 3.5 μM) [8], it was not replicated.

Zuclopenthixol showed a high anti-toxoplasmic activity with an IC 50 of 8 ± 1.8 μM (Table 2, Fig. 1). The IC 50 of haloperidol was beyond the range of concentration tested (0–20 μM) and could not be estimated.

Discussion

Our results are potentially important to improve our ability to prescribe antipsychotics or mood stabilizers based on etiological pathways. Choosing psychotropic drugs according to their anti-toxoplasmic activity might in the

future improve the efficacy of these treatments and thus the prognosis of toxo-positive patients. The number of tachyzoites, the solvents, the time of addition of drug versus parasite and the length of time post-infection until the assay was stopped were all different in previous studies [8, 12, 13]. As we chose to focus on the development phase of the parasite, we chose to reproduce Goodwin et al. [8] methodology. We confirmed that fluphenazine has a very good anti-toxoplasmic activity, but also that zuclopenthixol, another first-generation antipsychotic drug, has a high activity against the parasite development (with an IC 50 of 8 μM), which invalidates the previous hypothesis of a phenothiazine-specific class effect (zuclopenthixol belongs to the thioxanthene class) [8]. We also confirmed Goodwin et al. findings about haloperidol that has no anti-toxoplasmic activity in the 1–10 μM range.

Moreover, we performed a preliminary assay on several antipsychotics that have never been reported before (amisulpride, cyamemazine, loxapine, levopromazine and tiapride) which is important for further studies on the in vivo anti-toxoplasmic activity of antipsychotics in schizophrenia. Amisulpride, a second-generation antipsychotic, showed no anti-toxoplasmic effect, whereas cyamemazine levopromazine and loxapine (first-generation antipsychotics), frequently prescribed in treatment of acute psychotic agitation, have intermediate anti-toxoplasmic activity. As we did not have any soluble form at the moment of the experiments for clozapine, it was not tested, but Goodwin et al. [8] found no anti-toxoplasmic activity for this antipsychotic drug.

Another finding of interest is that valproate, a frequently prescribed mood stabilizer in bipolar or schizo-affective disorders, has no activity on parasite development, whereas it was found to have high anti-toxoplasmic activity on parasite invasion [13]. Given that invasion is likely a more elusive target in that development; valproate may probably be less efficient as anti-toxoplasmic agent in major psychiatric disorders.

Limits

None of these drugs, as far as we know, affect the tissue cyst stage of the parasite. We do not know also whether the drug concentrations used in vitro correspond to therapeutic concentration reached in vivo. The only available data concern plasma levels of antipsychotics in usual clinical doses [15, 25]. For example, zuclopenthixol blood levels vary from 4 to 50 ng/mL (0.01–0.12 μM) and that of fluphenazine from 1 to 10 ng/mL (0.02–0.2 μM), which is well below the concentrations reported here, as these antipsychotic are classified in "low-level drugs", contrary to thioridazine, the blood levels of which rank from 0.5 to 5.4 μM [15]. Thioridazine is a phenothiazine that was found by Goodwin et al. to have a high anti-toxoplasmic activity (IC 50 = 1.2 μM) but is not available in a soluble form in France and is unfortunately rarely prescribed. However, another important point is that antipsychotics may accumulate in the brain, and intracerebral concentrations may be much higher than plasmatic ones. Another limitation to the application of these in vitro findings is that these molecules give rise to many active derivatives when administered in humans, which can modify the drug anti-toxoplasmic activity.

Conclusion

We have confirmed one major finding of Goodwin et al., that is, that fluphenazine has a high anti-toxoplasmic activity, but also showed that this effect is not phenothiazine class specific. We found indeed that another first-generation antipsychotic, zuclopenthixol, has a high anti-toxoplasmic activity, which may be a finding of interest for further research on Toxoplasma in schizophrenia. This study raises some potentially important questions that could be the focus of future research. Further studies are warranted to determine whether these in vitro findings have potential implications in treatment of Toxoplasma infection among patients with schizophrenia and bipolar disorders. These findings may be of major interest for the choice of the first-line antipsychotic drug, in the context of emergent biomarkers for mental illnesses and the urgent need for developing personalized medicine.

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Conflict of interest Dr. G. Fond, Dr. A. Macgregor, Pr. M. Leboyer, Pr. R. Tamouza, Dr. N. Hamdani, Dr. A. Meary, and Dr. J. F. Dubremetz declare no conflict of interests in connection with the present study.

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